Collection of baseline data on humpback whale (*Megaptera novaeangliae*) health and causes of mortality for long-term monitoring in Western Australia

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Photo: D. Coughran

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Summary

Since 2008 an unprecedented number of humpback whales (*Megaptera novaeangliae*) have stranded in Western Australia (WA). Between 1989 and 2007 the mean number of humpback whales ashore was between 2 and 3 animals (range: 0-5). In 2008 there were 13 strandings followed by 46 in 2009 and 16 in 2010. The aim of this project was to initiate the collection of data by post-mortem examination of stranded whales in 2011 in order to: 1) identify and characterise factors associated with strandings; and 2) determine baseline and epidemiological information on disease and the nutritional status of stranded whales.

In 2011 there were 17 strandings consisting of 14 calves and 3 juveniles/sub-adults. Unlike the age categories reported for 1989 – 2009 (44% of strandings were calves of that year [i.e. calves born in that calendar year/breeding season], 37% were juveniles/sub-adults and 19% were adults) and in 2010 (31% of strandings were calves of that year, 63% were juveniles/sub-adults and 6% were adults) most of the strandings in 2011 were neonates with most animals thought to be less than 48 hours of age. Furthermore, there was no evidence of anthropogenic activity (e.g. ship strike/entanglement) associated with any of the 2011 strandings.

All reported strandings occurred between Exmouth and Stokes Inlet east of Esperance. Thus all stranded neonates were born at least 1000 km south of the currently known breeding grounds between Broome and the northern end of Camden Sound.

In February and March 2011, water temperatures off the south-western coast of WA rose to unprecedented levels due to an extremely strong *La Niña* event and a record strength Leeuwin Current. It is unknown if this unusual 'marine heat wave' had any impact on humpback whale calving site selection.

Post-mortem examinations were carried out on three of the stranded neonates in 2011. Significantly, two of the neonates were found to be in an extremely malnourished state as evidenced by severe generalised adipose hypoplasia. It is likely that these neonates were non-viable from birth due to the lack of lipids in their blubber. Blubber lipids are needed for

energy, thermoregulation and buoyancy. In addition to being malnourished, one of the neonates was also found to have severe interstitial pneumonia of unknown cause which would have compromised its ability to breathe and also contributed to its death.

In addition to the data collected from the three neonates that underwent post-mortem examination; photos, blubber samples, and blubber depth measurements were taken from an additional five neonates that stranded. It was thus possible to assess the nutritional status of eight neonates in total. The results of the visual assessment of body condition and analysis of blubber lipid content indicated that all but one of the eight neonates was in a state of severe malnutrition. Consequently these neonates were likely to be non-viable from birth due to a lack of energy reserves and a compromised ability to thermoregulate and control buoyancy. The malnutrition observed in the neonates is likely a reflection of the poor nutritional status of the mothers.

A number of theories could be postulated to account for the high proportion of neonate strandings in 2011:

1. Associated with increased population size and inherent high mortality rate in humpback calves.
2. Associated with parturition occurring in unsuitable areas outside of the known breeding grounds due to environmental conditions.
3. Associated with mothers in a poor nutritional state giving birth to malnourished non-viable calves.

Theory one is unlikely due to the population increasing gradually and 2011 standing out as atypical – given that in previous year's calves accounted for a much lower proportion of strandings. The extent to which theory two may have contributed to strandings in 2011 is difficult to say. The unusually warm water recorded along the WA coastline in 2011 could have potentially influenced humpback calving site selection resulting in calves being born in unsuitable areas. However, if calving site selection due to environmental conditions was the most significant factor we would expect that most calves would have been born in relatively good body condition. Given the malnourished condition of most of the stranded neonates examined in 2011, theory three is likely to have been the most influential factor in the high

proportion of neonates stranding in 2011. Furthermore, it is significant that the neonates were born, in some cases, several thousands of kilometres south of the current known breeding grounds. It could be speculated that a change in the abundance and distribution of feed in the Antarctic may have resulted in an expansion of foraging time and/or range which would have led to a delay or increase in migration times and subsequently reduced fat reserves in some pregnant cows. Consequently such animals would not make it in time to the calving grounds, resulting in calves born further south. The suboptimal fat reserves in some pregnant females would also result in the birth of calves in a poor nutritional state.

Acknowledgments

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1. Introduction

1.1 Disease in wildlife

Disease is defined as any impairment of the normal structure or physiological state of an animal. The manifestation of disease is often complex and not just the result of a single disease-causing agent but often includes responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; and inherent or congenital defects; or a combination of these factors (Wobeser 1997). There are three important epidemiological concepts of disease and these include:

1. Disease never occurs randomly
2. All diseases are multifactorial
3. Disease is an interaction between three main factors including host, agent and environment.

It is also important to recognise that disease may result not only in consequences for an individual but also the population and even the ecosystem. The consequence of disease at the individual level may be obvious, such as overt clinical signs or death, or may be more insidious such as a reduction in immune function, impaired reproduction, subtle behavioural changes, or decreased growth rate (Wobeser 2006). Diseases that affect many individuals within a population may result in an adverse population effect. This may be driven by multiple factors such as changes to birth rates, death rates, immigration and emigration. The population effect exerted by disease may in turn result in ecosystem scale consequences through changes in community composition (competitors, predators, prey), productivity and stability (Tompkins *et al* 2011).

The vast majority of disease and death that occurs in wildlife are said to go unrecognised (Wobeser 2006). Wobeser (2006, p 45) likens disease in wildlife to "...an iceberg in that only a tiny tip projects above the water to be visible while the bulk of disease is hidden from view".

1.2 Marine mammal strandings

Strandings offer a unique opportunity to study marine mammals and significant aspects of historical and current scientific literature on marine mammals have been gained through the investigation of stranded animals (Gulland *et al.* 1997; Colegrove *et al.* 2005; Bogomolni *et al.* 2010). Yet very few efforts to systematically investigate marine mammal strandings have been made (Bogomolni *et al.* 2010) including only a handful of studies attempting to investigate large whale strandings (Gulland *et al.* 2005; Uhart *et al.* 2009).

Investigations into stranding events can provide information on individual animal health but will not provide information relating to the health status of an overall population (Bogomolni *et al.* 2010). According to Wobeser (2006, p 51) "If inferences are to be drawn about the frequency of occurrence, distribution, or significance of a disease in a group of animals, any sample of animals that is examined must be representative of the group". Stranded marine mammals are not representative of the normal population, as stranded animals often include young, old, or severely diseased individuals. At best, investigations into stranding events will provide information relating to causes of mortality and will also provide insight into the types of diseases present within a population. Increased stranding rates also provide an indication of when the mortality rate has increased (Heide-Jorgensen *et al.* 1992; Harkonen *et al.* 2006).

1.3 Humpback whales

Humpback whales (*Megaptera novaeangliae*) have a global distribution and are found in all the major oceans. All but one of the subpopulations (that of the Arabian Sea) migrate between mating and calving grounds in tropical waters, and productive colder waters in temperate and high latitudes (Reilly *et al.* 2008).

Humpback whales are currently listed under the World Conservation Union's (IUCN) Red List as a species of Least Concern. In Australia humpbacks are listed as Vulnerable under the *Environmental Protection Biodiversity Conservation Act 1999*. Within Western Australia (WA) they are listed as a Schedule One species under the *Wildlife Conservation Act 1950*, Wildlife Conservation (Specifically Protected Fauna) Notice 2010, which includes fauna that is rare or likely to become extinct.

The International Whaling Commission (IWC) currently lists seven humpback breeding stocks in the Southern Hemisphere (A-G; Figure 1). In some instances these breeding stocks have been further subdivided to improve understanding in sub-structuring within these areas (Fleming and Jackson 2011). The seven stocks include:

A: southwestern Atlantic

B: southeastern Atlantic

- B1: Gabon
- B2: west South Africa

C: southwestern Indian Ocean

- C1: Mozambique
- C2: Comoros Archipelago
- C3: Madagascar
- C4: Mascarene Islands

D: southeastern Indian Ocean

E: southwestern Pacific

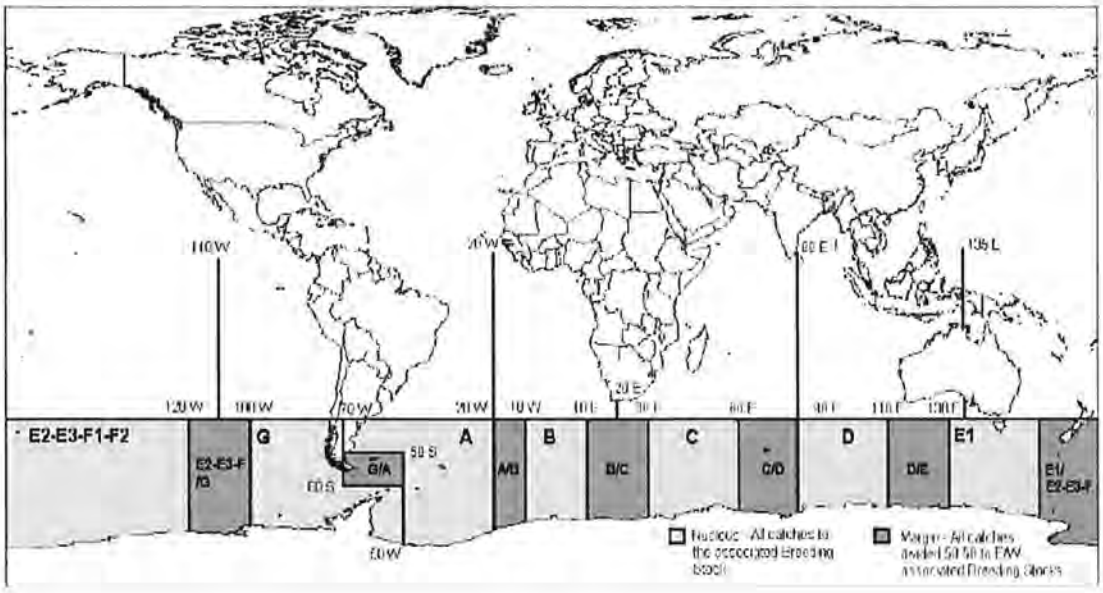
- E1: east Australia
- E2: New Caledonia
- E3: Tonga

E and F: Oceania

- F1: Cook Islands
- F2: French Polynesia

G: southeastern Pacific

Figure 1. Figure reproduced from IWC (In Press) and sourced from Fleming and Jackson (2011) showing Southern Hemisphere locations of Stocks A to G. Longitudinal boundaries encompass Antarctic feeding areas considered associated with those stocks.



1.3.1. Threats to humpback whales

Although whaling is no longer a current threat to humpback whale recovery, a variety of other anthropogenic factors may be of impact. Such factors include proximity to dense human populations, shipping traffic, oil and gas exploration, and fishing activities (Fleming and Jackson 2011). Detrimental effects may occur following human activities resulting in water pollution, increased noise levels, entrapment or entanglement in fishing gear, habitat degradation, ship strikes and whale watching and subsistence hunting (Fleming and Jackson 2011). In addition, climate and oceanographic change may have an impact by reducing reproductive output, survival and habitat availability (Fleming and Jackson 2011).

1.3.2 Humpback whales in Western Australia- Breeding Stock D

Breeding Stock D (BSD) was historically decimated by unsustainable whaling practices and by 1963 the population was thought to consist of less than 600 animals (Bannister 1964). The population has since made a remarkable recovery increasing at an annual rate of 10% (Bannister and Hedley 2001). The most recent estimate of the abundance of BSD calculated in 2008 was 21,750 (95% CI = 17,550-43,000) (Hedley *et al.* 2009).

The wintering grounds and coastal migratory routes of BSD are located between 15 and 35°S along the coast of WA, and the major calving grounds are located between 15 and 18°S in the Kimberley Region (Bannister and Hedley 2001; Jenner *et al.* 2001).

The exact timing of the migration of BSD from Antarctic waters is said to vary from year-to-year depending on water temperature, sea ice, predation risk, prey abundance and the location of the feeding ground (DEWR 2007). In general, humpback whales are often observed in southern Australian waters in May en route to the breeding grounds. The majority of whales are said to start their southward migration by October (DSEWPC 2010). During the northern migration, lactating females with weaning yearlings are the first to migrate, followed by immature males and females, followed by mature males together with resting females and then pregnant females. During the southern migration, mixed non-lactating females and immature males are first to migrate, followed by mature males and then cow-calf pairs (DSEWPC 2010).

As BSD depends on inshore areas along the WA coastline for migration, resting, and calving it is vulnerable to a wide spectrum of anthropogenic activities which have the potential to degrade important habitat (Department of the Environment and Heritage 2005). In 2005, as a component of the Humpback Recovery Plan 2005-2010, the Department of the Environment and Heritage (2005, p6) outlined the following anthropogenic activities of concern for habitat degradation in Australia:

- acoustic pollution (e.g. commercial and recreation vessel noise, and seismic survey activity);
- entanglement (e.g. in marine debris, fishing and aquaculture equipment);
- physical injury and death from ship strike;
- built structures that impact upon habitat availability and/or use (e.g. marinas, wharves, aquatic installations, mining or drilling infrastructure);
- changing water quality and pollution (e.g. runoff from land based agriculture, oil spills, outputs from aquaculture); and

- changes to water flow regimes causing extensive sedimentation or erosion or altered currents in near shore habitat (e.g. canals and dredging).

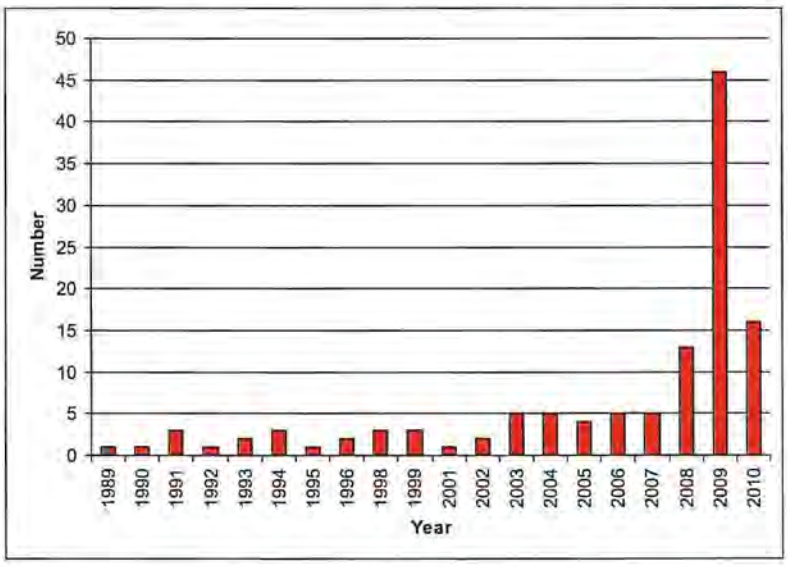
The threat of habitat degradation to BSD will increase as the coastally populated areas in WA grow rapidly, oil and gas exploration and development intensifies, and shipping activity increases.

1.3.3 Humpback strandings in Western Australia

Between 1989 and 2007 the mean number of humpback whales ashore was between 2 and 3 animals (range: 0-5) (Coughran and Gales 2010). In 2008 there were 13 strandings followed by 46 in 2009 and 16 in 2010 (Figure 2).

As the population of humpback whales increases it is reasonable to expect an increase in mortality events, however, the increase in strandings noted since 2008 (Figure 2) does not seem to follow the gradual increase in population size. Furthermore, the increase in humpback strandings recorded since 2008 is not believed to be influenced by any changes to reporting/monitoring practises or unusual weather conditions for that year (Coughran and Gales 2010).

Figure 2. Number of stranded humpback whales recorded on West Australian beaches between 1989 and 2010 (derived and adapted from Coughran and Gales 2010).



1.4 Aims and objectives of the project

The aims of this project are in accordance with a five year project and include:

1) identification and characterisation of factors associated with humpback whale strandings and;

2) determination of baseline and epidemiological information on disease and the nutritional status of stranded humpback whales.

Specific objectives include:

a) collect morphometric and life history data (e.g. size, sex, age class);

b) undertake partial and, where feasible, full post-mortem examinations to acquire information on causes of morbidity and mortality, and to collect tissues for pathogen identification, histopathology and toxicology;

c) quantify the nutritional status of stranded humpback whales by measuring blubber thickness, analysing blubber lipid content, and examining for the presence and degree of muscle and liver atrophy;

d) isolate and identify pathogenic viruses, bacteria, protozoa, or fungi from tissue samples (where appropriate);

e) archive tissue samples for long-term disease surveillance, toxicological monitoring, and retrospective study;

f) examine euthanized whales and provide feedback relating to the method of euthanasia;

g) identify and explore the possibility of using metabolites in skin and blubber biopsy samples as possible indicators of nutrition and health in free swimming humpback whales and;

i) establish beneficial collaborations within WA, nationally and internationally in order to enhance the information gained from the samples collected.

2. General materials and methods

2.1 Carcass condition

Carcasses were examined and classified according to the condition codes of Pugliares *et al.* (2007) in which code 1 is used to describe animals that are still alive; code 2 if the carcass is less than 24 hours post mortem; code 3 if there is a mild characteristic odour, the mucous membranes are dry and the eyes sunken, there may be bloating and the skin may be cracked and sloughing; code 4 (advanced decomposition) if there is a strong odor, the carcass is collapsed and the internal organs are liquefied; and code 5 when the carcass is mummified or there are skeletal remains.

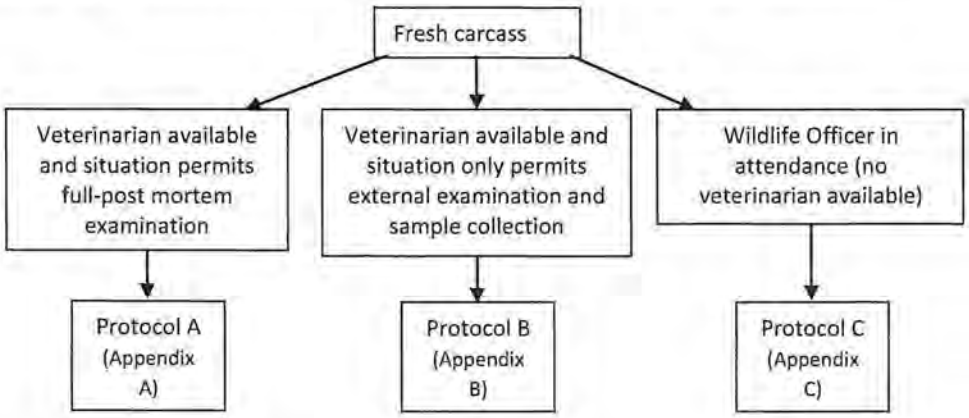
Decomposition severely affects the quality of samples and consequently post-mortem examination and sample collection for this project were limited to code 2 carcasses. Code 2 carcasses could further be split into 2a and 2b, with 2a encompassing carcasses that were fresh, as they had just died; and 2b encompassing carcasses exhibiting minimal bloating and slight haeme imbibition.

2.2 Development of post-mortem and sampling protocols

In order to ensure data collection from post-mortem examinations was done so in accordance with best practice numerous published protocols were consulted (Duignan 2000; Geraci and Lounsbury 2005; Higgins and Noad 2006; Pugliares *et al.* 2007). In addition, internationally renowned veterinarians with large whale post-mortem experience [including Drs: Frances Gulland (The Marine Mammal Center), Padraig Duignan (University of Calgary), Marcela Uhart (Wildlife Conservation Society) and Paul Jepson (Zoological Society of London)] were contacted for advice and access to non-published protocols.

Three sampling protocols were developed using a tiered approach as dictated by access to different levels of expertise (Figure 3; Appendices A,B,C).

Figure 3. Sampling protocols tiered in accordance with level of expertise available.



2.3 Stranding network

A network of volunteers was established to assist with full post-mortem examinations and currently there are ten volunteers.

2.4 Location and sampling

Prior to commencing the project it was decided that the jurisdiction for attempting a full post-mortem examination would be between Geraldton and Albany. This area was chosen in consideration of its proximity to Perth and the ability of project veterinarians to reach the carcasses in a timely manner.

Strandings that occurred outside of the area between Geraldton and Albany were attended by DEC regional staff.

The degree of sample collection and inspection of carcasses was based on consideration of the following factors: 1) the location of the carcass; 2) availability of personnel and resources; 3) safety of personnel; 4) degree of decomposition; 5) and social and cultural sensitivity.

3. Strandings in 2011

3.1 Introduction

3.1.1 Unreliability of carcass counts as an indicator of mortality rate

An increase in the number of individual strandings can indicate an increase in mortality rate (Heide-Jorgensen *et al.* 1992; Harkonen *et al.* 2006) but does equate to the actual mortality rate (Gulland and Hall 2007). Stranded dead cetaceans are said to be a poor representation of the true number of mortalities that occur within a species (Williams *et al.* 2011). The stranding of dead or sick marine mammals depends on many factors, including: behavioural response prior to death, proximity of the carcass to shore, decomposition factors, water temperature, wind, ocean currents, geography of the coastline and predation (Epperly *et al.* 1996; Faerber and Baird 2010; Williams *et al.* 2011).

Williams *et al.* (2011) examined historical carcass-detection rates for 14 cetacean species in the northern Gulf of Mexico and estimated that on average carcasses are recovered for only 2% of all deaths. Low detection rates have also been estimated for the carcasses of a number of species that occur near shore, including: gray whales (*Eschrichtius robustus*, <5%, Heyning and Dahlheim 1990), North Atlantic right whales (*Eubalaena glacialis*, 17%, Kraus *et al.* 2005), and harbour porpoises (*Phocoena phocoena*, <1%, Moore and Read 2008).

3.1.2 Humpback strandings in Western Australia: 1989-2010

Most strandings between 1989 and 2009 occurred during the southern migration (Coughran and Gales 2010) (Figure 4) while in 2010 there were more strandings during the northern migration (Table 1; Figure 4).

Figure 4. Number of live and dead humpback whales recorded on West Australian beaches during each month for the years 1989 – 2009 (from Coughran and Gales 2010).

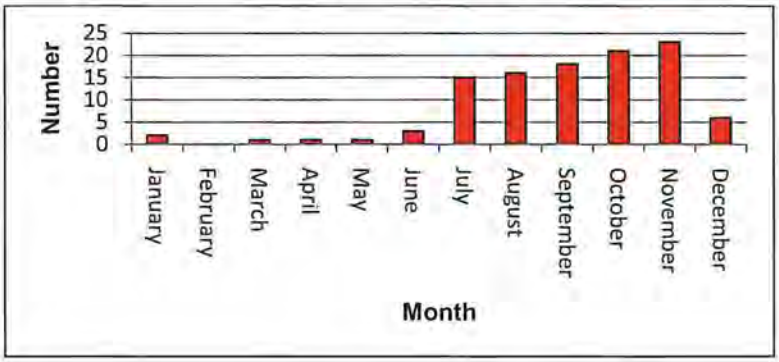
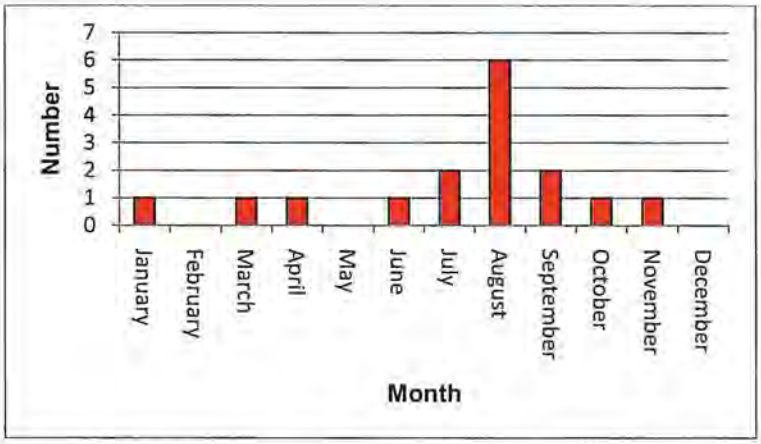


Table 1. Number of stranded humpback whales recorded in Western Australian in 2010 (Groom and Coughran, in review).

#	Date	Location	Length	Age	Sex
1	12 January	Cape Arid	12.7m	Adult	Male
2	30 March	Augusta	6m	Calf	Male
3	16 April	Walpole-Nornalup	9m	Juvenile/ sub-adult	Male
4	19 June	Coral Bay	Unknown	Calf	Unknown
5	16 July	Two Peoples Bay	9m	Juvenile/ sub-adult	Female
6	26 July	Geraldton	10m	Juvenile/ sub-adult	Unknown
7	2 August	Carnarvon	10m	Juvenile/ sub-adult	Unknown
8	7 August	80 Mile Beach	9.5m	Juvenile/ sub-adult	Unknown
9	9 August	Warroora Station	10m	Juvenile/ sub-adult	Male
10	14 August	Lancelin	4.5m	Neonate	Male
11	16 August	Dampier	10m	Juvenile/ sub-adult	Unknown
12	19 August	Albany	9.5m	Juvenile/ sub-adult	Unknown
13	16 September	Lucky Bay	4.2m	Neonate	Unknown
14	27 September	Favourite Island	9m	Juvenile/ sub-adult	Unknown
15	9 October	Carnarvon	10m	Juvenile/ sub-adult	Unknown
16	9 November	Rottnest Island	7m	Calf	Unknown

Figure 5. Number of stranded humpback whales recorded in Western Australia in 2010 per month.



The length of stranded humpback whales recorded between 1989 and 2009 ranged from 3.25 – 15m with a mean length of 8.0 ± 3.3m (mean ± sd) (Coughran and Gales 2010). Coughran and Gales (2010) used the recorded length of each individual to assign an age category, while acknowledging there is substantial overlap in length between calves of that year, juveniles/sub-adults and adults. Animals greater than 12m were deemed adults, those that were 7-12m were deemed juveniles/sub-adults and calves of that year included animals less than 7m (Coughran and Gales 2010). In accordance with these categories - between 1989 and 2009, 44% of strandings were calves of that year, 37% were juveniles/sub-adults and 19% were adults (Coughran and Gales 2010). In 2010 31% of strandings were calves of that year, 63% were juveniles/sub-adults and 6% were adults.

3.1.3 Aim of this section

The aim of this section is to outline the stranding data recorded for humpback whales in WA in 2011 and compare these with historical stranding data in order to identify and discuss any differences.

3.2 Methods

The West Australian Department of Environment and Conservation (DEC) has a well established and effective network for reporting strandings throughout the State and

maintains a database for all recorded marine mammal strandings (Groom and Coughran, in review). All humpback whale stranding data recorded by the DEC in 2011 were made available to this project.

The total length of each whale (measured on a straight line from the tip of the upper jaw to the deepest part of the fluke notch) was used to assign age class based on the categories outlined by Mazzuca *et al.* (1998).

3.3 Results

In 2011 there were 17 strandings consisting of 14 calves and 3 juveniles/sub-adults (Table 2). Of the 17 animals that stranded there were 6 females, 6 males and 5 were of unknown sex. Figure 6 shows the distribution of the strandings and Figure 7 depicts the number of strandings per month. The length of stranded animals ranged from 3.9 – 10m with a mean length of 5.2 ± 1.8m (mean ± sd).

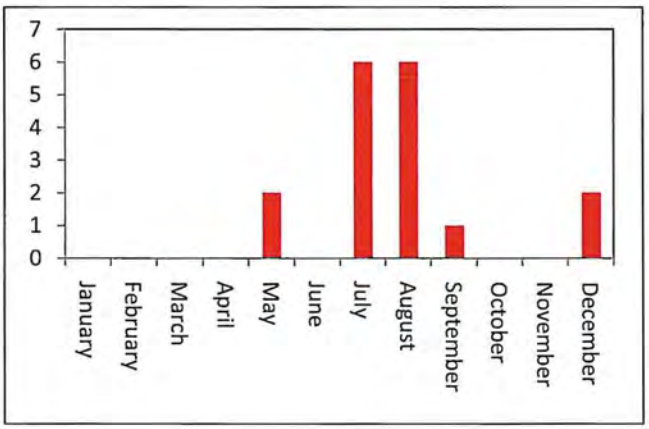
Table 2. Number of stranded humpback whales recorded in Western Australia in 2011.

#	Date	Location	Length	Age	Sex	Sampling and comments
1	May 19	Bremer Bay	4.8m	Neonate (thought to be less than 48 hours old)	Female	- Attended to by project veterinarians, full post-mortem examination conducted.
2	May 30	Lancelin	Unknown	Juvenile (likely calf from 2010)	Unknown	-Observed alive on May 27 (appeared extremely debilitated, skin lesions apparent). - Carcass came ashore May 30. - Carcass was still in the water and could not be removed. Had been heavily predated by sharks. - Only a genetic sample could be collected.
3	July 12	Exmouth	4.3m	Neonate	Unknown	-Euthanized. -DEC personnel took photos and collected samples of skin, blubber, eye and baleen.
4	July 19	Exmouth	4.2m	Neonate	Male	-Euthanized. -DEC personnel took photos, blubber depth measurements, and collected samples of skin, blubber, eye and baleen.
5	July 22	South of Kalbarri	5.4m	Neonate	Female	-No samples could be collected.
6	July 25	East of Augusta	4m	Neonate	Female	-Late notification by member of the public. -Moderately decomposed. -Carcass had been predated by sharks. -No samples collected.
7	July 27	Exmouth	Unknown	Neonate	Unknown	-Report of a live abandoned calf off the lagoon in Exmouth. Was last seen moving

						out to sea and being followed by several sharks.
8	July 28	Dongara	8.5m	Juvenile (likely calf from 2010)	Female	-Carcass approximately 2 weeks old (advanced decomposition) -No samples collected.
9	August 5	North of Geraldton	4.1m	Neonate	Male	-DEC personnel attended carcass August 6. Carcass was to be left on the beach. -Skin, blubber, eye, and baleen collected. Blubber depth measurements and photos taken.
10	August 6	Cape Arid	4.3m	Neonate	Unknown	-No samples could be collected.
11	August 17	Peaceful Bay, Walpole	4.8m	Neonate	Female	-Attended to by veterinarians from Denmark Veterinary Clinic, full post-mortem examination conducted.
12	August 24	Quinns Rocks, Perth	3.9m	Neonate	Female	-Attended to by project veterinarians, full post-mortem examination conducted.
13	August 25	Jurien	4.35m	Neonate	Male	-DEC personnel attended and collected Skin, blubber, eye, and baleen. Blubber depth measurements and photos taken
14	August 28	Bremer Bay	Approx 10m	Sub-adult	Male	-This carcass was found incidentally during aerial work. Photos indicated it was moderately decomposed. -No samples collected.
15	September 14	Gnaraloo	Approx 5m	Neonate	Male	-Photos taken but no samples could be collected due to the location. -From the photos the calf appears to be in poor body condition
16	December 12	Stokes Inlet	Unknown	Calf	Unknown	-Only a skull was recovered. -Given the appearance of the skull, and small amount of remanent tissue on the mandible, it is likely this is from a calf that was born early during the northern migration.
17	December 14	Belvidere Beach, Bunbury	5.64m	Calf	Male	- Due to location only a genetics sample was collected. -Photos suggest that the calf was in poor body condition.

Figure 6. Distribution of stranded humpback whales in Western Australia in 2011.

Figure 7. Number of stranded humpback whales recorded in Western Australia in 2011 per month.



3.4. Discussion

3.4.1 Stranding trend

The number of strandings recorded in 2011 has decreased since the peak in 2009. Unlike the age categories reported for 1989 – 2009 (44% of strandings were calves of that year, 37% were juveniles/sub-adults and 19% were adults [Coughran and Gales 2010]) and in 2010 (31% of strandings were calves of that year, 63% were juveniles/sub-adults and 6% were adults) most of the strandings in 2011 were neonates, with most animals thought to be less than 48 hours of age.

The Southern Kimberley between Broome and the northern end of Camden Sound are the current known calving grounds for BSD (Jenner *et al.* 2001). The neonates that stranded in 2011 were thus born very far south of the known breeding grounds. There are however historic reports of calves being born as far south as Albany (Chittleborough 1965) but it is unknown whether they survived. Chittleborough (1965) reported that following parturition in the Albany region the cows continued to move northwards during the first few weeks of lactation.

The most popular explanation for why mysticetes migrate to warmer climates during the winter is to reduce their thermoregulatory energy requirements when food resources are low at the poles (Brodie 1975; Shelden *et al.* 2004). New born calves are particularly vulnerable to heat loss because they have yet to acquire a sufficient layer of blubber (Rice and Wolman 1971) and have a high body surface area to mass ratio. Physiologically, the benefit of calves being born in warmer waters may be associated with two theories (which are not necessarily mutually exclusive), including 1) they are either physiologically incapable of living in polar waters and/or 2) warmer waters facilitate more rapid development, because energy that would otherwise be used for heat production by neonates in cold waters, is used in warm water for growth (Corkerson and Connor 1999). There is, however, currently little evidence to support these theories given a lack of data relating to neonate insulation capacity and metabolic rate (Corkerson and Connor 1999). The lower critical temperatures for mysticete neonatal survival are also unknown. A further theory as to why mysticetes migrate is associated with a preference for giving birth in calm water (Whitehead and Moore 1982; Payne 1995). Whitehead and Moore suggest that calm waters provide an

energy saving mechanism for calves whilst surfacing as it is more difficult to surface in stormy waters, which thus requires more energy.

An interesting change in migration and calving distribution trends have been noted for the eastern North Pacific stock of gray whales (*Eschrichtius robustus*) which migrate from feeding areas in the Arctic to warm, shallow lagoons in Mexico (Shelden *et al.* 2004). Prior to the mid-1970s, newborn calves were seen primarily in lagoons in Mexico, however, since 1980 there have been increased reports of calf sightings north of Mexico (Shelden *et al.* 2004). A one-week shift in the timing of the southbound migration since 1980 has been described (Rugh *et al.* 2002). According to Sheldon *et al.* (2004) a one week delay in the migration timing without a change in birthing dates would mean that calving would occur 1000km further north of the known calving lagoons in Mexico, assuming a constant travel rate of 147km/day (Swartz *et al.* 1987). Sheldon *et al.* 2004 offered two possible explanations (again, which are not necessarily mutually exclusive) for the shift in migration timing and increase in calves born outside of the known breeding grounds. The first relates to increased competition for food resources in the northern feeding areas as the population reaches carrying capacity. The second concerns a reduction in ice coverage in the Bering and Chukchi Seas, formerly primary feeding areas for this population of gray whales, which occurred after 1977. Both an increase in competition and reduction in ice coverage could result in a depletion of available prey species and an expansion in the foraging range of the gray whale - as has been noted (Rugh and Fraker 1981; Miller *et al.* 1985; Moore *et al.* 2003). Thus, as foraging pregnant females roam further in search of food, their migration south may be delayed or take longer. Shelden *et al.* (2004) suggested that these pregnant females may also be migrating with reduced fat reserves which could possibly explain the appearance of stranded calf carcasses along the migration corridor after 1977.

Also of interest is that a climatic regime shift occurred in the North Pacific during the winter of 1976 - 1977 resulting in unusually warm temperatures along the North American coast (Sheldon *et al.* 2004). Sheldon *et al.* (2004) suggested that because the North Pacific had warmed, calves likely experienced reduced thermoregulatory-stress when born along the migration route short of the breeding grounds.

In regard to humpback whale BSD, in 2011 there were anecdotal reports of mother and calf pairs observed off the south west coast of WA as early as May (Coughran 2011, pers. Comm., 20 May). Double *et al.* (2011) also reported many cow-calf pairs off the North West Cape during the north ward migration in 2011 and concluded that there are likely to be currently unidentified calving areas further south of the southern Kimberley region.

Humpback whale migration, feeding, resting, and calving site selection may be influenced by a number of factors such as ocean currents and water temperature (DSEWPC 2010). In February and March 2011, water temperatures off the south-western coast of WA rose to unprecedented levels (Pearce *et al.* 2011). This warming occurrence "...which coincided with an extremely strong *La Niña* event and a record strength Leeuwin Current, is viewed as a major temperature anomaly superimposed on the underlying long-term ocean-warming trend" (Pearce *et al.* p 1). It is unknown if this unusual 'marine heat wave' had any impact on the humpback whales from BSD but perhaps it could have influenced calving site selection.

It is also unknown whether there have been any recent shifts in the timing of the migration of humpback whale BSD. Long term, the global general ocean-warming trend may result in changes to migration timing, calving sites, resting areas and feeding sites and feed availability. For example, changes in feed abundance and distribution may result in an expansion of foraging range which may have further implications with regard to delaying or increasing migration times and reduced fat reserves.

3.4.2 Neonate mortality

Infant animals generally have poorer survivability compared to adults (Caughley 1966). There are no estimates of neonate (0-6 months) humpback survival currently available for the Southern Hemisphere (Fleming and Jackson 2011); however some estimates have been reported for Northern Hemisphere populations (Gabriele *et al.* 2001; Rosenbaum *et al.* 2002; Robbins 2007). Gabriele *et al.* (2001) estimated the mortality rate of humpback calves, in the central North Pacific Ocean, in their first 6 months by comparing photo-identification data between Hawaii (the wintering ground) and Alaska (the feeding ground). The estimated mortality rate in this study for the first 6 months of life was 0.182 (95% CI: 0.023-0.518; survival rate = 0.818) (Gabriele *et al.* 2001).

It is important to consider that the 2011 WA stranding records likely grossly underestimate the true number of mortalities that occurred given the remote nature of some of the WA coastline and the fact that most cetacean carcasses do not come ashore (Epperly *et al.* 1996; Faerber and Baird 2010; Williams *et al.* 2011).

3.4.3 Stranding distribution

The spatial distribution of strandings and the number of strandings reported is likely to be influenced by the distribution of the people rather than real mortality event distribution (Coughran and Gales 2010). Of the 46 humpback whale strandings reported in 2009, 75% occurred between Albany and Geraldton while in 2010 and 2011, 50% and 44% of strandings occurred in this area, respectively. This stretch of coastline is the most heavily inhabited area of WA and consequently it could be concluded that strandings are more likely to be reported in this area.

3.5 Conclusion

- The number of strandings recorded in 2011 has decreased since the peak in 2009.
- The mean length of stranded humpback whales recorded in 2011 (5.2 ± 1.9m [sd]) varied from that recorded between 1989 and 2009 (8.0 ± 3.3m [sd]).
- The representation of age categories of stranded animals in 2011 differed from that of age category data recorded between 1989 and 2010.
- The majority of strandings in 2011 were neonates that were less than 48 hours of age and born a considerable distance from the known breeding grounds.
- There was no evidence of anthropogenic factors (e.g. ship strike/entanglement) associated with any of the 2011 strandings.
- The stranding records are likely to grossly under represent the true number of humpback mortalities that occur.

4. Post mortem examinations

4.1 Introduction

Stranded whales are an important source of information on diseases given the difficulty and ethical dilemmas associated with collecting health related data from free ranging whales. Once diseases, lesions, and infectious agents are identified in stranded animals they can then be confirmed in free swimming animals. Stranded whales do not, however, represent the entire population (Aguilar and Borrell 1994) as disease is not evenly or fairly distributed in the population (Wobeser 2006). Thus it is not possible to assess the prevalence of disease and its impact on a population from data collected from stranded animals, as the sample is skewed towards animals with disease.

Like all mammals, marine mammals can be affected by a wide range of infectious and non-infectious disease. There is, however, an extreme lack of information relating to disease in humpback whales, and mysticetes in general. Table 3 is a list of some of the diseases that have been reported in mysticetes.

Table 3. Diseases reported in mysticetes.

Disease/pathogen	**Species**	**Reference**
Viral		
Unknown type of morbillivirus	Fin whale (*Balaenoptera physalus*)	Blixenkrone-Møller *et al.* (1996); Jauniaux *et al.* 1998; Jauniaux *et al.* 2000
Dolphin morbillivirus	Fin whale (*Balaenoptera physalus*)	Mazzariol *et al.* 2012
Poxvirus	Bowhead whale (*Balaena mysticetus*)	Bracht *et al.* (2006)
Bacteria		
Brucella spp.	Minke whale (*Balaenoptera acutorostrata*) Bryde's whale (*Balaenoptera edeni*)	Clavareau *et al.* (1998); Ohishi *et al.* (2003)

Parasites		
Protozoa		
Toxoplasma gondii	Fin whale (*Balaenoptera physalus*) Humpback whale (*Megaptera novaeangliae*)	Mazzariol *et al.* 2012 Forman *et al.* (2009)
Nematodes		
Crassicauda boopis	Blue whale (Balaenoptera musculus) Fin whale (*Balaenoptera physalus*) Humpback whale (*Megaptera novaeangliae*)	Lambertsen (1992)
Anisakis simplex	Gray whale (*Eschrichtius robustus*)	Dailey *et al.* 2000
Bolbosoma balanae	Gray whale (*Eschrichtius robustus*)	Dailey *et al.* 2000
Trematode		
Ogmogaster spp.	Gray whale (*Eschrichtius robustus*)	Dailey *et al.* 2000
Crustaceans		
Pennella spp.	Fin whale (*Balaenoptera physalus*)	Mazzariol *et al.* 2012
Cyamus scammoni	Gray whale (*Eschrichtius robustus*)	Dailey *et al.* 2000
Biotoxins		
Domoic acid	Gray whale (*Eschrichtius robustus*)	Gulland *et al.* 2005
Saxitoxin	Humpback whale (*Megaptera novaeangliae*)	Geraci *et al.* 1989

4.1.1 Aim of this section

The aim of this section is to outline the post-mortem findings of all humpback whales examined in 2011.

4.2 Materials and Methods

A full post-mortem examination and implementation of Protocol A was carried out on three of the humpback neonates that stranded in 2011:

1. Bremer Bay neonate 19.5.2011: examination was performed approximately 24 hours post-death.
2. Peaceful Bay neonate 17.8.2011: examination was performed three hours after euthanasia.
3. Quinns Rock neonate 24.8.2011: examination was performed approximately 24 to 36 hours post-death.

Histological examination was carried out by Dr Nahiid Stephens, Department of Anatomic Pathology, Murdoch University.

4.2.1 Blood collection

In the case of the Peaceful Bay neonate blood was collected from a gun shot hole in the head immediately following euthanasia. Blood was collected into 5 ml EDTA tubes (Sarstedt, Australia Pty Ltd) for haematology, 5 ml Lithium Heparin tubes (Sarstedt, Australia Pty Ltd) for biochemistry, and 10 ml plain serum tubes (Sarstedt, Australia Pty Ltd) for serology and serum banking. Several blood smears were also made.

In the case of the Quinns Rock neonate clotted/haemolysed blood was collected from the heart 24-36 hours post death and stored in a plain serum tube.

4.2.2 Haematology and Biochemistry

Haematology was performed by Vetpath Laboratory Services while biochemistry was performed by the Animal Health Laboratory, Department of Agriculture and Food. Clinical biochemistry was interpreted against data for blood from Puls (1994) - note these are generic "adult whale" parameters and both age and species variations are likely.

Furthermore, the Puls (1994) reference ranges are likely to have been calculated from a small sample population given the width of some reference ranges.

Biochemistry was also performed on the vitreous humor of some animals.

4.2.3 Ancillary tests

Bacteriology and serology for *Toxoplasma gondii,* Influenza A, *Brucella abortus* and *B. melitensis* was performed by the Animal Health Laboratory, Department of Agriculture and Food. Leptospira serology was performed by Queensland Health.

Toxoplasma immunohistochemistry was performed by the Animal Health Laboratory, Department of Agriculture and Food on sections of the optic vascular rete from the Bremer Bay (11/292), Quinns Rocks (12/135) and Peaceful Bay (12/136) neonates, as well as a section of cerebrum from the Quinns Rocks (12/135) neonate.

Morbillivirus immunohistochemistry was performed by the Australian Animal Health Laboratory, Geelong on sections of lung from the Bremer Bay neonate (11/292).

Trace element and nutrient analysis was performed on frozen liver by the Animal Health Laboratory, Department of Agriculture and Food; this was done for the Bremer Bay (11/292), Quinns Rocks (12/135) and Peaceful Bay (12/136) neonates.

4.3 Results

4. 3.1 Bremer Bay neonate 19.5.2011

For all gross and histological findings refer to Appendix D.

4.3.2 Peaceful Bay neonate 17.8.2011

For all gross and histological findings refer to Appendix E. Biochemistry results are listed in Table 4 and Appendix E along with bacteriological and serological results. Haematology results are listed in Table 5 and Appendix E.

4.3.3 Quinns Rocks neonate 24.8.2011

For all gross and histological findings refer to Appendix F. Biochemistry results are listed in Table 4 and Appendix F along with bacteriological results.

4.3.4 Trace elements and nutrient analysis of liver and biochemistry results for vitreous humor and urine

Refer to Appendix G-1 for the levels of Vitamin E and A recovered from frozen liver samples from the Bremer Bay, Peaceful Bay and Quinns Rocks neonates. Biochemistry results for vitreous humour of five neonates are also presented in Appendix G-1. Refer to Appendix G-2 for the corresponding identification of each sample.

Table 4. Biochemistry values results from serum collected from the Peaceful Bay and Quinns Rock humpback neonates and reference ranges from other cetacean species.

	Glucose mmol/L (mg/dL)	Glycerol mmol/L	Total protein g/L	Urea mmol/L (mg/dL)	Creatinine umol/L (mg/dL)	Total Bilirubin umol/L (mg/dL)	Conjugated bilirubin umol/L	Cholesterol mmol/L (mg/dL)	GSHPx in RBC U/g Hb	Haptoglobin mg/mL	Albumin g/L
Peaceful Bay Humpback neonate	3.71 (66.78)	0.28	43.1	22.4 (62.72)	130 (1.469)	1 (0.0584)	0	4.83 (186.4)	172	4.39	22.2
Quinns Rock Humpback neonate		1.25	72.5	29.6 (82.88)	125 (1.41)	6 (0.3504)	2	6.22 (240.1)		2.66	38.1
Adult whale[a]	5.2-6.4		52-73	17.9-32.1	26.5-265.2	3.4-10.3		3.9-77.6			
Gray Whale, *Eschrichtius robustus* (n= 1, sample= 14)[b]	(47-147)			(21-75)	(1.0-2.0)	(0-0.2)		(136-1470)			
Fin Whale, *Balaenoptera physalus* (n changed with variable but ranged from 49 to 18)[c]			56-80	16.4-30.7	70-278			2.0-11.4			26-44
Beluga, *Delphinapterus*	4.1-9.5		54-106	1.7-33.2		0-0.0146	0-0.0062	1.3-9.8			29-53

leucas (n= 151, sample number varied with parameter)[d]											
Free ranging beluga whale, *Delphinapterus leucas* (n= 145, sample= 145)[b]	(108-114)			(52-55)	(0.3-3.0)	(0.2-0.4)		(174-194)			
Killer whales, *Orcinus orca* (n= 19, sample= 1761)[b]	(110-135)			(30-50)	(0.8-2.0)	(0.1-0.2)		(140-280)			
Free-ranging bottlenose dolphins, *Tursiops truncatus* (n= 36, sample= 36)[b]	(62-170)			(45-72)	(1.0-2.1)	(0.1-0.4)		(137-235)			
Free ranging juvenile bottlenose dolphin, Tursiops truncatus (n= 96)	(56.43-172.62)		62-81.4	(41.82-77.49)	(0.68-1.49)	(?-0.21)					38.6-48.8

	Albumin Globulin ratio	BHB mol/L	ALT U/L	GGT U/L	CK U/L	GLDH U/L	Mg mmol/L	Cu mg/L	Zn mg/L	Ca mmol/L (mg/dL)	P mmol/L (mg/dL)	Fe umol/L (µg/dL)	Vit A mg/L	Vit E mg/L
Peaceful Bay Humpback neonate	1.10	0	101	3	41170	21	1.72	0.93	0.91	1.74 (6.96)	3.15 (9.77)	3.9 (21.8)	0.03	14.69
Quinns Rock Humpback neonate	1.10	0.12	108	40	5970	3357	2.94		8.82	2.23 (8.92)	11.76 (36.46)	25.3 (141.4)		
Adult whale[a]			5-18		50-120		0.8-1.9			2.2-3.0	1.5-3.2	19.7-71.6		
Gray Whale, *Eschrichtius robustus* (n=1, sample = 14)[b]			3-12	2-52	107-255					(8.0-11.0)	(3.7-9.0)	(54-328)		
Fin Whale, *Balaenoptera physalus* (n changed with variable but ranged from 49 to 18)[c]							0.5-3.2			1.5-3.75	0.52-3.25			
Beluga, *Delphinapterus leucas* (n= 151, sample number varied with parameter)[d]	0.7-1.8			0-62			0.57-1.32			1.03-3.54	1.75-3.69	0-265		
Free ranging beluga whale, *Delphinapterus leucas* (n= 145, sample 145)[b]			7-15	15-18	149-175					(10.4-10.8)	(7.9-8.3)	(438-551)		
Killer whales, *Orcinus orca* (n=19, sample = 1761)[b]			10-40		60-230					(8.9-9.5)	(5.0-7.0)	(50-130)		
Free-ranging bottlenose dolphins,			9-33		ND					(8.2-9.4)	(3.2-7.2)	(74-		

Tursiops truncatus (n= 36, sample= 36)[b]												176)		
Free ranging juvenile bottlenose dolphin, Tursiops truncatus (n= 96)[e]			?-48.45	10.41 - 30.03	47.58-455.87		0.62-0.89			(9.49-9.38)	(3.49-6.64)			

[a] Reference ranges from Puls (1994)

[b] Reference ranges from Reidarson (2003)

[c] Reference ranges from Kjeld (2001)

[d] Reference ranges from St Aubin *et al.* (2001)

[e] Reference ranges from Hall *et al.* (2007)

Table 5. Haematology results from blood collected from the Peaceful Bay humpback neonate and reference ranges from other cetacean species.

	HB g/L	HCT L/L	PCV L/L	RBC $x10^{12}/L$	MCHC g/L	MCH pg	MCV fL	WBC $x10^{9}/L$	Platelets $x10^{9}/L$
Peaceful Bay Humpback neonate	40	0.100	0.10	0.8	369	50	135	0.2	30
Beluga, *Delphinapterus leucas* (n= 151, sample number varied with parameter)[a]	165-260	0.485-0.675		1.7-5.20	229-448	34-86	118-203	1.8-25.7	
Free ranging juvenile bottlenose dolphin, Tursiops truncatus (n= 96)[b]	129.7-15.98			3.12-4.00	323.4-365.2	37.23-44.29	107.23-129.89	5.72-15.53	11.752-244.922

[a] Reference ranges from St Aubin *et al.* (2001)

[b] Reference ranges from Hall *et al.* (2007)

4.4. Discussion

A significant finding for both the Bremer Bay neonate and Quinns Rocks neonate was severe generalised adipose hypoplasia - in contrast to the Peaceful Bay neonate, which had normal blubber characterised by the presence of mature (albeit small and somewhat variably-sized) adipocytes. This finding correlated with the body condition scores assigned by visual assessment. The blubber of the Bremer Bay and Quinns Rocks neonates was thus almost completely devoid of any lipids and consequently it is unlikely they could have survived. Whilst the adipose connective tissue of many neonatal animals when born is relatively devoid of lipid, only filling quickly during the lactation period (Iverson, 2009), neonate mysticetes are said to be born with a blubber layer that is several centimetres thick (Iverson, 2009.). Humpbacks require blubber lipid for energy, thermoregulation and buoyancy (Iverson 2009a) - see section 5 for a more comprehensive discussion. One would therefore normally expect to see relatively well developed adipose connective tissue present within the blubber (and presumably also internally at other sites e.g. peri-renal, heart base etc), even in a neonate; however in two of the three individuals examined both grossly and histologically (Bremer Bay and Quinns Rocks neonates) the connective tissue occupying the areas where one would normally expect adipose was instead a loose, immature, poorly differentiated connective tissue characterised by the presence of immature precursor mesenchymal cells and ground substance. Due to the estimated age of both individuals, this is likely to represent adipose *hypoplasia*; as one would expect to have seen serous atrophy of formed mature adipocytes histologically if it were instead a case of *atrophy* due to excessive post-natal mobilisation. Given the severity of the change (particularly in comparison to the Peaceful Bay neonate, the only one examined post-mortem that was considered to be in good body condition and which had a significantly higher blubber lipid content in comparison to these two) and the likelihood that these two neonates were no more than 48 hours old (based on other observations), it is likely that this adipose hypoplasia reflects insufficient nutrient supply in utero (i.e. poor maternal nutritional status).

In contrast to the Bremer Bay and Quinns Rocks neonates, histological examination of the blubber from the single neonate in good body condition (Peaceful Bay) showed it to be relatively well developed; with the presence of mature adipocytes, each of which possessed

a prominent intracytoplasmic lipid vacuole. The lipid vacuoles were diffusely slightly small and there was increased variability in the size of the lipid vacuoles between adipocytes; this reduction in size of individual adipocytes and the variability in inter-adipocyte lipid vacuole size likely reflects the fact that significant further development and accumulation of intracytoplasmic lipid occurs during the lactation phase. It is therefore likely that this Peaceful Bay individual more truly approaches the normal neonate in terms of its blubber lipid content and blubber histological appearance. Furthermore, the histological appearance of the blubber of this more normal individual matched the histological description by Elfes (2008) of the blubber from a yearling humpback whale, as well as blubber from other species by various other authors (see 5.1.1 Blubber for more details). Concurring with Elfes (2008) findings, the superficial blubber of the Peaceful Bay neonate contained larger numbers of adipocytes with small bundles of collagen interspersed, with the collagen bundle number and size increasing gradually (and numbers of adipocytes between them decreasing) moving deeper into the blubber; however there were no clear delineations that might be termed strata or layers. Interestingly, evident deep in the section from the Peaceful Bay neonate the blubber transition to skeletal muscle (attached to the adipose connective tissue by loose fibrovascular connective tissue) was also a gradual one, with numerous depots of adipose connective tissue strewn amongst the skeletal muscle even deep within the section.

In addition to being malnourished, the Bremer Bay neonate had moderate to severe, acute, multifocal to coalescing serofibrinous alveolitis and interstitial pneumonia (evident to varying extents/severity in samples from multiple areas of both lungs). Following this type of extensive alveolar insult (representing damage to the type I pneumocytes or the endothelium of the alveolar septa), repair by proliferation of type II pneumocytes is observable initially at 2-3 days post injury. Given no type II pneumocyte hyperplasia was seen, this case was still in the acute exudative phase (i.e. insufficient time had elapsed to mount a reparative response). Grossly the lungs of this neonate appeared consolidated and had not collapsed, there was also froth in the airways including the trachea. It is likely that this neonate would have had significant difficulty breathing which would have (in addition to the fact that it also exhibited severe generalised adipose hypoplasia) contributed to its death. The cause of the serofibrinous alveolitis and interstitial pneumonia remains

unknown. The histological changes observed are not dissimilar to some of those seen with acute cetacean morbillivirus infection; however the typical 'Warthin-Finkeldey' type multinucleated giant syncytial cells and inclusions were not seen (Stone *et al.* 2011). Furthermore, there was no evidence of lymphoid depletion as is usually seen in acute morbillivirus infection (Stone *et al.* 2011). Immunohistochemistry for morbillivirus was performed on samples from this neonate and positive staining was not seen, hence testing did not indicate the presence of morbillivirus. The presence of the gram negative coccobacilli (whose morphology is not consistent with post-mortem saprophytic invaders, although they too appear to be present; thus caution is warranted in interpretation) is interesting, particularly as there are rare examples of bacterial engulfment by phagocytes; it is possible they represent terminal secondary/opportunistic invaders. Foetal squames were seen; they are normally present in amniotic fluid and thus can normally be seen as an incidental finding in the lungs of stillborn or neonatal mammals. Bacteriology isolated several *Clostridia* spp. and a *Bacillus* sp. This is consistent with contamination and/ or post-mortem overgrowth (i.e. not significant and not the cause of the lung changes). However, wet prep smears at AHL and histological examination have documented the presence of numerous inflammatory cells occasionally containing phagocytosed bacteria, so it is possible that an unidentified bacterial agent was present (i.e. perhaps the bacteriology result was adversely affected by the fact that the tissue was frozen and thawed prior to culture) that may be responsible for, or at least contributing to (i.e. they may simply be secondary opportunists following a primary, as yet unidentified, viral insult) the pulmonary changes. Generally speaking, using examples found in many mammalian species (Caswell *et al*, 2006), the possible scope for underlying cause is wide – many infectious agents infect type I pneumocytes (e.g. parainfluenza viruses, herpesviruses, adenoviruses, respiratory syncytial viruses and *Toxoplasma gondii*) although no such agents nor changes characteristic or their presence were seen. Various non-infectious agents can cause injury to type I pneumocytes (e.g. various toxins, near-drowning, drug reactions) and ventilator injury can cause damage to alveolar septa. Likewise, a wide variety of systemic syndromes (such as acute hypersensitivity pneumonitis, shock, disseminated intravascular coagulation/DIC, uraemia and pancreatitis) can trigger extensive to diffuse alveolar damage. Obviously, many of these differentials are highly unlikely in this species given its environment. Additionally, there was

no other gross/histologic evidence to suggest that syndromes such as DIC, uraemia or pancreatitis were present.

All three of the neonates had congested livers with marginal multifocal micro/macro-vesicular vacuolar hepatopathy. It is likely that this pathology is a consequence of stranding on land resulting in compression of the thoracic and abdominal vasculature and compromised blood flow to the liver and is a not unexpected finding, being common in stranded cetaceans (Jaber *et al.* 2004). The vacuolar changes were marginal, multifocal and non-specific in aetiology, although may reflect an early degenerative change secondary to hepatocyte hypoxia (oxygen deficiency due to poor bloody supply).All three of the neonates also had evidence of inflammation in the connective tissue of the optic vascular rete; the significance and aetiology of which is unknown. Given the optic nerve itself (and retina, where it had not degenerated) was unremarkable, it is likely to represent an incidental finding (i.e. not causing visual impairment and hence not clinically significant). The predominant type of inflammatory cells observed (eosinophils) are usually associated with parasitic infection and larval helminthiasis (larval migrans) could potentially explain this inflammation however no such agents were found. Additionally, it is not known whether such helminth parasites can be transmitted trans-placentally in baleen whales, which would have to be the case given this animal's age. *Toxoplasma gondii* has also been reported as a cause of endophthalmitis in a number of mammalian species (Wilcock, 2006) and histologic lesions can be seen not only in the retina and uvea, but also in the extraocular tissues e.g. muscles; however it tends to be necrotising with granulomatous to lymphocyte rich inflammation (rather than eosinophilic).

Disseminated necrotising infection by *T. gondii* (particularly involving encephalitis) has been demonstrated in pinnipeds, sirenians and odontocetes (Inskeep *et al.* 1990; Dailey 2001) however has not been documented in mysticetes. There is evidence, however, that mysticetes may be affected (presumably from contact with water contaminated by oocysts, given they are primarily plankton feeders, supplemented with small fish), with seropositivity documented in a humpback whale from British waters (Forman *et al.* 2009) and molecular (PCR) and immunohistochemical techniques documenting the presence of *T. gondii* in various tissues from a fin whale (*Balaenoptera physalus*) (Mazzariol et al 2012). Due to the

state of decomposition the histology of these tissues was not described in the latter report, so it is unknown what lesions the apicomplexan was associated with, however this individual was also RT-PCR positive for morbillivirus and was additionally reported to have high organochlorine (OC) pollutant levels in blubber; so it is postulated by the authors to have been immunocompromised.

Immunohistochemistry (IHC) for *T. gondii* for all three neonates was performed at AHL DAFWA, however definitive staining of organisms was not detected; hence testing did not indicate the presence of *Toxoplasma gondii. T. gondii*, in any case, was unlikely to be a cause of the eosinophilic inflammation seen in the optic vascular rete, particularly as necrotising myelitis/encephalitis was not a feature in any of the neonates, nor were the organisms themselves seen. Additionally, indirect fluorescence antibody testing (IFAT) for *T. gondii* antibodies in blood from the Peaceful Bay neonate (the only individual tested in this manner) was negative at AHL. A consideration that must be taken into account is that the use of *T. gondii* monoclonal antibody for IHC is not currently validated for use in cetaceans; however all of the testing done indicates that *T. gondii* was not present in the three neonates. The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete therefore remains unknown. Larval helminthiasis (larval migrans) could potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found.

The low GGT recorded in both the Peaceful Bay and Quinns Rocks neonates indicate that both animals received little or no colostrum. Consequently the serology performed on the Peaceful Bay neonate's bloods would not be indicative of the status of the mother. A further consideration, although speculative, given the severely malnourished state of the Quinns Rocks neonate may have been a reflection of the poor nutritional status of the mother; it is possible that the mother may not have been able to lactate (i.e. agalactia - this may also have been the case for the Bremer Bay neonate).

It is interesting to note that both the neonates in poor body condition (Bremer Bay and Quinns Rocks neonates) exhibited lower liver levels of Vitamins A and E in comparison to the Peaceful Bay neonate, which was in good body condition. This was particularly noticeable in

the Bremer Bay neonate. These differences in these figures may represent variations in maternal vitamin status. However, intake of fat soluble vitamins also occurs in milk and this source must be considered in the Peaceful Bay neonate, even though the low GGT recorded in this neonate indicates that this animal is likely to have received little or no colostrum.

The Quinns Rocks neonate exhibited incidental lesions involving the superficial umbilicus and surrounding skin - severe, acute to subacute, focally extensive necrosuppurative omphalitis, omphalophlebitis and dermatitis with intralesional bacteria. Certainly some inflammation and necrosis of these tissues is expected as a result of normal umbilical involution; however the inflammatory and necrotic changes seen in the skin at the edge of the umbilicus were more florid than one would expect with normal/physiologic umbilical involution in the neonate. Additionally, the bacteria seen were of a single morphologic type (gram negative coccobacilli), less consistent with simple bacterial contamination, and were furthermore observed phagocytosed in places. The thrombosed internal umbilical artery (taken from the umbilical stump immediately within the peritoneal cavity) showed similar changes to the peri-umbilical skin, again with inflammatory and necrotic changes much more inflammatory than normally expected of physiologic involution, and with the presence of bacteria. Unfortunately the umbilicus was not cultured. However, given the abdominal fluid swab failed to culture any bacterial growth at all, and given the liver and spleen did not demonstrate histological changes consistent with sepsis, the bacteria seen and the associated omphalitis, omphalophlebitis and dermatitis are unlikely to be significant (i.e. it is unlikely that sepsis was a factor in this individual's death). Similarly, it is unlikely to have significantly compromised to the animal's well-being, had it lived.

The Quinns Rocks neonate also returned interesting brain swab bacteriology results, however the isolates are unlikely to be clinically relevant, given the brain and meninges appeared grossly (apart from vascular changes suggestive of hyperaemia or post-mortem hypostasis/ blood pooling) and histologically (apart from minimal cerebral gliosis unlikely to be related to the bacteriology results) unremarkable. That is to say, there was no evidence of significant brain/ meningeal inflammation that could be due to the presence of the bacteria found on brain swab. Given two isolates (haemolytic *Escherichia coli* and *Plesiomonas shigelloides*) were detected; it is more than likely that they represent

contaminants or post-mortem invasion. Even so, the presence of *Plesiomonas shigelloides* is an interesting result for the fact that it has never to our knowledge been isolated from this species before. *Plesiomonas shigelloides* is an environmental bacteria, however it can be an opportunistic pathogen; it is more common in freshwater than marine species and is generally isolated from fish (Dr Shane Besier, personal communication). Typing on the *E. coli* isolate shows that it lacks most of the common virulence factors (Vero-toxin, Shiga toxin, hlyA and eaeA) and is not serotype O157 (Dr Shane Besier, personal communication).

The Peaceful Bay neonate exhibited two incidental findings, neither of which would have been sufficient to cause death or significant compromise to the animal's well-being, had it lived. One of its corneas was grossly affected by a focal opacity confirmed histologically to be a moderate, acute to subacute, focal eosinophilic and ulcerative keratitis. No aetiological agents were evident on routine and special histochemical stains. The cause remains unknown as the changes were aetiologically non-specific; physical trauma may simply have been the cause. Certainly it was a small lesion, limited to the outer one-third of the corneal stroma; and showing signs of early reparative response – more than likely had the animal lived, it would have healed without incident. Additionally, this same neonate histologically demonstrated mild to moderate, acute to subacute, multifocal to coalescing eosinophilic gastritis (pyloric/C3 stomach) and mild, acute to subacute, multifocal to coalescing eosinophilic enteritis (small intestine).The most likely cause of eosinophilic gastritis and enteritis is likely to be infestation by nematodes (e.g. family Anisakidae), although no nematodes were found grossly or histologically to enable confirmation and identification. It is also not known whether these nematodes can be transmitted trans-placentally, which would have to be the case given this animal's age. Given the mucosal surface of the alimentary tract appeared grossly normal, this is unlikely to have been significant in terms of causing clinical disease. The cause of death in this individual is not known; maternal abandonment remains a possibility.

The haematology results for the Peaceful Bay neonate should be regarded as unreliable due to the method of collection of blood from the gun shot hole.

4.5 Conclusion

- The Bremer Bay and Quinns Rocks neonates were in a severely malnourished state. It is likely that these neonates were non-viable from birth, due to the lack of lipids in the blubber. Blubber lipids are needed for energy, thermoregulation and buoyancy.
- The malnourished state of the Bremer Bay and Quinns Rocks neonates may reflect poor maternal nutritional status.
- The low GGT measured in the Peaceful Bay and Quinns Rocks neonates suggest that these calves had received little or no colostrum.
- The lower liver levels of Vitamins A and E in the two neonates in poor body condition (Bremer Bay and Quinns Rocks neonates) in comparison to those from the Peaceful Bay neonate may be a reflection of maternal vitamin status (assuming the higher levels seen in the Peaceful Bay neonate were not due to milk ingestion; although as discussed, the low GGT indicates this is unlikely).
- The Bremer Bay neonate had moderate to severe, acute, multifocal to coalescing serofibrinous alveolitis and interstitial pneumonia, which would have compromised its ability to breathe and contributed to its death. The aetiology is unknown.
- All three neonates had evidence of predominantly eosinophilic inflammation in the connective tissue of the optic vascular rete, the significance and aetiology of which is unknown.
- A number of incidental (i.e. not clinically significant) findings were noted - severe, acute to subacute, focally extensive necrosuppurative omphalitis, omphalophlebitis and dermatitis with intralesional bacteria (Quinns Rocks neonate); moderate, acute to subacute, focal eosinophilic and ulcerative keratitis; and mild to moderate, acute to subacute, multifocal to coalescing eosinophilic gastroenteritis (the last two in Peaceful Bay neonate). The aetiology of all three findings are unknown.
- In euthanized calves blood should not be collected from the gun shot site. Blood should be collected immediately following verification of death from one of the two recommended sites:
    - Removal of an eye followed by collection of blood that pools in the eye socket (P. Duignan, Personal Communication, March 2011)

- Slash to the tail stock area followed by collection of the blood from the wound (M. Uhart, Personal Communication, February 2011)

5. Baseline information on the nutritional status of stranded humpback whales

5.1 Introduction

Humpback whales undertake some of the longest migratory movements ever recorded (Clapham 2009). The migratory path of humpback whales from BSD covers more than 3,600 nautical miles (Jenner *et al.* 2001) and during the migration they are thought to ingest very little food - if any (Chittleborough 1965). According to Chittleborough (1965) the total food intake is negligible for at least four month of each year and during this time the whales utilise fat reserves (Chittleborough 1965) mainly stored in the blubber (Iverson 2009a).

5.1.1 Blubber

Blubber is a specialised layer of hypodermis found only in marine mammals. Blubber has several functions including: providing insulation; contributing to buoyancy; biomechanically providing support during locomotion; increasing streamlining of the body surface; and is the primary site for lipid and thus also energy storage (Iverson 2009a). Blubber is composed of adipocytes (fat cells), collagen, elastic fibers and blood vessels (fibrovascular connective tissue). Prior to filling with fat, adipocytes are composed of mostly protein and water (Iverson 2009a). The fat content of adipocytes is dynamic and they can alternately fill and empty with lipid, resulting in great changes in size (Iverson 2009a). In many species of cetaceans there is distinct stratification of blubber into an inner, middle, and outer layer based on the size, shape, and metabolic characteristics of the adipocytes, as well as on the lipid and collagen content (Iverson 2009a). The amount, depth, and chemical composition of blubber have been shown to vary with species, age, nutrition, and reproductive status. During times of nutritional stress or fasting, lipid is mobilised from adipocytes; resulting in reduced blubber thickness and lipid content (Iverson 2009a).

To date, very limited information is available on the structure and lipid content of blubber in humpback whales. Elfes (2008) analysed the structure and lipid content of full-thickness blubber samples collected from a single male yearling humpback that was found stranded in Truro, Massachusetts, in 1998. The animal was reported to be in a moderate state of

decomposition and cause of death was thought to be via entanglement in fishing gear. The carcass was found in May which usually constitutes the beginning of the feeding season in the Northern Hemisphere; hence this animal would have been fasting for a prolonged period of time.

Histological examination of full depth segments of blubber taken from the dorsal flank posterior to the dorsal fin revealed that the upper portion of the hypodermis was composed mainly of adipocytes with small bundles of collagen fibres interspersed (Elfes 2008). As depth increased, there was a gradual decrease in adipocytes and increase in collagen fibres, both in size and overall quantity. This pattern is in agreement with previous histological work in fin (*Balaenoptera physalus*), sei (*Balaenoptera borealis*), minke (*Balaenoptera acutorostrata*) and northern right whale (*Eubalaena glacialis*) blubber (Solokov, 1960; Ackman, 1975). According to Elfes (2008 p21): "the increase in collagen fibres was gradual and there were no clear, abrupt changes within the hypodermis that delineated what might be called 'layers'."

On histological examination the outer sections had the highest lipid and lowest collagen percent values while the inner subsections had the lowest lipid and highest collagen percent (Elfes 2008). Chemical analysis, however, showed no difference in lipid percent between outer and inner layers. The lipid content at Site 3 (dorsal flank just posterior to the dorsal fin) was found to be 61% for both the outer and inner layers. Elfes (2008) could not account for the difference between histological and chemical analyses and suggested that a greater sample size was needed to help elucidate the nature of lipid variation throughout depth in humpback whales.

There seems to be little consensus in the literature for baleen whales regarding the lipid variation between outer and inner blubber layers. Ackman *et al.* (1975b) reported higher lipid content in the outer versus inner layer in a female humpback blubber sample examined chemically. The dorsal blubber sample described by Ackman *et al.* (1975b) had 47.3% and 18% lipid in the outer versus inner layers, respectively (the site from which the blubber sample was collected was not specified). Lockyer *et al.* (1984) and Ackman *et al.* (1965, 1975b) found that fin whales had a higher lipid content in outer versus inner layers. In

contrast, Aguilar and Borrell (1991) did not find a significant difference in the lipid content of outer and inner layers in the fin whales they examined. Gauthier et al (1997) did not find a significant difference in the lipid content of blubber layers of blue (*Balaenoptera musculus*) and minke whales.

5.1.2 Humpback whale - a fasting adapted species

The humpback whale is a fasting adapted species, however very little is known about the physiological processes associated with fasting whales (Castellini and Rea 1992). An understanding of the processes involved in successful fasting versus terminal starvation in whales can perhaps be gleamed from an examination and comparison of the biochemistry of fasting in non-adapted species with that of adapted marine mammal species for which information is available (for example certain species of seals).

5.1.2.1 Biochemical processes of fasting

Phase I: Glucose is the critical fuel for the central nervous system (CNS) and levels are usually maintained within a tight range. During the first few days of food deprivation, hepatic glycogen reserves are almost completely utilised in an effort to maintain glucose levels (Castellini and Rea 1992). The mobilisation of stored lipids is activated as the body starts to switch to fat oxidation and reduces protein catabolism. Metabolism also slows down (Castellini and Rea 1992).

Phase II: This stage involves increased oxidation of lipids, the production of ketone bodies and the partial sparing of protein. Lipids cannot cross the blood-brain barrier, but ketone bodies (a product of lipid catabolism) can cross the barrier and act as a fuel source for the CNS. As a result of lipid oxidation, both circulating lipids (as nonesterified fatty acids; NEFA) and ketone bodies increase in their concentration in blood (Castellini and Rea 1992). In species not adapted to fasting, the high production of ketone bodies alters the acid-base balance and metabolic acidosis (ketosis) may occur. During Phase II, protein catabolism is reduced but still occurs. Protein catabolism to amino acids provides the precursors for gluconeogenic production of glucose. Therefore, during Phase II fasting, ketones from lipid catabolism and glucose derived from protein provide energy for the CNS, while NEFA provide energy for the rest of the body (Castellini and Rea 1992). After a prolonged

period of fasting the body may still have an adequate store of lipid to use, but protein from skeletal muscle starts to become limited. Cardiac muscle is one of the first sources of protein that is then utilised and this may in some instances result in sudden death (Castellini and Rea 1992).

Phase III: or terminal starvation occurs when 30-50% of the body protein has been wasted. The function of vital organs may become compromised as lipid catabolism decreases and circulating ketones decline (Castellini and Rea 1992).

5.1.2.2 Fasting adapted species

The biochemical processes associated with food deprivation are similar for both fasting and non-fasting adapted species (Castellini and Rea 1992). In both groups, during fasting metabolic rate and protein utilisation decline, fat oxidation increases and gluconeogeneis and ketone body production provide fuel for glucose dependent tissues (Castellini and Rea 1992). In fasting adapted species the protein sparing phase is, however, very efficient and nitrogen wastes are minimal. Ketone bodies also do not accumulate to levels that induce ketosis in fasting adapted species (Castellini and Rea 1992).

Seals appear to be able to preferentially select the use of reserves from different parts of the body (e.g. blubber versus core fat and proteins) during different stages of the fast. It has been suggested that this occurs in order to ensure an adequate layer of blubber is preserved for thermoregulation. Long-chain fatty acids (chain length >C18) are thought to be the main source of fuel during the fast (Castellini and Rea 1992).

Evidence of phase III (terminal) fasting has not been conclusively observed in studied marine mammals such as seals. It seems under normal fasting conditions the fast is terminated before phase III fasting is reached. Evidence of phase III fasting has been observed in penguins, but only after the penguins were forcibly fasted past their natural departure times to feeding grounds (Castellini and Rea 1992). Thus the natural behaviour of a fasting adapted species appears to be that the fast is timed to end before increased utilisation of protein becomes necessary.

If phase III fasting and thus starvation occurs, some animals will die rapidly from hypothermia and exhaustion, while others die after a period of illness caused by immunocompromise brought about by chronic malnutrition and complicated by factors such as hypothermia, dehydration and electrolyte imbalance, hormonal disturbances, and infection by parasites and opportunistic pathogens (Geraci and Lounsbury 2002).

5.1.3 Body condition

The body condition of an animal is said to correspond with its energetic state (Millar and Hickling 1990). For example an animal in good body condition has more energy reserves than an animal in poor condition. Animals with larger energy stores may have better resilience and higher survival than individuals with small reserves (Millar and Hickling 1990). Decreases in body condition may have an effect at both the individual and population level (Millar and Hickling 1990) as variation in fat reserves in mammals has been shown to influence reproductive performance by affecting the onset of sexual maturity, inter-birth intervals, pregnancy rates, foetal growth rates and the onset of menopause (Aguilar and Borrell 1990; Trites 1991; Guinet *et al.* 1998; Evans *et al.* 2003).

5.1.4 Aim of this section

The aim of this section is to assess the body condition and nutritional status of stranded humpback whales via:

- Visual assessment of body condition
- Measuring the total lipid content of blubber samples
- Measuring blubber depth

5.2 Materials and Methods

5.2.1 Visual assessment of body condition

A visual assessment of body condition was made based on the anatomical locations and scoring system outlined by Bradford *et al.* (2012). The scoring system developed by Bradford *et al.* (2012) was intended for gray whales (*Eschrichtius robustus*), however it is partially based on the findings and scoring system of Pettis *et al.* (2004) whom initially provided a scoring system for evaluating post-cranial subcutaneous fat reserves in North Atlantic Right

Whales (*Eubalaena glacialis*). It is assumed the scoring system outlined by Bradford *et al.* (2012) is also applicable to humpback whales.

According to Bradford *et al.* (2012), the relative amount of subcutaneous fat can be visually assessed in three body regions: the post-cranial area, the scapular region, and the lateral flanks. Apparent reductions in body mass in these regions results in three diagnostic features, respectively: 1) a post-cranial depression, 2) a subdermal protrusion of the scapula, and 3) a depression along the dorsal aspect of the lateral flanks bilaterally.

As outlined by Bradford *et al.* (2012) the following scoring system was applied to the humpback samples:

Post-cranial Cranial Condition Score

3 = whales with flat or rounded backs

2 = whales with a slight to moderate post-cranial depression

1 = whales with a significant post-cranial depression such that a pronounced 'hump' is visible posterior to the blowholes

Scapular Condition Score

2 = whales with rounded sides over the shoulder blades

1 = whales with a subdermal protrusion of the scapula

Lateral Flank Condition Score

2 = whales with rounded sides from the post-cranial area to the start of the caudal peduncle

1= whales with a depression along the dorsal aspect of the lateral flanks bilaterally

The post-cranial condition score is said to be the region most indicative of overall body condition and is also the site where a discernable loss of mass first occurs (Bradford *et al.* 2012).

Composites of the postcranial (P), scapular (S) and later flank (L) condition scores are used by Bradford *et al.* (2012) to assign overall body condition (i.e. good, fair, or poor). In

consideration that P is most indicative of body condition scores of 3SL are classified as good body condition, 2SL as fair body condition, and 1SL as poor body condition, unless both the scapular and lateral condition scores are poor. In those cases (i.e. composites of 311 and 211) the condition score is brought down a rating level (i.e. to fair and poor, respectively) (Bradford *et al.* 2012). If a region is unable to be scored, it is given an X. A score of X does not change the overall body condition rating unless an X is assigned to the postcranial score (Bradford *et al.* 2012). In summary, the possible composites within each body condition category are (Bradford *et al.* 2012):

Good- 322, 321, 32X, 312, 31X, 3X2, 3X1, 3XX

Fair- 311, 222, 221, 22X, 212, 21X, 2X2, 2X1, 2XX

Poor- 211, 122, 121, 12X, 112, 111, 11X, 1X2, 1X1, 1XX

Unknown- X22, X21, X2X, X12, X11, X1X, XX2, XX1, XXX

Adequate photos for a visual assessment of body condition were available for only seven of the humpback whales that stranded in 2011 (Appendix J).

5.2.2 Sites for blubber thickness measurements

The sites for blubber thickness measurements (Figure 8) follow those used by the Southern Right Whale Health Monitoring Program in Argentina.

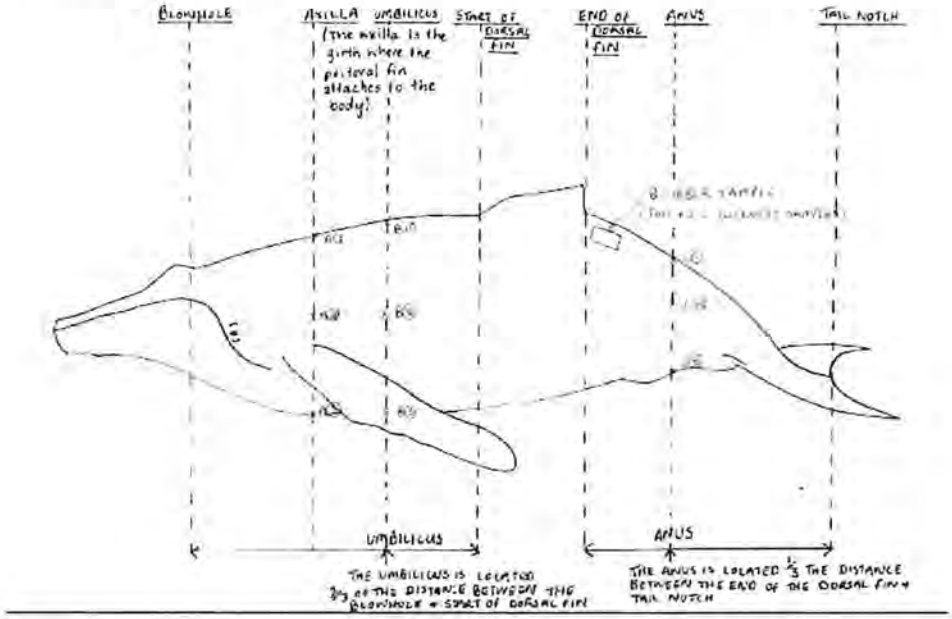
Blubber thickness measurements were collected for five humpback whales. One confounding factor was that in the extremely emaciated animals in which the blubber was largely devoid of adipose content (and comprised largely of the fibrovascular component), the division between blubber and underlying musculature was very poorly demarcated, making accurate blubber measurement difficult.

5.2.3 Site for blubber sample collection

Full thickness blubber samples were collected immediately posterior to the dorsal fin in a dorsolateral position (Figure 8). This location was selected for future comparative purposes given that biopsy samples are collected from this site in free swimming whales.

Blubber samples were collected from seven humpback whales in 2011. For comparative purposes, a blubber sample collected opportunistically from a sub-adult humpback whale, in fair body condition, euthanized in 2010 was also included in the analyses. This humpback live stranded on a sandbank in Princess Royal Harbour, Albany on the 19th of August 2010 and was subsequently euthanized on the 2nd of September 2010.

Figure 8. Sites for blubber thickness measurements and blubber sample.



5.2.4 Analysis of blubber for total lipid content

Each blubber sample was divided into three equal sections: an outer, middle and inner section. Approximately 0.5g of blubber was then taken from each section (Figure 9). Lipid was extracted using a modified Folch *et al.* (1957) procedure employing chloroform and methanol. Instead of grinding the samples in a mortar an ultraturrex (30 seconds) was used to break up the samples. The samples were then left overnight at 4°C to complete the extraction.

Figure 9. Sampling blubber according to outer, inner and middle areas.

5.3 Results

All but one of the neonates examined were found to be in poor body condition (Table 6). Blubber thickness measurements were found to vary with location and between individuals (Table 7). The blubber lipid content of the sub-adult was much higher than that of most of the neonates (Table 8). All but one of the neonates had very low blubber lipid content. There appeared to be stratification of lipid content between the sections but the trend (in terms of the top section containing more lipids than the lower section and vice versa) varied between individuals (Table 8).

Table 6. Visual assessment of body condition based on the scoring system of Bradford *et al.* (2012).

Date	Location	Length	Age	Sex	Postcranial	Scapular	Lateral flank	Condition score
May 19	Bremer Bay	4.8m	Neonate	Female	1	1	1	Poor
July 12	Exmouth	4.3m	Neonate	Unknown	1	1	1	Poor
July 19	Exmouth	4.2m	Neonate	Male	1	1	1	Poor
August 5	North of Geraldton	4.1m	Neonate	Male	1	1	1	Poor
August 6	Cape Arid	4.3m	Neonate	Unknown	1	1	1	Poor
August 17	Peaceful Bay, Walpole	4.8m	Neonate	Female	3	2	2	Good
August 24	Quinns Rocks, Perth	3.9m	Neonate	Female	1	1	1	Poor

Table 7. Blubber thickness measurements in centimetres.

Date	Location	Length	Age	Sex	Dorsal Axilla	Lateral Axilla	Ventral Axilla	Dorsal Umbilicus	Lateral Umbilicus	Ventral Umbilicus	Dorsal Anus	Lateral Anus	Ventral Anus
May 19	Bremer Bay	4.8m	Neonate	Female	2.7	1.8	3.8	3.6	3.7	3.9	3.5	3.7	3.3
August 5	North of Geraldton	4.1m	Neonate	Male	3.4	4.5	3.8	5.4	4.7	6.0	4.1	3.8	8.7
August 17	Peaceful Bay, Walpole	4.8m	Neonate	Female	4.6	5.3	3.3	7.3	6.4	5.8	5.0	4.1	4.6
August 24	Quinns Rocks, Perth	3.9m	Neonate	Female	3.4	3.8	3.2	4.0	3.2	3.4	8.9	3.2	3.7
August 25	Jurien	4.35m	Neonate	Male	4.8	4.6	2.2	3.0	2.8	2.7	2.6	2.3	2.0

Table 8. Blubber lipid content.

Year	Date	Location	Length	Age	Sex	Blubber location	% Lipid	Body Condition
2010	August 19	Albany	9.5m	Sub-adult	Unknown	Top Mid Lower	66.84 87.67 79.12	Fair
2011	May 19	Bremer Bay	4.8m	Neonate	Female	Top Mid Lower	12.44 2.57 2.56	Poor
2011	July 12	Exmouth	4.3m	Neonate	Unknown	Top Mid Lower	2.45 0.71 1.29	Poor
2011	July 19	Exmouth	4.2m	Neonate	Male	Top Mid Lower	4.11 3.65 1.40	Poor
2011	August 5	North of Geraldton	4.1m	Neonate	Male	Top Mid Lower	4.10 1.06 0.44	Poor
2011	August 17	Peaceful Bay, Walpole	4.8m	Neonate	Female	Top Mid Lower	45.42 49.38 50.00	Good
2011	August 24	Quinns Rocks, Perth	3.9m	Neonate	Female	Top Mid Lower	3.26 0.75 0.43	Poor
2011	August 25	Jurien	4.35m	Neonate	Male	Top Mid Lower	10.30 5.58 2.06	Unknown

5.4 Discussion

Neonate baleen whales are said to be born with a blubber layer that is several centimetres thick (Iverson 2009), however it is unknown what the average lipid content is or whether the body condition score outlined by Bradford *et al.* (2012) is applicable to neonates. The condition score of the Peaceful Bay neonate that stranded August 17, in conjunction with the histology findings, blubber lipid content and blubber thicknesses recorded; compared to the assessment scores, blubber lipid contents and blubber thicknesses of the other neonates, suggests that the Bradford *et al.* (2012) scoring system is applicable to neonatal humpbacks. The Peaceful Bay humpback was the only neonate that appeared to be in good body condition and had a blubber lipid content that was much higher than any of the other neonates. It is, however, unknown if the lipid content and condition score of the Peaceful Bay neonate is representative of that of a normal calf. It could be speculated that humpback calves are usually born in good body condition with a blubber lipid content similar or higher than that of the Peaceful Bay neonate.

The poor body condition scores and extremely low blubber lipid content in all but one of the humpback neonates indicate they were in a state of malnutrition and had virtually no fat reserves. It is highly likely that the ability of the neonates to thermoregulate and maintain buoyancy would have been compromised. In addition the post-mortem findings and blood results of two of the humpback neonates indicate that they received little, if any, colostrum. Calves born without any fat reserves that are then deprived of milk would die quickly. Humpback milk is highly nutritious and contains 20.4-41.3% fat, 10.7-13.6% protein, 0.2-1.7% lactose, and 40.6-65.4% water (Yablokov *et al.* 1974). A minimum of 43 kg of milk may be consumed by a calf daily (Winn and Reichley 1985).

The malnutrition observed in the neonates is likely a reflection of the poor nutritional status of the mothers. In mammalian species there is a correlation between body condition and reproductive success in females (Loudon *et al.* 1983). The nutritional status of a breeding female may impact on every aspect of reproduction, including the timing of reproduction, probability of pregnancy,

embryonic absorption, offspring mass, offspring survival, and progeny sex ratio (Bradford *et al.* 2012). According to Bradford *et al.* (2012) the relationship between body condition and reproductive success in whales is not well understood but if a reproductive female has insufficient body reserves one of the following is likely to occur: she may fail to ovulate, fail to conceive, fail to give birth, or fail to nurse. Calves born to malnourished mothers are vulnerable from birth and often their longevity is compromised early in development (Geraci and Lounsbury 2009).

A decrease in prey abundance has been associated with reduced reproduction in some baleen whales (Lockyer 1987; Leaper *et al.* 2006). Southern right whales (*Eubalaena australis*) breeding off the coast of Argentina have been found to have increased failure late in pregnancy or early in lactation following periods of warm sea surface temperature in the feeding grounds in South Georgia in the preceding year (Leaper *et al.* 2006). An inverse relationship between krill density and sea surface temperature at South Georgia has been identified (Trathan *et al.* 2003).

The sub-adult humpback whale that stranded in Albany in August 2010 was likely on the southward migration back to the feeding grounds and thus would have been fasting for a considerable period of time. This animal was stuck on a sand bank for 15 days prior to euthanasia and despite appearing in a debilitated state (its skin had begun to slough and it had a severe cyamid burden) it was still in a fair body condition. Thus the blubber lipid content recorded for this individual is representative of a sub-adult humpback that has been fasting for months and was in a debilitated state [the cause of the stranding/debilitation remains unknown but most cetaceans that strand singularly are thought to be diseased (Geraci and Lounsbury (2009)].

There is insufficient data to interpret the blubber thickness measurements although there is a notable difference in the blubber thickness at the dorsal and lateral umbilicus of the Peaceful Bay neonate (which was in good body condition) compared to the other neonates in poor body condition. Konishi (2006) found that blubber depth measurements taken at the dorsal and lateral umbilicus and laterally beneath

the dorsal fin in minke whales (*Balaenoptera bon*aerensis) provided the best indication of body condition as blubber at these sites changed in thickness in relation to fattening. In contrast anterior blubber in minke whales appears not to store energy but rather serves the purpose of maintaining streamlining and/or insulation (Konishi 2006).

5.5 Conclusion

- Data was available to assess the body condition and blubber lipid content of eight humpback neonates. This data indicated that all but one of the neonates was in a state of malnutrition.
- The malnutrition observed in the neonates reflects the poor nutritional status of the mothers.
- The contrast between the condition score and associated histology findings, blubber lipid content and blubber thicknesses of the Peaceful Bay neonate in comparison with the other neonates suggests that the visual body assessment score outlined by Bradford *et al.* (2012) is applicable to humpback neonates.
- It is currently not possible to interpret the blubber thickness measurements as more data is needed in order to identify trends and make comparisons.

6. Fatty Acids

6.1 Introduction

6.1.1 Why study fatty acids?

Fatty acids can be used to study foraging ecology and food webs (Budge *et al.* 2006) as many long chain fatty acids that are specific to individual prey items are transferred from prey to predator with insignificant alterations (Worthy 2008). Thus, by examining changes in fatty acids of the predator, it is possible to ask qualitative questions about spatial or temporal variations in diet, both among and within individuals or populations (Budge *et al.* 2006).

6.1.2 Fatty Acids in Marine Mammals

Lipids in blubber are stored predominantly as triacylglycerols, which consist of three fatty acids linked by an ester bond to a glycerol molecule (Iverson 2009b). Most marine fatty acids are synthesised by phytoplankton and other organisms at low trophic levels and undergo little biochemical change when passed up the food chain (Dalsgaard *et al.* 2003; Iverson 2009b).

Vertical stratification of fatty acid composition in blubber has been reported in a number of cetacean species (Budge *et al.* 2008). Although the patterns of stratification differ among species, a trend seems to exist in those species examined in that higher monounsaturated fatty acids 14:1n-5, 16:1n-7 and occasionally 18:1n-9, and lower saturated fatty acids 16:0 and 18:0 occur more in the outer than in the inner blubber layers (Budge *et al.* 2008). Possible explanations for stratification of fatty acids include: a particular arrangement of fatty acids may enhance membrane fluidity in the outer blubber layers; fatty acid stratification might improve the insulation properties of blubber; or the distribution of fatty acids may be due to both the mobilisation and replenishment of dietary polyunsaturated fatty acids from the inner layer of blubber, with an accumulation of readily biosynthesised fatty acids (e.g. 14:0, 14:1n-5, 16:0, 16:1n-7 and 18:0) in the outer layer (Budge *et al.* 2008).

Raclot (2003) reported that selective mobilisation of fatty acids from fat stores depends on fatty acid length and saturation status. A fatty acid is more readily mobilised if its carbon chain is shorter and more saturated (Raclot 2003). Highly mobilised fatty acids are said to include 16-20 carbon atom fatty acids with 4-5 double bonds, whereas weakly mobilised fatty acids include 20-24 carbon atom fatty acids with 0-1 double bond, with moderately mobilised fatty acids including all others (Raclot 2003).

Budge *et al.* (2008) examined the fatty acid composition of bowhead whales (*Balaena mysticetus*) and found no significant differences in fatty acid composition among inner blubber layers sampled at different body locations on the same animal. Additionally, there was no significant difference between genders. In contrast, significant differences in fatty acid composition were found between inner and outer layers of blubber at the same body site (see above paragraph for explanation); furthermore, fatty acid composition varied significantly with age. According to Budge *et al.* (2008), although milk fatty acid signatures arise predominantly from the mothers' diet the actual fatty acid signature of the milk is likely to be somewhat different from the prey. Season and year were all also found to have significant effects on fatty acid composition (Budge *et al.* 2008). The yearly variations in fatty acid composition in the bowhead whales were likely due to yearly variations in plankton fatty acid profiles (Budge *et al.* 2008). Yearly climatic variation will affect the distribution of phytoplankton species, most of which have species-specific fatty acid compositions (Budge *et al.* 2008), thus resulting in shifts in the fatty acid profiles of the phytoplankton. When phytoplankton is consumed, these variations are passed to the zooplankton grazers and, in turn, to carnivorous zooplankton and filter-feeding whales, such as the bowhead (Budge *et al.* 2008). Budge *et al.* (2008) concluded that given the close link of filter-feeding whales to the base of the food web, such species could be used to monitor the effects of climate change on lower trophic levels and production processes in the Arctic.

6.1.3 Humpback whale diet in the Southern Hemisphere

Southern hemisphere humpback whales are thought to feed almost exclusively on Antarctic krill (*Euphausia superba*). Krill have a long life span (>6 years) and Friedlaender *et al.* (2008) found that humpbacks around the Antarctic Peninsula (IWC Management Area I) mostly forage for krill greater than two years of age. In waters around the Antarctic Peninsula a positive relationship between krill density and humpback abundance has also been identified (Friedlaender *et al.* 2006).

The summer feeding grounds of BSD are located in the eastern Antarctic (>56°S, 80-110°E) (Chittleborough 1965) in IWC Management Area IV. There is currently a lack of data regarding humpback prey interactions in this region (IWC In Press).

6.1.4 The diet of Krill (Euphausia superba)

All life stages of krill have been shown to be capable of adapting to locally available food (Nichol 2006). Larvae are thought to be highly dependent on ice algae and the under-ice microbial community (Quetin and Ross 1991). Adults are less dependent on the underside of the ice than the larvae and will consume detritus and heterotrophic material in winter (Perisonotto *et al.* 2000). All stages are thought to consume pelagic food sources throughout the year (Quetin and Ross 1985).

Phleger *et al.* (2002) found interannual changes in krill fatty acid composition collected from the oceanographic region near Elephant Island reflecting variation in prey between years at this location.

6.1.5 Aims of this section

The fatty acid profiles of humpback whales will reflect the fatty acid profiles of their prey. Spatial and temporal differences in the fatty acids of krill will thus be reflected in the humpbacks and hence could potentially be used to: 1) discriminate between individual humpbacks feeding in different areas within Area IV; and 2) changes in feeding ecology of krill - which in turn may be used to monitor for oceanographic and climate change.

The aim of this section is to capture the blubber fatty acid profiles of stranded humpback whales from 2011 as a baseline for future monitoring.

6.2 Material and Methods

The same eight blubber samples measured for total lipid content (see section 5.2.3) were also analysed for fatty acids.

Following lipid extraction using the Folch *et al.* (1957) method (see section 5.2.4) transesterification (formation of FA methyl esters or FAME) was initiated using an acidic catalyst (Hilditch reagent) (Budge *et al.* 2006; Appendix 4). Gas Chromatography was used for FA determination (Budge *et al.* 2006; Appendix 4).

6.3 Results

In general the most abundant fatty acids for the sub-adult juvenile were also the most abundant fatty acids for the neonates (Table 9). The fatty acid profiles for the two neonates known not to have received any milk were similar to the fatty acid profiles of the other neonates for which it was unknown whether they had received any milk.

There appears to be vertical stratification in fatty acid composition between blubber sections (Table 9).

Table 9. Fatty acid composition of blubber samples collected.

		% of total FAME												
Humpback	**Blubber layer**	**C8:0**	**C10:0**	**C11:0**	**C12:0**	**C13:0**	**C14:0**	**C14:1n5**	**C15:0**	**C15:1**	**C16:0**	**C16:1n7**	**C17:0**	**C17:1**
August 19 2010	Top	0.0	0.07	0.0	0.11	0.04	10.25	2.04	0.45	0.0	13.56	23.89	0.21	0.0
Albany	Mid	0.0	0.04	0.0	0.14	0.05	12.96	1.11	0.47	0.0	18.59	19.18	0.22	0.0
Sub-adult	Lower	0.0	0.05	0.0	0.14	0.05	13.37	0.96	0.43	0.0	19.82	17.31	0.22	0.0
May 19 2011	Top	0.0	0.30	0.0	0.25	0.07	7.15	3.13	0.31	0.0	15.49	39.12	0.08	0.0
Bremer Bay	Mid	0.0	1.70	0.0	0.44	0.17	10.79	3.42	0.35	0.0	31.13	2.85	0.18	0.0
neonate	Lower	0.0	1.41	0.0	0.47	0.18	9.76	2.55	0.26	0.0	38.28	2.75	0.21	0.0
July 12 2011	Top	0.0	2.23	0.0	0.28	0.11	6.27	4.34	0.33	0.0	20.69	4.75	0.21	0.0
Exmouth	Mid	0.0	2.53	0.0	0.14	0.06	4.53	1.19	0.23	0.0	16.62	14.92	0.64	0.0
neonate	Lower	0.0	4.74	0.0	1.28	0.24	4.57	0.76	0.12	0.0	17.79	11.50	1.17	0.0
July 19 2011	Top	0.0	1.15	0.0	0.44	0.11	7.65	4.91	0.40	0.0	13.30	4.52	0.16	0.0
Exmouth	Mid	0.0	2.05	0.0	0.82	0.13	7.04	2.60	0.22	0.0	20.24	19.39	0.41	0.0
neonate	Lower	0.0	2.21	0.0	0.41	0.11	7.21	2.57	0.29	0.0	27.54	2.24	0.57	0.0
August 24 2011	Top	0.0	0.66	0.0	0.27	0.13	11.72	0.26	0.47	0.0	22.60	3.69	0.40	0.0
Quinns Rocks	Mid	0.0	2.52	0.0	0.72	0.05	4.92	1.48	0.24	0.0	14.12	14.83	0.36	0.0
neonate	Lower	0.0	0.79	0.0	1.23	0.00	3.65	0.54	0.24	0.0	14.92	9.23	0.72	0.0
August 5 2011	Top	0.0	1.49	0.0	0.34	0.08	7.12	0.05	0.39	0.0	22.19	5.26	0.17	0.0
North Geraldton	Mid	0.0	3.68	0.0	0.51	0.10	3.87	1.86	0.80	0.0	17.51	19.76	1.30	0.0
neonate	Lower	0.0	2.42	0.0	1.45	0.00	2.69	0.80	0.20	0.0	17.81	12.95	0.31	0.0

August 17 2011	Top	0.0	0.12	0.0	0.02	0.09	10.61	0.22	0.43	0.0	17.03	27.22	0.08	0.0
Peaceful Bay	Mid	0.0	0.14	0.0	0.02	0.04	10.83	0.23	0.44	0.0	16.36	27.95	0.07	0.0
neonate	Lower	0.0	0.14	0.0	0.02	0.05	11.38	0.23	0.46	0.0	19.02	25.53	0.11	0.0
August 25	Top	0.0	0.50	0.0	0.08	0.02	7.18	0.11	0.34	0.0	15.65	40.98	0.02	0.0
Jurien	Mid	0.0	1.31	0.0	0.10	0.10	8.09	0.07	0.25	0.0	23.32	26.22	0.16	0.0
neonate	Lower	0.0	2.05	0.0	0.48	0.10	5.95	2.07	0.19	0.0	21.28	19.69	0.21	0.0

		% of total FAME									
Humpback	**Blubber layer**	**C18:0**	**C18:1 cis=trans**	**C18:2 trans 9**	**C18:2n 6**	**C18:3n6**	**C18:3n3**	**C18:4n3#**	**C20:0**	**C20:1**	**C20:2**
August 19 2010	Top	1.89	23.16	0.0	2.37	0.13	0.64	0.18	0.0	1.19	0.19
Albany	Mid	2.32	21.62	0.0	2.25	0.16	0.60	0.18	0.0	1.73	0.12
Sub-adult	Lower	2.60	21.90	0.0	2.80	0.15	0.54	0.15	0.0	1.79	0.18
May 19 2011	Top	1.42	16.49	0.0	0.85	0.06	0.15	0.06	0.0	0.67	0.08
Bremer Bay	Mid	3.91	23.68	0.0	0.78	0.08	0.13	0.04	0.0	0.77	1.47
neonate	Lower	4.99	19.03	0.0	2.28	0.05	0.11	0.00	0.0	0.59	1.36
July 12 2011	Top	4.25	29.69	0.0	1.96	0.09	0.15	0.00	0.0	0.95	1.97
Exmouth	Mid	8.22	21.96	0.0	0.93	0.29	0.38	0.00	0.0	1.08	2.19
neonate	Lower	10.71	20.29	0.0	1.07	0.30	0.33	0.00	0.0	1.17	1.29
July 19 2011	Top	2.59	31.35	0.0	1.96	0.09	0.21	0.00	0.0	0.86	1.91
Exmouth	Mid	4.46	24.25	0.0	1.71	0.06	0.00	0.00	0.0	0.71	1.23
neonate	Lower	6.54	26.34	0.0	2.52	0.11	0.29	0.33	0.0	1.12	2.25

August 24 2011	Top	5.44	19.94	0.0	3.16	0.14	0.88	0.00	0.0	0.80	0.76
Quinns Rocks	Mid	8.20	24.55	0.0	2.32	0.29	0.16	0.00	0.0	0.60	0.61
neonate	Lower	12.82	23.48	0.0	2.79	0.00	0.22	0.00	0.0	0.66	0.76
August 5 2011 North	Top	3.41	33.99	0.0	1.98	0.09	0.14	0.09	0.0	0.86	2.04
Geraldton neonate	Mid	6.43	23.64	0.0	1.69	0.36	0.29	0.00	0.0	0.69	0.85
	Lower	14.01	21.53	0.0	1.61	0.10	0.45	0.00	0.0	0.40	0.61
August 17 2011	Top	2.93	9.38	0.0	2.73	0.14	0.12	0.12	0.0	0.26	0.14
Peaceful Bay	Mid	2.45	9.40	0.0	2.92	0.15	0.12	0.19	0.0	0.23	0.06
neonate	Lower	3.13	9.71	0.0	2.42	0.17	0.14	0.20	0.0	0.22	0.04
August 25	Top	1.77	11.76	0.0	1.16	0.08	0.11	0.12	0.0	0.48	0.86
Jurien	Mid	2.76	17.01	0.0	1.88	0.06	0.16	0.17	0.0	0.63	0.96
neonate	Lower	5.70	17.56	0.0	1.63	0.15	0.17	0.25	0.0	0.61	1.16

		% of total FAME									
Humpback	**Blubber layer**	**C21:0**	**C20:3n6**	**C20:4n6**	**C20:3n3**	**C22:0**	**C20:5n3**	**C22:1n9**	**C22:2**	**C23:0**	**C22:4n6#**
August 19 2010	Top	0.0	0.40	0.76	0.05	0.0	4.98	0.21	0.0	0.0	0.22
Albany	Mid	0.0	0.39	0.65	0.06	0.0	5.59	0.29	0.0	0.0	0.15
Sub-adult	Lower	0.0	0.40	0.64	0.05	0.0	5.01	0.30	0.0	0.0	0.16
May 19 2011	Top	0.0	0.67	0.77	0.0	0.0	2.15	0.17	0.0	0.0	0.30
Bremer Bay	Mid	0.0	1.79	1.43	0.0	0.0	1.55	0.00	0.0	0.0	0.47
neonate	Lower	0.0	1.39	1.35	0.0	0.0	1.59	0.00	0.0	0.0	0.37

July 12 2011	Top	0.0	1.69	2.52	0.0	0.0	2.33	0.00	0.0	0.0	0.79
Exmouth	Mid	0.0	2.19	4.70	0.0	0.0	3.47	0.24	0.0	0.0	3.28
neonate	Lower	0.0	2.11	6.45	0.0	0.0	3.35	0.00	0.0	0.0	0.94
July 19 2011	Top	0.0	1.24	1.67	0.0	0.0	1.40	0.18	0.0	0.0	0.74
Exmouth	Mid	0.0	1.52	2.10	0.0	0.0	1.22	0.00	0.0	0.0	0.53
neonate	Lower	0.0	1.82	2.64	0.0	0.0	1.23	0.04	0.0	0.0	0.74
August 24 2011	Top	0.0	0.65	2.67	0.0	0.0	5.33	0.00	0.0	0.0	0.71
Quinns Rocks	Mid	0.0	0.58	4.68	0.0	0.0	4.96	0.31	0.0	0.0	0.83
neonate	Lower	0.0	0.81	8.08	0.0	0.0	5.89	0.43	0.0	0.0	1.08
August 5 2011 North	Top	0.0	1.25	1.70	0.0	0.0	1.71	0.17	0.0	0.0	0.55
Geraldton neonate	Mid	0.0	1.68	3.04	0.0	0.0	1.95	0.00	0.0	0.0	0.51
	Lower	0.0	0.00	8.70	0.0	0.0	2.99	0.00	0.0	0.0	1.15
August 17 2011	Top	0.0	0.42	1.03	0.0	0.0	8.61	0.04	0.0	0.0	0.31
Peaceful Bay	Mid	0.0	0.43	1.04	0.0	0.0	8.68	0.04	0.0	0.0	0.28
neonate	Lower	0.0	0.42	0.99	0.0	0.0	8.33	0.00	0.0	0.0	0.27
August 25	Top	0.0	0.17	0.97	0.0	0.0	3.66	0.05	0.0	0.0	0.48
Jurien	Mid	0.0	0.10	1.12	0.0	0.0	1.90	0.00	0.0	0.0	0.51
neonate	Lower	0.0	1.14	3.32	0.0	0.0	3.30	0.20	0.0	0.0	0.87

		% of total FAME				
Humpback	**Blubber layer**	**C24:0**	**C22:5n3#**	**C24:1**	**C22:6n3**	**Total mg-100g as received**
August 19 2010	Top	0.0	6.57	0.0	6.41	77452
Albany	Mid	0.0	5.02	0.0	6.12	74457
Sub-adult	Lower	0.0	5.03	0.0	5.97	76476
May 19 2011 Bremer	Top	0.0	6.52	0.0	3.73	73988
Bay neonate	Mid	0.0	9.09	0.0	3.76	55656
	Lower	0.0	7.21	0.0	3.80	59088
July 12 2011	Top	0.0	11.46	0.0	2.92	46883
Exmouth	Mid	0.0	7.02	0.0	3.18	35994
neonate	Lower	0.0	6.70	0.0	3.13	15079
July 19 2011	Top	0.0	14.02	0.0	3.13	53758
Exmouth	Mid	0.0	7.04	0.0	2.26	24033
neonate	Lower	0.0	8.21	0.0	2.66	47602
August 24 2011	Top	0.0	13.97	0.0	5.35	48113
Quinns Rocks	Mid	0.0	9.46	0.0	3.22	48649
neonate	Lower	0.0	9.07	0.0	2.60	42724
August 5 2011 North	Top	0.0	10.04	0.0	4.86	48590
Geraldton neonate	Mid	0.0	7.02	0.0	2.46	41442
	Lower	0.0	7.06	0.0	2.76	35192
August 17 2011	Top	0.0	9.07	0.0	8.88	67068

Peaceful Bay	Mid	0.0	8.75	0.0	9.17	65644
neonate	Lower	0.0	8.52	0.0	8.50	65418
August 25	Top	0.0	8.89	0.0	4.56	74856
Jurien	Mid	0.0	9.79	0.0	3.35	44253
neonate	Lower	0.0	8.18	0.0	3.74	26849

6.4 Discussion

Statistical analysis of the fatty acid results was not possible given the small sample size (n=8) and consequently in-depth analysis was not possible. This data however serves as an important baseline for 2011 and additional future data will allow for more significant interpretation.

There appeared to be vertical stratification between the blubber sections but further data and analysis is needed to confirm this. If vertical stratification is confirmed it will have important implications for measuring fatty acid profiles from biopsy samples collected from free-ranging whales.

Two of the neonates were known to have received little to no colostrum/milk and the status of the other neonates was unknown. It is unknown whether the fatty acid composition of the blubber of a neonate which had not received any milk would provide any information as to the mother's diet.

Fatty acids can be divided into two groups including non-essential fatty acids which are readily biosynthesized in mammalian systems and essential fatty acids which are acquired through the diet (Iverson *et al.* 1995). Iverson *et al.* (1995) found that the fatty acid composition of new born hooded seal (*Cystophora cristata*) pups was notably different to that of the mother. It was concluded that foetal deposition of fatty acids in the blubber was likely due to a combination of both foetal synthesis and direct placental transfer of maternal circulating fatty acids.

7. General discussion

7.1 Climate change, Antarctic krill and humpback whales as a sentinel species

Ninety percent of the world's great whales feed in the Antarctic predominantly on krill (*Euphausia superba*) (Simmonds and Isaac 2007). There is increasing concern that global warming is resulting in ecosystem scale changes in the Antarctic and these changes combined with an expanding krill fishery may result in a considerable reduction in the biomass of krill available to whales (Nicol and Foster 2003). For example, juvenile krill are dependent on access to a constant source of sea ice algae in order to survive (McMinn 2011). Climate change is likely to reduce the seasonal extent of sea ice which will in turn impact on the quantity of sea ice algae present. This decline will effect juvenile krill survival and consequently the abundance of all species that rely on krill for a food source (McMinn 2011). Additionally, it is unknown how significantly krill biomass will be altered by an expanding krill fishery although increased krill fishing in conjunction with climate warming has already been linked to population changes in penguin populations in Antarctica (Trivelpiece *et al.* 2011). A reduction in food may result in an expansion of whale foraging time and range which may have further implications with regard to delaying or increasing migration times and reduced fat reserves, as was noted for gray whales in the Bering and Chukchi Seas (Rugh and Fraker 1981; Miller et al. 1985; Moore *et al.* 2003).

A species, such as the humpback whale, given its close link to the base of the Antarctic food chain, could be used as a sentinel species for the health of the Southern Ocean and Antarctic ecosystem. Furthermore, small changes in baleen whale birth and death rates may have a significant effect on the Southern Ocean ecosystem through a reduction in primary productivity via a decreased supply of iron into surface water (Wiedenmann *et al.* 2011). Iron is a limiting micronutrient in the Antarctic and baleen whale faeces is thought to be an importance source of iron (Wiedenmann *et al.* 2011). As an abundant species in the Antarctic, humpback whales are likely to significantly contribute to the iron supply in the Antarctic.

7.2 Increase in strandings recorded in Western Australia

In 2010 Coughran and Gales (2010) reported that the increase in humpback strandings reported in WA in 2008 and 2009 most likely represented a 'real spike in mortality'. They proposed the following three hypotheses as possible explanations (Coughran and Gales 2010, p 3):

1. The peak in mortalities does not represent an increase in mortality rate in BSD, but is an artefact of other features such as search effort and coastal oceanography.
2. The peak in mortalities represents a transient increase in mortality rate in BSD driven by unknown cause(s) that may be associated with processes on the feeding grounds, breeding grounds, or both.
3. The peak in mortalities represents the start of an increasing trend in mortality rates in BSD driven by unknown cause(s) that may be associated with processes on the feeding grounds, breeding grounds, or both.

Coughran and Gales (2010) deemed hypothesis one to be the least plausible, especially in consideration of the scale of change in strandings recorded over such a short period of time. They were however unable to discriminate the plausibility between hypotheses two and three due to the need for further data but suggested that if hypothesis three is correct then it may "...signify a threshold change in carrying capacity for this population of humpback whales in the Southern Ocean" (Coughran and Gales 2010, p 3).

The number of strandings reported in 2010 and 2011 indicate that the increased stranding trend first noted in 2008 and 2009 has continued for another two years.

7.2.1 Carrying capacity

The abundance of a population is determined generally by the availability of resources (e.g. food, a particular range of temperature, appropriate breeding and resting sites etc) mediated through changes in growth rate (i.e. changes in reproduction, immigration, mortality, and emigration) (Wobeser 2006). According to Wobeser (2006, p 155):

"As the population density increases, the *per capita* availability of resources declines, the rate of increase slows, and eventually a level is reached at which population growth levels off. This level is often referred to as the "carrying capacity" (*K*). At this level, additions to the population through reproduction and immigration approximate losses through mortality and emigration."

Australian populations of humpback whales are thought to have been reduced to 3.5 – 5% of pre-whaling abundance prior to the IWC imposed ban on humpback whaling in the southern hemisphere in 1963 (Department of the Environment and Heritage 2005). The population of BSD prior to exploitation is difficult to estimate but is thought to be between 16,000 and 30,000 (Department of the Environment and Heritage 2005; IWC 2007).

The most recent population estimate for BSD from 2008 (Hedley *et al.* 2008) of 21,750 (95% CI = 17,550-43,000) suggests that the population is likely to be at estimates of pre-exploitation population size. *K,* however, is not a constant; it is dynamic, and represents a maximum population level consistent with environmental conditions at the time (Wobeser 2006). Substantial ecological changes are likely to have occurred throughout the range of BSD since pre-whaling times and as a result *K* is likely to have shifted.

Following the spike in strandings in WA in 2009 the IWC (In Press) noted that:
"...continued monitoring of strandings in this region is important, and might inform as to whether this event was related to unusual climatic or oceanographic conditions in the preceding year, or the dynamics of a population approaching carrying capacity."
It should be noted that the impact of unusual climatic/ oceanographic conditions and the dynamics of a population nearing *K* should not necessarily be viewed as mutually exclusive factors that may have influenced the stranding rate. If the population is at or nearing carrying capacity the impact of unusual climatic and oceanographic conditions on humpback food resources may act as a limiting factor which influences *K*.

The factors that regulate the abundance and growth rate of BSD are not well understood. There are however, an array of anthropogenic factors that should be considered as potentially having an influence either currently or in the future, including:

- Resumption of commercial whaling and/or the expansion of scientific whaling (Department of the Environment and Heritage 2005).
- Habitat degradation which may result in reduced occupancy and/or exclusion of individual whales from suitable habitat, compromised reproductive success, and mortality (Department of the Environment and Heritage 2005).
- Climate and oceanographic change which may result in a two fold effect:

    1. Change in habitat availability - migration, feeding, resting, and calving site selection may be influenced by factors such as ocean currents and water temperature. A change in habitat availability may influence reproductive success and mortality (Department of the Environment and Heritage 2005).

    2. Change in food availability - changes to climate and oceanographic processes may lead to decreased productivity and different patterns of prey distribution and availability (Department of the Environment and Heritage 2005).
- Prey depletion due to over harvesting (Department of the Environment and Heritage 2005).

7.2.2 Significance of the high proportion of strandings in 2011 being neonates

A number of theories could be postulated to account for the high proportion of neonate strandings in 2011:

1. Associated with increased population size and inherent high mortality rate in humpback calves.

2. Associated with parturition occurring in unsuitable areas outside of the known breeding grounds due to environmental conditions.

3. Associated with mothers in a poor nutritional state giving birth to malnourished non-viable calves.

Theory one is unlikely due to the population increasing gradually and 2011 standing out as atypical – given that in previous year's calves accounted for a much lower proportion of strandings. The extent to which theory two may have contributed to strandings in 2011 is difficult to say. The unusually warm water recorded along the WA coastline in 2011 could have potentially influenced humpback calving site selection resulting in calves being born in unsuitable areas. However, if calving site selection due to environmental conditions was the most significant factor we would expect that most calves would have been born in relatively good body condition. Given the malnourished condition of most of the stranded neonates examined in 2011, theory three is likely to have been the most influential factor in the high proportion of neonates stranding in 2011. Furthermore, it is significant that the neonates were born, in some cases, several thousands of kilometres south of the current known breeding grounds. It could be speculated that a change in the abundance and distribution of krill in the Antarctic may have resulted in an expansion of foraging time and/or range which would have led to a delay or increase in migration times and subsequently reduced fat reserves in some pregnant cows. Consequently such animals would not make it in time to the calving grounds, resulting in calves born further south. The suboptimal fat reserves in some pregnant females would also result in the birth of calves in a poor nutritional state.

8. Recommendations

In order to better understand humpback whale strandings in WA the following recommendations are suggested:

1. Continue the current project and the collection of baseline data from stranded whales for at least a five year duration.

Significant information has been gained from the first year of this project and it appears that the data collection has been initiated when the population of BSD is in a period of flux. Anthropogenic threatening factors acting both locally along the WA coastline and in the Antarctic are likely to have an increasing impact on BSD in the future and the collection of data from stranding events will help to elucidate the impact such factors are having.

2. Research is needed into the relationship between krill and humpback whales in Area IV.

Research is needed on annual changes in krill abundance and distribution and the factors which influence this. Furthermore, research is needed in relation to how changes in krill abundance and distribution affect humpback whales in terms of body condition, calving rates and migration timing.

The difficulty associated with conducting such research is appreciated but nevertheless similar research has been conducted elsewhere for baleen whales (as demonstrated by the examples drawn upon throughout this report).

3. Research is needed into monitoring the nutritional status of free swimming whales.

A research project entitled '*Development of a non-lethal method for evaluation of nutritional condition in humpback whales; facilitating chemical and environmental*

risk assessment' has been initiated at Griffith University, Queensland with funding from the Ocean Foundation. If funding was available samples could be collected from BSD for inclusion in this research.

4. Research into biomarkers of immune status, stress and contaminants exposure in free swimming whales.

Such research would provide further information in relation to the overall health status of BSD.

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Appendix A. Maximum sample protocol and check-list for fresh humpback carcasses (for veterinarians)

1. BACKGROUND INFORMATION

Date:	
Name of attending veterinarian	
Location (in relation to the nearest named place):	
When was the whale found:	Date: Time:
Sex (male/female/unknown):	
Age class (calf, yearling, lactating female, adult):	
Carcass condition (Circle) 1. Live (becomes code 2 at death) 2a. Extremely fresh (as if just died, no bloating, meat is considered by most edible) 2b. Slight decomposition (slight bloating, haeme imbibition visible) 3. Moderate decomposition (moderate bloating, skin peeling, penis may be extended in males, organs still intact, excluding post-mortem damage) 4. advanced decomposition (major bloating, skin peeling, penis extended in males, organs beyond recognition, bones exposed due to decomposition) 5. Indeterminate (mummified carcass or skeletal remains, no organs present)	
Remarks (circumstances of stranding) (For example - was the whale seen prior to stranding? If so - describe the whale's behaviour prior to stranding?):	
Weather around time of stranding:	
Other background information:	

2. PHOTOGRAPHS

All photos should be taken at right angles as much as possible (and not obliquely).

Photo					
1. Whole animal, side on (include 1m scale)	Left		Right		
2. Whole animal with beach/coast in frame (include 1m scale)					
3. Whole animal, from the head					
4. Tail flukes					
5. Dorsal fin	Left		Right		
6. Head	Left		Right		
7. Head from above (may only be possible for calves)					
8. Area around blowholes					
9. Baleen					

10. Whole ventral surface (if possible)	
11. Genital slit, anus and umbilicus (all in one shot)	
12. Scars, wounds, injuries, colour pattern variation (use a ruler or the scale stick to provide an indication of the size) (all lesions should also be drawn onto the whale sketch and the photo# recorded next to it; if samples are collected also record the photo# in the sample collection check-list table below).	
13. External parasites (Congregations of parasites should be drawn onto the whale sketch and the photo# recorded next to it; if parasites are collected also record the photo# in the sample collection check-list table below).	

3. EXTERNAL ASSESSMENT

Body condition (For a description refer to the 'Visual assessment of body condition' section in the Additional Notes)	
1. Post-cranial condition score (1,2 or 3):	
2. Scapular condition score (1 or 2):	
3. Lateral flank condition score (1 or 2):	

Evaluation of skin condition:
1. Is the skin intact or blistering or pealing? (describe the distribution on the body and estimate % of surface area affected)
2. Does the whale appear to have black skin or gray skin?
3. Is there any evidence of entanglement injuries? If so describe: Photo #:
4. Are there any wounds possibly associated with vessel strike? If so describe: Photo#
5. Describe any scars, wounds, lesions or colour irregularities (refer to the notes on how to describe lesions). Mark any lesions on the whale drawing provided and write the photograph # next to each labelled lesion.

Parasites:
1. Are there no or few cyamids? (yes/no)
2. Are the blowholes significantly covered with cyamids?
3. If there are extensive aggregations of cyamids describe the distribution on the body and estimate % of surface area covered:
Note the location of parasites on the whale drawing and photograph all parasites collected.

4. MORPHOMETRICS

All measurements should be taken in a straight line (i.e. **not** following the contour of the body) and recorded in centimetres.

(Note: if you have limited time the two bolded measurements are the most important to collect.)

Measurement	Centimetres
1. Total length (tip of upper jaw to deepest part of fluke notch):	
2. Tip of upper jaw to eye:	
3. Length of gape (tip of upper jaw to corner of mouth):	
4. Tip of upper jaw to blowhole:	
5. Tip of upper jaw to anterior insertion of flipper:	
6. Tip of upper jaw to tip of dorsal fin:	
7. Tip of upper jaw to of anus:	
8. Girth at axilla (may only be able to do half measurement)	
9. Girth at umbilicus (may only be able to do half measurement)	
10. Maximum girth (may only be able to do half measurement)	
11. Girth at anus (may only be able to do half measurement)	
12. Length of flipper (anterior to tip):	
13: Width of flipper (maximum):	
14. Width of tail flukes (tip to tip):	
15. Depth of notch between flukes:	
16. Height of dorsal fin (tip to base):	

5. BLUBBER THICKNESS MEASUREMENTS

(See associated diagram)

Location	Measurement (cm)
A. Axilla	
1 Doral:	
2. Lateral:	
3. Ventral a. Top of ventral groove b. Bottom of ventral groove	
B.Umbilicus	

1. Doral:	
2. Lateral:	
3. Ventral:	
C. Anus	
1. Doral:	
2. Lateral:	
3. Ventral:	

6. <u>GROSS PATHOLOGICAL EXAMINATION (</u>adapted from UK Cetacean Strandings Investigation)

Encircle the appropriate category: *NE = not examined*
NAD = nothing abnormal detected
A = abnormal

EXTERNAL EXAMINATION

NE NAD A -body orifices
NE NAD A -fins and flukes
nutritional state: good / moderate / poor

INTEGUMENT

NE NAD A -epidermis
NE NAD A -blubber
NE NAD A -subcutaneous tissue
NE NAD A -mammary glands

MUSCULOSKELETAL SYSTEM

NE NAD A -skull
NE NAD A -other bones
NE NAD A -back muscle mass
NE NAD A -other muscles

NERVOUS SYSTEM

NE NAD A -brain
NE NAD A -spinal cord
NE NAD A -peripheral nerves

CARDIOVASCULAR SYSTEM

NE NAD A -pericardial sac
NE NAD A -myocardium
NE NAD A -valves
NE NAD A -arteries, veins

RESPIRATORY SYSTEM

NE NAD A -nasal cavity
NE NAD A -sinuses
NE NAD A -trachea, bronchi
NE NAD A -lungs

NE NAD A -pleura/pleural cavity

ALIMENTARY SYSTEM

NE NAD A -mouth
NE NAD A -oesophagus
NE NAD A -cardiac section stomach
NE NAD A -fundic section stomach
NE NAD A -pyloric section stomach
NE NAD A -intestine
NE NAD A -anus
NE NAD A -liver
NE NAD A -pancreas
NE NAD A -peritoneum/peritoneal cavity

UROGENITAL SYSTEM

NE NAD A -kidneys
NE NAD A -ureters
NE NAD A -urinary bladder
NE NAD A -urethra
NE NAD A -ovaries/testes
NE NAD A -uterus
NE NAD A -vagina/penis
NE NAD A -vulva/preputium

LYMPHATIC AND ENDOCRINE SYSTEMS

NE NAD A -adrenal glands
NE NAD A -thyroid gland
NE NAD A -spleen
NE NAD A -thymus
NE NAD A -lymph nodes

7. HISTOLOGY SAMPLES (tick if taken and assign a pot number)

Lung		Blubber		Kidney	
Trachea		Subcut Fat		Ureter	
Heart		Peri-renal fat		Urethra	
Aorta		Muscle		Urinary bladder	
Pulmonary artery		Oesophagus		Mammary gland	
Thymus		Stomach		Prepuce	
Salivary gland		Ileum		Testis/Ovary	
Thyroid		Colon		Uterus	
Tongue		Pancreas		Vagina	
Tonsil		Liver		Cervix	
Adrenal		Mesenteric LN		Brain	
Mediastinal LN		Eye		Spinal cord	

8. MAXIMUM SAMPLE CHECK-LIST

Sample	**Purpose** (Items in red need processing as soon as possible)	**Collected**
Blood		
EDTA tube	Haematology Buffy coat - RNA later	
Lithium heparin tube	Biochemistry	
Plain serum tube	Endocrinology; serology; biotoxins	
Blood smear	Cell differential counts; haemoparasites	
Skin and blubber (Collected where biopsy samples usually taken - posterior and ventral to the dorsal fin)		
RNA later	Virology; biomarker	
10 % formalin	IHC for CYP	
Freeze x 2	Genetics	
Blubber (Full thickness- posterior and ventral to the dorsal fin)		
Aluminium foil in zip lock bag- freeze	Contaminants	
Zip lock bag- freeze	Lipid composition; fatty acid analysis	
Formalin	Histology	
Muscle (Collect below where blubber sample collected)		
Formalin	Muscle atrophy (body condition)	
Frozen	Heavy metals; stable isotopes	
Baleen (Collect from the longest section of baleen (5cm x full length))		
Zip lock bag (freeze or air temp)	Stable isotopes	
Eye		

Freeze	Aging, Aqueous humour	
Blowhole swab		
Sterile swab with transport media	Microbiology (anaerobic and aerobic bacterial culture)	
Plain sterile swab- RNA later	Virology	
Parasites		
70% ethanol	Parasitology	
Faeces		
Freeze	Parasitology, biotoxins, hormones	
Urine		
Room temperature	Ketones; urinalysis	
Freeze	contaminants; biotoxins	
Thyroid		
Freeze	Archive	
Lung		
Freeze -80C	Archive	
RNA later	Virology	
Liver		
Plastic -20C	Heavy metals	
RNA later	Virology; biomarker	
Kidney		
Plastic -20C	Heavy metals; archive	
RNA later	Virology	
Spleen		
Freeze	Archive	
RNA later	Virology	
Adrenal gland		
Freeze	Archive	
Gonad		
Freeze	Archive	
Stomach contents		
Freeze	Biotoxins	
Brain (representative sections of cerebrum, cerebellum and brain stem)		
RNA later	Virology	
Freeze	Archive	
Mediastinal LN		
RNA later	Virology	
Freeze	Archive	
Mesenteric LN		
RNA later	Virology	
Freeze	Archive	
Milk		
Freeze	Contaminants	
Bone (humerus preferred otherwise section of 10th rib)		
Freeze	Archive, lead	

Formalin	Histology of bone marrow - body condition	

9. BACTERIOLOGY

Samples collected	Comments/indications

Appendix B. Minimum sample protocol and check-list for fresh humpback carcasses (for veterinarians)

1. BACKGROUND INFORMATION

Date:	
Name of attending veterinarian	
Location (in relation to the nearest named place):	
When was the whale found:	Date: Time:
Sex (male/female/unknown):	
Age class (calf, yearling, lactating female, adult):	
Carcass condition (Circle) 1. Live (becomes code 2 at death) 2a. Extremely fresh (as if just died, no bloating, meat is considered by most edible) 2b. Slight decomposition (slight bloating, haeme imbibition visible) 3. Moderate decomposition (moderate bloating, skin peeling, penis may be extended in males, organs still intact, excluding post-mortem damage) 4. advanced decomposition (major bloating, skin peeling, penis extended in males, organs beyond recognition, bones exposed due to decomposition) 5. Indeterminate (mummified carcass or skeletal remains, no organs present)	
Remarks (circumstances of stranding) (For example - was the whale seen prior to stranding? If so - describe the whale's behaviour prior to stranding?):	
Weather around time of stranding:	
Other background information:	

2. PHOTOGRAPHS

All photos should be taken at right angles as much as possible (and not obliquely).

Photo					
1. Whole animal, side on (include 1m scale)	Left		Right		
2. Whole animal with beach/coast in frame (include 1m scale)					
3. Whole animal, from the head					
4. Tail flukes					
5. Dorsal fin	Left		Right		
6. Head	Left		Right		
7. Head from above (may only be possible for calves)					
8. Area around blowholes					
9. Baleen					

10. Whole ventral surface (if possible)	
11. Genital slit, anus and umbilicus (all in one shot)	
12. Scars, wounds, injuries, colour pattern variation (use a ruler or the scale stick to provide an indication of the size) (all lesions should also be drawn onto the whale sketch and the photo# recorded next to it; if samples are collected also record the photo# in the sample collection check-list table below).	
13. External parasites (Congregations of parasites should be drawn onto the whale sketch and the photo# recorded next to it; if parasites are collected also record the photo# in the sample collection check-list table below).	

3. EXTERNAL ASSESSMENT

Body condition (For a description refer to the 'Visual assessment of body condition' section in the Additional Notes)	
1. Post-cranial condition score (1,2 or 3):	
2. Scapular condition score (1 or 2):	
3. Lateral flank condition score (1 or 2):	

Evaluation of skin condition:
1. Is the skin intact or blistering or pealing? (describe the distribution on the body and estimate % of surface area affected)
2. Does the whale appear to have black skin or gray skin?
3. Is there any evidence of entanglement injuries? If so describe: Photo #:
4. Are there any wounds possibly associated with vessel strike? If so describe: Photo#
5. Describe any scars, wounds, lesions or colour irregularities (refer to the notes on how to describe lesions). Mark any lesions on the whale drawing provided and write the photograph # next to each labelled lesion.

Parasites:
1. Are there no or few cyamids? (yes/no)
2. Are the blowholes significantly covered with cyamids?
3. If there are extensive aggregations of cyamids describe the distribution on the body and estimate % of surface area covered:
Note the location of parasites on the whale drawing and photograph all parasites collected.

4. MORPHOMETRICS

All measurements should be taken in a straight line (i.e. **not** following the contour of the body) and recorded in centimetres.

(Note: if you have limited time the two bolded measurements are the most important to collect.)

Measurement	**Centimetres**
1. Total length (tip of upper jaw to deepest part of fluke notch):	
2. Tip of upper jaw to eye:	
3. Length of gape (tip of upper jaw to corner of mouth):	
4. Tip of upper jaw to blowhole:	
5. Tip of upper jaw to anterior insertion of flipper:	
6. Tip of upper jaw to tip of dorsal fin:	
7. Tip of upper jaw to of anus:	
8. Girth at axilla (may only be able to do half measurement)	
9. Girth at umbilicus (may only be able to do half measurement)	
10. Maximum girth (may only be able to do half measurement)	
11. Girth at anus (may only be able to do half measurement)	
12. Length of flipper (anterior to tip):	
13: Width of flipper (maximum):	
14. Width of tail flukes (tip to tip):	
15. Depth of notch between flukes:	
16. Height of dorsal fin (tip to base):	

5. BLUBBER THICKNESS MEASUREMENTS

(See associated diagram)

Location	**Measurement (cm)**
A. Axilla	
1 Doral:	
2. Lateral:	
3. Ventral a. Top of ventral groove b. Bottom of ventral groove	

B.Umbilicus	
1. Doral:	
2. Lateral:	
3. Ventral:	
C. Anus	
1. Doral:	
2. Lateral:	
3. Ventral:	

6. MINIMUM SAMPLE COLLECTION CHECK-LIST (in order of priority)

Label all samples with 'HB' and the date (dd/mm/yy)

Tick box	Sample	Collection method	#samples needed	Photo #	Storage method	Test	Lab/ researcher
	Blood	-EDTA tube -Lithium heparin tube -Plain serum tube -Blood smear (Ideally air-dried thin smears should be made from fresh or blood recently added to EDTA)	2 1 5 3		Fridge, 4°C (should be processed at the lab within 48 hours) For plain serum tubes: allow clot to form and then centrifuge (at 3000 rpm for 10 minutes) and separate clot from serum. Keep both the blood clot and serum in separate labelled tubes. Freeze the serum and blood clots at -20°C also store 1 tube of serum and 1 blood clot at -80 C.	Haematology, biochemistry, archiving (endocrinology, serology, biotoxins)	1 X EDTA to Vetpath for CBC (clinic number 942) 1 x EDTA centrifuge, remove buffy coat (WBC) and place in RNA later 1 x Lith Hep AHL for biomchem etc Serum for serology AHL Serum for archiving Archive blood clots
	Skin sample	Placed into a small tube containing either saturated salt + 10% DMSO (or in a sterile container and freeze) or just freeze	2		Fridge, 4°C	Genetics	Archive
	Blubber 5cm x 5cm full thickness	Wrapped one sample in aluminium foil, then in plastic/	2		Freeze, -20°C	Contaminants	Archive for Susan Bengtson-Nash

	with skin (collect posterior and ventral to the dorsal fin- see associated diagram)	ziplock bag. Place the second sample straight into a plastic/ziplock bag			Freeze, -20°C	Lipid composition	(Griffith University) Archive for Chemistry Centre
	Biopsy of skin lesions (only biopsy lesions that <u>do not</u> appear to be the result of abrasions from stranding or from sun burn) Mark these lesions on the whale diagram- photograph and describe.	10 x vol 10% buffered formalin (important to also include normal surrounding tissue in biopsies). If lesion small use a yellow top pot and decant some formalin into it. If several large lesions use large white formalin pot for all lesions sampled. Collect an addition biopsy of any lesions and place in a yellow top pot If the appearance of the lesion suggests that it may be an abscess cut it out and place it into a yellow top pot.	Any		Room temperature Freeze, -20°C Fridge, 4°C (should be processed at the lab ASAP)	Histology Molecular work Microbiology (anaerobic and aerobic bacterial culture; fungal culture)	Murdoch University, Anatomic Pathology (Nahiid Stephens) Archive DAFWA
	Baleen	Collect from the longest section of baleen (5cm x full length) and place in a plastic/ ziplock bag			Freeze, -20°C	Stable isotopes	Archive
	Blowhole swab	Sterile swab with transport media. Swab as deep as possible inside the blowhole	1		Fridge, 4°C (should be processed at the lab ASAP)	Microbiology (anaerobic and aerobic	DAFWA

		and place it in accompanying transport.				bacterial culture)	
	Eye	Remove entire eye and place in a ziplock bag Remove the other eye and place it in a large white pot of 10 x 10% buffered formalin	2		Freeze, -20°C Room temperature	Aging, ketones Histology	Archive Murdoch University, Anatomic Pathology (Nahiid Stephens)
	External parasites	Collect into yellow top pots and decant 70% ethanol until parasite(s) are covered .	Any		Room temperature	Parasitology	Archive
	Faeces	Attempt to collect per-rectum: Place approx 5 grams into a pot with10 x vol 10% buffered formalin Place another 5 grams into an empty yellow top pot .	2		Room temperature Freeze, -20°C	Parasitology Biotoxins, hormones	DAFWA Archive

Appendix C. Sample protocol for Wildlife Officers

PHOTOGRAPHS

All photos should be taken at right angles as much as possible (and not obliquely).

Photo					
1. Whole animal, side on (include 1m scale)	Left		Right		
2. Whole animal with beach/coast in frame (include 1m scale)					
3. Whole animal, from the head					
4. Tail flukes					
5. Dorsal fin	Left		Right		
6. Head	Left		Right		
7. Head from above (may only be possible for calves)					
8. Area around blowholes					
9. Baleen					
10. Whole ventral surface (if possible)					
11. Genital slit, anus and umbilicus (all in one shot)					
12. Scars, wounds, injuries, colour pattern variation (use a ruler or the scale stick to provide an indication of the size)					
13. External parasites					

GIRTH MEASUREMENTS

Location	**Measurements (cm)** indicate if only half measurements were taken
Axilla girth	
Umbilicus girth	
Maximum girth	
Anus	

BLUBBER THICKNESS MEASUREMENTS (see diagram)

Location	**Measurement (cm)**
A. Axilla	
1 Doral:	
2. Lateral:	
3. Ventral a. Top of ventral groove b. Bottom of ventral groove	
B.Umbilicus	
1. Doral:	
2. Lateral:	
3. Ventral:	
C. Anus	
1. Doral:	
2. Lateral:	
3. Ventral:	

SAMPLES (Wear gloves and mask)

Label all samples with the date and location of the stranding

Tick box	Sample	Collection method	Storage method
	Skin sample	1cm x 1cm x full thickness Placed into a small tube containing saturated salt + 10% DMSO and store at room temperature Or if there is no salt + DMSO store in a container/ziplock bag and freeze	 Room temperature If no access to salt + DMSO then freeze
	Blubber 5cm x 5cm full thickness with skin (collect posterior and ventral to the dorsal fin)	Two 5cm x 5cm full thickness with skin (see diagram for location) Wrap samples in aluminium foil, then in plastic/ ziplock bag	Freeze
	Eyes	Remove entire eye and place in a ziplock bag (If possible: remove the other eye and place it in a large pot of 10 x 10% buffered formalin - possibly obtain 10% buffered formalin from local vets or veterinary/medical pathology laboratory)	Freeze Room temperature
	Baleen	Collect from the longest section of baleen (5cm x full length) and place in a plastic bag	Freeze

Appendix D: Post-mortem report for Bremer Bay neonate

School of Veterinary & Biomedical Sciences
South St, Murdoch
Western Australia 6150
Telephone: (61-8) 9360 2356
Facsimile: (61-8) 9310 4144
Duty Pathologist: 04202 77743

MURDOCH UNIVERSITY
PERTH, WESTERN AUSTRALIA

ANATOMIC PATHOLOGY NECROPSY REPORT

Pathology No:

11/292

Date In: 19/05/2011
Pathologist: Dr Nahiid Stephens (with assistance from Dr Shane Besier, AHL/DAFWA; and Dr Louise FitzGerald)

Owner's Details: **C/O Dr Carly Holyoake (Conservation Medicine)** Ph: **0407 335 262**	Consulting Veterinarian: ***Drs Carly Holyoake and Nahiid Stephens*** Date of Consult: **20/05/2011**
Patient's Details: Clinic Number: **146215** Name: **Anatomic Pathology (Humpback Whale) – Bremer Bay neonate** D.O.B: **Unknown**	Species: **Humpback Whale (Megaptera novaeangliae)** Breed: **N/A** Gender: **Female** Current Age: **Neonate**

History: A humpback calf was found alive in the surf at Bremer Bay by a member of the public (Ann Gadsby; ph: 9837 4063; mob: 0427 812 106) at 6am on Thursday May 19, 2011. The photo below was taken by A. Gadsby. The humpback was reported to turn upside down and drown at 9:30am. Following verification of death, it was transported to a council holding area for necropsy early on 20/05/2011, with assistance from Dean Jolly, Shire Ranger; mob: 0429 351 022.

Submission: A female neonatal Humpback Whale in poor body condition (see table below for condition scores), estimated to be <48 hours old.
Status: Post natural death and movement off public beach to Bremer Bay council holding area.
Post mortem interval: Approximately 24 hours (necropsy commenced early morning 20/05/2011).
Post mortem decomposition: Carcase condition code at commencement of post-mortem examination – 2a to 2b.
Identifying features: Dark grey skin. No identifying wounds/entanglements.
Morphometrics:

Measurement	**Centimetres**
Total length (tip of upper jaw to deepest part of fluke notch)	~480 (tail distorted as in contact with fence; cross-reference with DEC field measurements)
Tip of upper jaw to centre of eye	101
Length of gape (tip of upper jaw to corner of mouth)	91
Tip of upper jaw to blowhole	76.5
Tip of upper jaw to anterior insertion of pectoral fin	147
Tip of upper jaw to tip of dorsal fin	231
Tip of upper jaw to centre of anus	344
Maximum girth (reaching by doubling the half of maximal girth measurement)	256
Length of pectoral fin (anterior to tip)	144
Width of pectoral fin (maximum)	37
Width of tail flukes (tip to tip)	120
Depth of notch between flukes	3.5
Height of dorsal fin (tip to base)	Unable to measure as trapped under body
Blubber thickness at axilla	Dorsal = 2.7
	Lateral = 1.8
	Ventral = 3.8 (to top of throat groove)

Blubber thickness at umbilicus	Dorsal = 3.6
	Lateral = 3.7
	Ventral = 3.9
Blubber thickness at anus	Dorsal = 3.5
	Lateral = 3.7
	Ventral = 3.3
Post-cranial condition score (1,2 or 3)	1
Scapular condition score (1 or 2)	1
Lateral flank condition score (1 or 2)	1

□ **External examination system**
See Below

□ **Skin and subcutis**
See below

□ **Body cavities**
No visible lesions

□ **Respiratory system**
See below

□ **Cardiovascular system**
See below

□ **Alimentary system**
See below

□ **Lymphoreticular**

No visible lesions

□ **Urogenital system**
No visible lesions

□ **Endocrine system**
No visible lesions

□ **Musculoskeletal system**
No visible lesions

□ **Nervous system**
No visible lesions

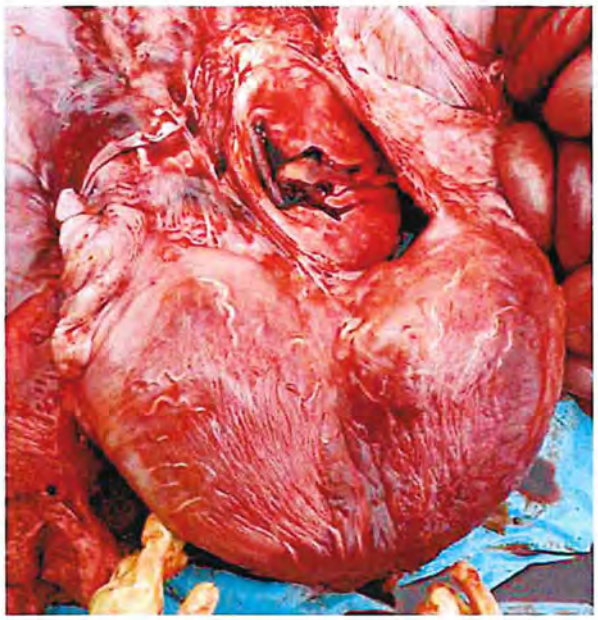
Visible lesions:

Significant External Findings

1. External examination: The female calf was in poor body condition with reduced body mass, visually and palpably evidenced by a postcranial depression, protrusion/ prominence of the scapula, and bilateral depressions along the dorsolateral aspects of the flanks. Each of these sites was therefore awarded a condition score of 1 (see table above), using the scoring system developed by Bradford (2011). See photo above (taken on the beach at site of stranding) – the scapular prominence is particularly evident.
2. Skin and subcutis: There was no external evidence of trauma nor were there any identifying scars/ barnacles; the skin was dark grey. Several linear to curvilinear superficial epidermal excoriations were present scattered over the body, particularly the dorsum and lateral flanks (inter-individual interaction marks, excoriations sustained during stranding).
   The blubber appeared diffusely sparse, pale pink and fibrous in consistency (i.e. prominent fibrovascular component with poor adipose stores). See table above for blubber thickness measurements (and Gross Comment for discussion of these).
   At the level of the genital slit and surrounding it the subcutis was expanded by a focal area of gelatinous material (subcutaneous oedema).

Significant Internal Findings

1. Cardiovascular system: There was virtually no adipose connective tissue present at the heart base and that present was smooth, gelatinous and translucent yellow (adipose hypoplasia versus serous adipose atrophy). A probe patent ductus arteriosus was present. The photo below documents the lack of heart base adipose.



The umbilicus appeared fresh and moist and was trailing a 60 x 100mm segment of mottled red-purple (hyperaemia, haemorrhage) moist connective tissue containing blood vessels (neonate). The external surface of this tissue as well as the surface of the umbilicus itself appeared clean/ unremarkable; incision into these tissues found only similarly fresh clean tissue which did not exhibit any exudate/ evidence of inflammation. Deep to this the internal vascular structures appeared unremarkable and no overt intraluminal thrombi could be found (i.e. death likely to have occurred prior to the significant formation of physiological thrombosis secondary to umbilical involution).

2. Respiratory system: The lungs were diffusely non-collapsed and exhibited linear slightly raised pink areas alternating with linear white to pale pink depressions (the latter corresponding to rib impressions) (failure to collapse – pulmonary oedema, inflammatory cellular infiltrate, acellular deposits, neoplasia). The pulmonary tissue floated upon immersion. The lungs were heavy and felt wet (pulmonary oedema, consolidation).

Copious amounts of red-tinged frothy fluid oozed out of the bronchi (see photo next page) and the distal one-third of the trachea (pulmonary oedema versus terminally-aspirated water, mixed with surfactant). The surrounding parenchyma on cut surface appeared meaty in consistency and consolidated (pulmonary oedema versus inflammatory cellular infiltrate).

The pleural diaphragmatic surface exhibited a linear 140 x 40mm band comprised of multifocal to coalescing flat red discolourations, each ranging from 1-5mm in size (petecchial and ecchymotic haemorrhage).

3. Alimentary system: The entire alimentary tract was devoid of ingesta and there was no colostrum/ milk in the forestomach/ glandular / pyloric stomach. A small amount of viscous sticky to tarry green-brown faeces was present in the terminal colon/ rectum (meconium).

Gross Summary:

1. **Body as a whole: severe generalised adipose hypoplasia (ddx: adipose atrophy).**
2. **Cardiovascular system: probe patent ductus arteriosus.**
3. **Lungs: severe acute diffuse interstitial pneumonia (ddx: pulmonary oedema).**
4. **Pleural surface of diaphragm: mild acute multifocal to coalescing petecchiae and ecchymoses.**
5. **Blubber surrounding vaginal slit: mild acute focal subcutaneous oedema.**

Gross Comment: The size of this animal was consistent with it being a neonatal calf. Given the fact that the umbilicus appeared fresh, the ductus arteriosus was probe-patent and there still appeared to be meconium within the terminal colon/ rectum; it is likely to have been born a relatively short time prior to stranding/ presentation (likely <48 hours prior). Furthermore, given the alimentary tract was devoid of milk/ ingesta, it does not appear to have fed (i.e. likely not to have received colostrum).

In retrospect it is felt the blubber thickness was measured incorrectly, owing to the fact that there was very little adipose content and the layers were therefore misinterpreted at time of measurement. One would normally expect to see relatively well developed adipose connective tissue present within the blubber, even in a neonate.

Assuming the calf was indeed <48 hours old, the most likely interpretation would be a lack of adequate failure in utero (i.e. adipose hypoplasia) rather than depletion of stores post-natally (i.e. adipose atrophy). Hopefully histological examination will help determine if this is indeed the case.

The pulmonary changes are interesting. The fact they floated on immersion is consistent with them having been inflated prior to death (i.e. the calf was not stillborn; rather it had breathed air following birth). However, one expects normal lungs to collapse with death, particularly upon opening the thoracic cavity resulting in loss of normal negative intrathoracic pressure. These lungs, however, had failed to collapse appropriately and instead felt wet, heavy and exhibited rib impressions consistent with something holding the tissue up such that it could not collapse. This could be seen with interstitial pneumonia (i.e. inflammatory infiltrate) or also with pulmonary oedema. It is hoped histopathology may help establish a possible aetiology; frozen lung has also been retained in case of the need for future investigation.

The diaphragmatic haemorrhages and peri-vaginal subcutaneous oedema are likely to be agonal (peri-mortem) changes and are incidental findings.

Ancillary Tests: A portion of frozen lung was sent to AHL for bacteriology, see results below; one isolate was sent to PathWest for identification. Liver was submitted to AHL for trace elements/ nutrient analysis; see Appendix at end for grouped preliminary results on this. *Toxoplasma gondii* immunohistochemistry on histology blocks J and B (optic vascular rete and lung) is pending (AHL). Morbillivirus IHC is pending on block B (lung) (AAHL). The results are on the next page(s) (some are outstanding/ongoing). See Appendix at end for grouped urine/ vitreous humor ketone results and blubber lipid analysis.

ANIMAL HEALTH LABORATORIES

LABORATORY REPORT

Department of Agriculture and Food

3 Baron-Hay Court South Perth, WA 6151 • Tel: (08) 9368 3351 • Fax: (08) 9474 1581
444 Albany Highway, Albany, WA 6330 • Tel: (08) 9892 8444 • Fax: (08) 9892 8564

Case Number: AS-12-0808-F-V1 **Final Report**

Date: 19-APR-2012 Your Ref: Not Supplied
Enquiries: Dr Shane Besier(Pathology Perth)

To: Dr Nahiid Stephens
School of Vet.& Biomedical Sciences
South Street
Murdoch
WA 6150

cc.

Owner:
Project: Animal sample testing - non disease investigation
Species: Cetacead - Humpback Whale
Samples Received: 4 blocks; 1 fresh - added 20/3/12
Date Collected: 16-MAR-2012 Date Received: 16-MAR-2012 Submission Number:

History
Frozen lung from a Humpback calf received for bacterial culture and paraffin blocks for Toxoplasma immunohistochemistry.

Histopathology
Four blocks received for Toxoplasma IHC. Note several sections have fragmented tissue following processing. Positive and negative controls performed as expected.

Definitive staining of organisms was not detected. A very small amount of non-specific or background stain was detected.

Comments
Immunohistochemistry did not indicate the presence of *Toxoplasma gondii*.

Bacterial culture of the lung detected several *Clostridium* species and a *Bacillus* sp. These were considered to be contaminants in this case. Occasional acid fast staining was noted on smear examination.

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444 Albany Highway, Albany, WA 6330 • Tel: (08) 9892 8444 • Fax: (08) 9892 8564

Case Number: AS-12-0808-F-V1 **Final Report**

Bacteriology Results

Spec No.	Spec ID	Spec Description	Bacillus sp. likely contaminant	Routine culture	ZN stain examination	Smear examination
5	11-292	Lung	1 colony	Significant growth	occasional acid fast bacilli seen	very large numbers of inflammatory cells seen

Spec No.	Spec ID	Spec Description	Clostridium bifermentans	Clostridium sporogenes	Clostridium sordellii	Anaerobic culture
5	11-292	Lung	moderate growth	moderate growth	moderate growth	Significant growth

Spec No.	Spec ID	Spec Description	Erysipelothrix rhusiopathiae
5	11-292	Lung	neg

Yours faithfully

Dr Shane Besier
VETERINARY PATHOLOGIST

Histopathological findings: H12-0187A-N

Kidney (A): No significant changes are noted. The glomeruli are foetal in morphology, being small with peripheral capillaries.

Spleen (A): The red pulp appears diffusely diminished such that the reticuolendothelial network appears prominent; however the splenic architecture is otherwise normal. Occasional individual megakaryocytes (often accompanied by several immature nucleated eythrocytes; splenic extramedullary haematopoiesis) are seen scattered throughout the parenchyma. The white pulp appears unremarkable.

Liver (A): There is diffuse sinusoidal hyperaemia throughout all zones of the lobule/acinus. The majority of the hepatocytes exhibit a single intracytoplasmic microvesicular to macrovesicular vacuole.

Adrenal, thyroid (B): There is diffuse hyperaemia of the vasculature. No significant changes are noted.

Lung (B): The vast majority of alveoli are filled with copious amounts of multifocal to coalescing eosinophilic (proteinaceous) amorphous material which rarely in some places appears fibrillar to lace-like (modified transudate-oedema with fibrin exudation). Numerous alveolar macrophages, lesser numbers of lymphocytes and occasional admixed neutrophils accompany the intra-alveolar oedema/fibrin (acute exudative interstitial pneumonia). Numerous small coccobacilli are interspersed amongst the alveoli, particularly those worst affected by exudation and alveolar histiocytosis. Rare macrophages exhibit 1-2 phagocytosed bacteria within their cytoplasm. There is multifocal to coalescing alveolar septal hyperaemia and the septa also appear hypercellular (alveolitis). Syncytia and inclusions (both intracytoplasmic and intranuclear) typical of morbillivirus are not seen, however. Occasional alveoli and terminal bronchioles contain numerous foetal squames and keratin flakes. Gram Twort histochemistry shows the majority of the bacteria to be small gram-negative coccobacilli, although occasional larger bacilli which are gram positive are also present (likely *Clostridia* spp. – contaminants, opportunistic pathogens, PM change). Martius Scarlet Blue histochemistry confirms the presence of rare small strands of intra-alveolar fibrin. A Ziehl-Neelsen stain failed to demonstrate any intracellular bacteria.

Ovary (C): The ovary appears unremarkable however is foetal/neonatal in morphology, with numerous primordial follicles and little intervening stroma present. Rare follicles show slight enlargement (presumably under the influence of maternal hormones in utero). There is diffuse hyperaemia.

Skeletal muscle (C): No significant changes are noted.

Heart and heart base adipose connective tissue (C): No significant changes are noted in the myocardium and the portion of adjacent artery. The adipose connective tissue supporting the adjacent artery is scant and appears unusual histologically. Instead of normal adipocytes containing cytoplasmic fat vacuoles, there is instead very loose, paucicellular connective tissue comprised of small groups of poorly differentiated mesenchymal cell precursors accompanied by scant amounts of pale fibrillar eosinophilic material and a marked increase in interstitial space separating the individual precursor mesenchymal cells (ground substance). No well-formed adipose tissue with normal well-developed cytoplasmic vacuoles is seen (adipose hypoplasia).

Pancreas (D): No significant changes are noted. There is diffuse hyperaemia.

Lymph node (D): No significant changes are noted. There is diffuse hyperaemia.

Forestomach (C1 stomach) (D): No significant changes are noted. There is diffuse hyperaemia.

Main/glandular stomach (C2 stomach) (D): There is moderate to marked mucosal autolysis. No significant changes are noted.

Pyloric stomach (C3 stomach), colon (E) (portion of colon also in F): There is moderate to marked mucosal autolysis. There is diffuse hyperaemia. No significant changes are noted.
Urethra (E): There is moderate mucosal sloughing. There is diffuse hyperaemia. No significant changes are noted.
Peripheral nerve, TS and LS (site unknown) (F): No significant changes are noted.
Cerebrum (G): No significant changes are noted.
Skin including blubber (H): The epidermis and the immediately subjacent dermal fibrous connective tissue are unremarkable. The blubber is unusual in its appearance as the normal expected adipose connective tissue component is non-existent. Instead of adipocytes separating collagenous bundles (the latter of which comprise the fibrous structurally supportive portion of the blubber), there is instead very loose, paucicellular connective tissue comprised of small groups of poorly differentiated mesenchymal cell precursors accompanied by scant amounts of pale fibrillar eosinophilic material and a marked increase in interstitial space separating the individual precursor mesenchymal cells (ground substance). No well-formed adipose tissue with normal well-developed cytoplasmic vacuoles is seen (adipose hypoplasia).
Baleen, TS and LS (I): No significant changes are noted.
Optic nerve within vascular rete (J and K): The optic nerve itself is unremarkable. Within the connective tissues of the vascular rete (most evident in the section contained in J) there is minimal to mild, patchy inflammatory infiltration. The inflammatory cell population is predominantly comprised by eosinophils with slightly lesser numbers of lymphocytes and large granular lymphocytes (the latter possessing round nuclei and intensely eosinophilic intracytoplasmic granules), with occasional histiocytes and neutrophils admixed. No aetiologic agents are overtly visible in the sections examined and the vessels themselves are unremarkable.
Iris and cornea, retina (L): No significant changes are noted.
Additional lung sections (M, N): The changes are similar to as described in B but less extensive and florid; multifocal to coalescing areas up to 30% of the tissue in M and 15% of the tissue in N are affected as previously described. These confirm the lesion is extensive, however varying in its severity depending on site.

Final Diagnosis:

1. **Body as a whole: severe generalised adipose hypoplasia.**
2. **Lung: moderate to severe, acute, multifocal to coalescing serofibrinous alveolitis and interstitial pneumonia with alveolar histiocytosis.**
3. **Connective tissue of the optic vascular rete: minimal to mild, subacute, multifocal eosinophilic and lymphocytic inflammation.**
4. **Liver: marked, acute, diffuse hyperaemia (congestion) with mild multifocal to coalescing microvesicular to macrovesicular vacuolar hepatopathy.**
5. **Cardiovascular system: probe patent ductus arteriosus.**
6. **Pleural surface of diaphragm: mild acute multifocal to coalescing petecchiae and ecchymoses.**
7. **Blubber surrounding vaginal slit: mild acute focal subcutaneous oedema.**

Final Comment: Please also refer to the 'Gross Comments' section above. The presence of foetal glomeruli (present for a short time postpartum) in the kidney is consistent with the estimation of the individual's age (recent neonate). The very fresh nature of the umbilicus grossly also correlates with this.

It is interesting to note that the lungs of this individual appeared grossly congested and had not collapsed; furthermore, rib impressions were noted on their

pleural surface, indicative of parenchymal expansion by some sort of material (e.g. cellular infiltrate, acellular infiltrate such as fluid). This gross clinical suspicion is confirmed by histological examination. The changes within the section examined are indicative of an acute exudative interstitial pneumonia. These changes are not dissimilar to those seen with acute cetacean morbillivirus infection; however the typical 'Warthin-Finkeldey' type multinucleated giant syncytial cells and inclusions are not seen (Stone et al. 2011). Furthermore, the splenic white pulp (and the germinal centres of the lymph node in D) in A does not exhibit the marked lymphocytolysis and lymphoid depletion as one would expect to see in acute infection (Stone et al. 2011). To be sure, samples have been sent to AAHL for Morbillivirus IHC (and also to AHL for *T. gondii* IHC, see further on). The presence of the gram negative coccobacilli (whose morphology is not consistent with post-mortem saprophytic invaders, although they too appear to be present; thus caution is warranted in interpretation) is interesting, particularly as there are rare examples of bacterial engulfment by phagocytes; it is possible they represent terminal secondary/opportunistic invaders. Foetal squames are normally present in amniotic fluid and thus can normally be seen as an incidental finding in the lungs of stillborn or neonatal mammals. Further lung sections from samples collected from different areas (M and N) show it to be an extensive change, although less severe in the later 2 sections; given the changes described are indicative of the majority of the lung, it is likely that this calf would have been suffering respiratory distress which may have contributed to its death. Full bacteriology is pending; however preliminary cultures have isolated various *Clostridia* spp. This is consistent with contamination and/ or post-mortem overgrowth. However, wet prep smears at AHL and histo have documented the presence of numerous inflammatory cells and phagocytosed bacteria, so there may as yet be an unidentified bacterial agent present that may be responsible for the pulmonary changes.

Histological examination of multiple sites has confirmed a generalised lack of adequate mature adipose connective tissue stores. Whilst the adipose connective tissue of many neonatal animals when born is relatively devoid of lipid, only filling quickly during the lactation period (Iverson, 2009), neonate baleen whales are said to be born with a blubber layer that is several centimetres thick (Iverson, 2009.). One would normally expect to see relatively well developed adipose connective tissue present within the blubber (and presumably also internally at other sites e.g. peri-renal, heart base etc), even in a neonate; however in this individual the connective tissue occupying the areas where one normally expects adipose is instead a loose, immature, poorly differentiated connective tissue characterised by the presence of immature precursor mesenchymal cells and ground substance. Due to the estimated age of this individual, this is likely to represent adipose hypoplasia. Given the severity of the change (particularly in comparison to the Peaceful Bay neonate [12/0136], the only one examined post-mortem that was considered to be in good body condition and which had a significantly higher blubber lipid content) and the likelihood that the neonate was no more than 48 hours old, it is possible that this adipose hypoplasia reflects insufficient nutrient supply in utero (i.e. poor nutritional status of the dam); as one would expect to see serous atrophy of formed mature adipocytes histologically if it were instead a case of atrophy due to excessive post-natal mobilisation.

The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete is unknown. *Toxoplasma gondii* has been reported as a cause of endophthalmitis in a number of mammalian species and histologic lesions can be seen not only in the retina and uvea, but also in the extraocular muscles; however it tends to be necrotising with granulomatous to lymphocyte rich inflammation (rather than eosinophilic; although the infiltrate seen in this individual is almost equally

lymphocytic as eosinophilic, compared to 12/0135). Retinal sections were unremarkable. Disseminated necrotising infection by *T. gondii* (particularly involving encephalitis) has been demonstrated in pinnipeds, sirenians and odontocetes (Dailey, M.D. 2001; Inskeep, W. et al 1990) however has not been documented in mysticetes. There is evidence, however, that mysticetes may be affected (presumably from contact with water contaminated by oocysts, given they are primarily plankton feeders, supplemented with small fish), with seropositivity documented in a humpback whale from British waters (Forman, D. et al 2009) and molecular (PCR) and immunohistochemical techniques documenting the presence of T. gondii in various tissues from a Fin whale (Mazzariol, S. et al 2012). Due to the state of decomposition the histology of these tissues was not described in the latter report, so it is unknown what lesions the apicomplexan was associated with, however this individual was also RT-PCR positive for Morbillivirus and was additionally reported to have high organochlorine (OC) pollutant levels in blubber; so it is postulated by the authors to have been immunocompromised. Immunohistochemistry (IHC) of this case is pending (block J); however *T. gondii* is considered unlikely to be a cause of the eosinophilic to lymphocytic inflammation seen, particularly as encephalitis is not a feature of this case. Furthermore, the use of *T. gondii* monoclonal antibody is not currently validated for use in cetaceans. Larval helminthiasis (larval migrans) could also potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found. *T. gondii* can also infect type I pneumocytes to cause serofibrinous alveolitis and interstitial pneumonia, so despite the fact that no organisms were seen in various sections of lung, block B (lung) has also been submitted for IHC (results pending).

During stranding, pressure causing compression of the thoracic and abdominal vasculature compromises blood flow leading to acute to subacute passive hepatic hyperaemia (liver congestion) and subsequent hepatocellular hypoxia (Jaber et al. 2004) and hepatocellular injury. Acute hepatocellular necrosis typical of shock is not seen in the centrilobular areas, although is commonly a sequela to prolonged hepatic hypoxia. This fact, as well as the fact that erythrophagocytosis and evidence of haeme breakdown is not seen, indicates the vascular changes are acute in this case, reflecting changes occurring shortly prior to death. The vacuolar changes are marginal, multifocal and non-specific in aetiology, although may reflect an early degenerative change secondary to hepatocyte hypoxia.

Extrapolating from the fact that the spleen of the *Tursiops truncatus* (bottlenose dolphin) has a thick capsule (fibrous externally and muscular internally) with the presence of thick smooth muscle trabeculae (extending from the muscular internal capsule) (Cowan et al. 1999), and assuming that Humpbacks are similar (as they appear to be both grossly and histologically), it is possible that splenic contraction can occur following catecholamine release under circumstances requiring blood volume redistribution (e.g. circulatory shock). Extramedullary haematopoiesis (EMH) is reported to be uncommon in cetaceans (Rommel et al. 2001), however it is not surprising to see EMH in a neonate, given erythropoiesis is a major function of the foetal mammalian spleen, and is known to persist in other neonatal mammals (e.g. horses and ruminants) for several weeks postpartum (Press et al. 2006).

In summary, this neonate was in exceptionally poor body condition. It is likely that lack of adequate blubber adipose stores adversely impacted this individual by (a) being inadequate for insulation and thermoregulation, (b) being inadequate for buoyancy, and (c) being inadequate as an alternative energy source. Hypothermia and lack of buoyancy leading to being unable to keep pace with the dam/ mother is likely to have led to this individual becoming separated from her, and subsequently becoming

weak and eventually stranding (assuming the mother did not die herself first, leaving the calf orphaned). The real question is how a calf could come to be born with such inadequate and poorly developed blubber adipose stores in the first place; it is possibly a reflection of insufficient nutrient supply in utero, and may be associated with poor nutritional status of the dam/ mother. Furthermore, the pulmonary changes are likely to also have had an adverse impact on this individual. The pulmonary changes were moderate to severe and extensive, involving both lungs, and as such are likely to have caused respiratory insufficiency. This would also have meant the calf is unlikely to have been able to keep pace with the dam/ mother.

Addendum 25th May, 2012:

Immunohistochemistry for *Toxoplasma gondii* was carried out at AHL (DAFWA) on sections from the optic vascular rete; definitive staining of organisms was not detected, hence testing did not indicate the presence of *Toxoplasma gondii* (see AHL case number AS-12-0808-F-V1 above; nb: contains IHC results for all 3 neonates). The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete therefore remains unknown. Larval helminthiasis (larval migrans) could potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found.

Morbillivirus immunohistochemistry was performed on lung sections by the Australian Animal Health Laboratory, Geelong; definitive staining was not detected, hence testing did not indicate the presence of morbillivirus.

Liver was sent to AHL (DAFWA) to measure the concentration of various nutrients/trace elements, the results are as follows:

- Vitamin A (mg/kg) ar – 1.6
- Selenium (mg/kg) – 2.25
- Copper (mg/kg dw – 30
- Vitamin E (mg/kg ar) – 2.3
- Zinc (mg/kg dw) – 186

However, the significance of these figures is unknown as normal reference ranges for Humpback Whales are not known. It is interesting to note that the figures for Vitamins A and E are significantly lower than those from the Peaceful Bay neonate (12/136), which was in good body condition whereas this individual was in poor body condition. These figures may represent variations in maternal vitamin status.

Yours Sincerely,

Nahiid Stephens BSc BVMS (Hons) MANZCVSc (Vet Path)
Associate Lecturer in Veterinary Pathology

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Checklist: (√ = no gross lesions; **H** = sample for histology; **C** = culture; **P** = photographed)									
	Eyes		Lungs		Stomach		Adrenals		Other organs
	Skin		Bronch LN		S. intestine		Testes		(list)
	Head LN		Heart		Caecum		Ovaries		
	Tongue		Liver		Colon		Meninges		
	Oesophagus		Gall bladder		Mesent LN		Brain		
	Thyroid		Spleen		Kidneys		Bone Marrow		
	Parathyroid		Pancreas		Bladder				
	Thymus		Forestomachs						
Frozen samples: □ Liver □ Fat □ Kidney □ Brain □ Other (list)									

Disease Process	**DYS**	**Disease Process**	**INA**

1		2	
System 1	**SYS**	**System 2**	**RES**
General Cause 1		**General Cause 2**	
Aetiology 1		**Aetiology 2**	
Common Name 1	**Severe generalised adipose hypoplasia**	**Common Name 2**	**Serofibrinous alveolitis and interstitial pneumonia with alveolar histiocytosis**

Dr. Nahiid Stephens

Appendix E: Post-mortem report for Peaceful Bay neonate

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MURDOCH UNIVERSITY
PERTH, WESTERN AUSTRALIA

ANATOMIC PATHOLOGY NECROPSY REPORT

Pathology No:

12/0136

Date In: 17/08/2011
Pathologist: Dr Nahiid Stephens (with assistance from Dr Shane Besier, AHL/DAFWA)

Owner's Details: **C/O Dr Carly Holyoake (Conservation Medicine)** Ph: **0407 335 262**	Consulting Veterinarian: ***Dr Dave Edmonds*** Date of Consult: **17/08/2011**
Patient's Details: Clinic Number: **146215** Name: **Anatomic Pathology (Humpback Whale) – Peaceful Bay, Walpole neonate** D.O.B: **Unknown**	*Species:* ***Humpback Whale (Megaptera novaeangliae)*** *Breed:* ***N/A*** Gender: **Female** Current Age: **Neonate**

History: A humpback neonate came ashore alive in shallow waters in Peaceful Bay near Walpole 17/08/2011. Once its position had been stabilised on the beach it was euthanased by DEC personnel (firearm). Two vets from Denmark (headed by Dr Dave Edmonds), who are a part of our stranding network, were on location and collected blood samples shortly after euthanasia from the euthanasia wound; they carried out a necropsy 3 hours following euthanasia and post-mortem transport to secure location and sent the samples collected to MU.

Submission: A female neonatal Humpback Whale in good body condition (see table below for condition scores), estimated to be between 48-96 hours old.
Status: Post firearm euthanasia.
Post mortem interval: 3 hours.
Post mortem decomposition: Carcase condition code at commencement of post-mortem examination - 2a.
Identifying features: Dark grey skin. No identifying wounds/entanglements.

Morphometrics:

Measurement	Centimetres
Total length (tip of upper jaw to deepest part of fluke notch)	480
Tip of upper jaw to centre of eye	117
Length of gape (tip of upper jaw to corner of mouth)	97
Tip of upper jaw to blowhole	74
Tip of upper jaw to anterior insertion of pectoral	142

fin	
Tip of upper jaw to tip of dorsal fin	318
Tip of upper jaw to centre of anus	352
Maximum girth (reaching by doubling the half of maximal girth measurement)	Not measured
Length of pectoral fin (anterior to tip)	140
Width of pectoral fin (maximum)	34.5
Width of tail flukes (tip to tip)	108.5
Depth of notch between flukes	6.5
Height of dorsal fin (tip to base)	7.7
Blubber thickness at axilla	Dorsal = 4.6
	Lateral = 5.3
	Ventral = 3.3
Blubber thickness at umbilicus	Dorsal = 7.3
	Lateral = 6.4
	Ventral = 5.8
Blubber thickness at anus	Dorsal = 5
	Lateral = 4.1
	Ventral = 4.6
Post-cranial condition score (1,2 or 3)	3
Scapular condition score (1 or 2)	2
Lateral flank condition score (1 or 2)	2

□ **External examination system**
See Below

□ **Skin and subcutis**
See below

□ **Body cavities**
No visible lesions

□ **Respiratory system**
No visible lesions

□ **Cardiovascular system**
See below

□ **Alimentary system**
See below

□ **Lymphoreticular**
No visible lesions

□ **Urogenital system**
No visible lesions

□ **Endocrine system**
No visible lesions

□ **Musculoskeletal system**
No visible lesions

□ **Nervous system**
No visible lesions

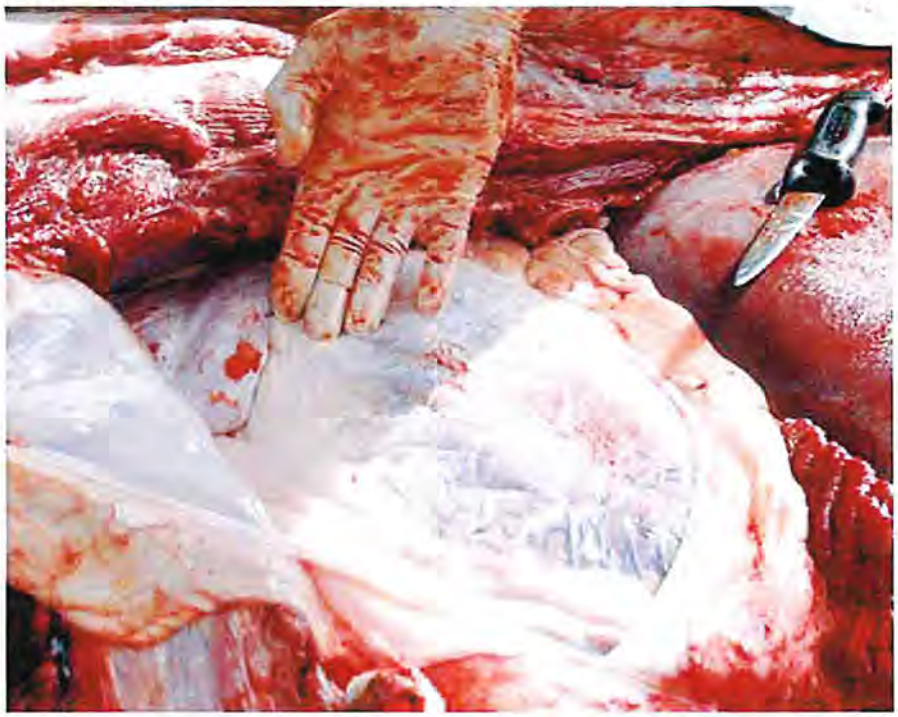
Visible lesions:
Significant External Findings

3. External examination: The female calf was in good body condition with good body mass, based on the 3-site scoring system developed by Bradford (2011) for visual assessment. See table above for body condition scores.
   One of the eyes (side not specified) had a 2-3mm ovoid paracentral corneal opacity (corneal ulcer +/- keratitis, corneal oedema). See photo below.

4. Skin and subcutis: There were no identifying scars/ barnacles; the skin was dark grey. Numerous linear to curvilinear superficial epidermal excoriations were present disseminated over the body, particularly the dorsum, ventrum and lateral flanks (inter-individual interaction marks, excoriations sustained during stranding). Several irregularly-shaped superficial red (acute) abrasions were present multifocally on the ventrum, ranging from approximately 50-100mm in width (abrastions sustained during stranding in the surf zone; these are evident in the photos of the calf in situ on the beach prior to death). Numerous cyamids were present, particularly on the ventrum around the vaginal slit.

Significant Internal Findings

1. Cardiovascular system: Adequate amounts of unremarkable adipose connective tissue was present at the heart base and within the coronary groove; consistent with the 'good' body condition score of this individual, see image below.



The umbilicus was still prominent however was clean and dry with no fresh vessels/ connective tissue trailing from it.

2. Alimentary system: The intestines appeared mildly distended (gaseous dilation). A very small amount (<20mL) of thick flocculent creamy material was present in the main (glandular) stomach (mucoid glandular discharge versus very small amount of milk). The alimentary tract was otherwise empty and there was viscous sticky to tarry green-brown faeces (meconium) in the large intestine.

Gross Summary:

1. **Cornea: mild acute focal ulcerative keratitis with focal oedema.**

Gross Comment: Although a neonate, this individual may have been up to several days old owing to the appearance of the umbilicus, likely between 48-96 hours old. The alimentary tract was empty apart from meconium and a very small amount of material in the main (glandular) stomach, which could well have been mucoid discharge. It could also have been milk – however if this were the case the calf had only ingested a negligible amount, as humpback calves consume many litres of milk daily (i.e. one would expect to see the stomach full of mik had it fed properly recently prior to death). Of the 3 neonates examined by necropsy post-mortem, this individual is the only one in good body condition and likely represents the normal adipose stores a calf could be expected to be born with. The cause of the corneal lesion is unknown as such a lesion is a non-specific response to multiple aetiologies; histology may reveal a cause and is pending.

Ancillary Tests: Blood was sent to AHL and VetPath, see results on the next 4 pages. The CBC particularly should be interpreted with caution given the method of blood collection. *Toxoplasma gondii* immunohistochemistry on histology block E (optic vascular rete) is pending (AHL). Liver was submitted to AHL for trace elements/ nutrient analysis; see Appendix at end for grouped preliminary results on this. See Appendix at end for grouped urine/ vitreous humor ketone results and blubber lipid analysis.

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LABORATORY REPORT
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444 Albany Highway, Albany, WA 6330 • Tel: (08) 9892 8444 • Fax: (08) 9892 8564

Case Number: AS-11-2101-F-V2 **Final Report**

Date: 3-APR-2012 Your Ref: Humpback Whale Calf
Enquiries: Dr Shane Besier(Pathology Perth)

To: Dr Carly Holyoake

cc.

42 West View Blvd
Mullaloo
WA 6027

Owner:
Project: Animal disease diagnosis
Species: Cetacead - Humpback whale calf
Samples Received: 1 animal - 2 swabs; 10 blood
Date Collected: Not Supplied Date Received: 18-AUG-2011 Submission Number:

History
Blowhole swabs and blood samples from a beached female Humpback whale calf.

Aetiological diagnoses
See comments

Comments
Serology for *Toxoplasma gondii*, Influenza A, *Brucella abortus* and *B.melitensis* was negative.

Leptospira serology was performed at Queensland Health (WHO, OIE and FAO Reference Centre for Leptospirosis). MAT testing was negative for *Leptospira* serovars Pomona, Hardjo, Tarassovi, Grippotyphosa, Celledoni, Australis, Zanoni, Robinsoni, Canicola, Kremastos, Szwajizak, Medanensis, Bulgarica, Cynopteri, Arborea, Bataviae, Djasiman, Javanica, Panama, Shermani, Topaz and Icterohaemorrhagica.

In a neonate antibodies against these organisms are likely to be maternally derived, and reflect maternal exposure, rather than reflecting infection status of the calf itself. If colostral transfer has not occurred these results may not be accurate indicators of maternal exposure.

Clinical biochemistry was interpreted against data from Puls, R "Mineral Levels in Animal Health" 1994. Note these are generic "adult whale" parameters and both age and species variations are likely. Values will be re-interpreted if more appropriate reference intervals become available. Where no reference interval is given, comments were made with regard to typical domestic livestock levels.

The marked CK and ALT elevations suggest marked subacute muscle injury. This is presumed to reflect trauma during beaching and the pre-beaching period. The relatively high GSHPx (selenium indicator) and vitamin E suggest nutritional myopathy is unlikely to be contributing to muscle parameter elevations.

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GGT is usually used as an indicator of biliary tract injury. As large amounts of GGT are secreted by the mammary gland, and very high levels persist for several weeks, a marked GGT elevation can also be used to indicate colostral intake in neonates. Such a low GGT level may indicate a lack of colostral transfer if the calf sampled was less than 2 months old.

Total protein is decreased. In this case both albumin and globulins appear low although globulins are lower than albumin (increased A:G ratio). This may support inadequate globulin intake.

Haptoglobin is moderately increased. This is an acute phase reactant and suggests an inflammatory process is present. It is possible this reflects the degree of inflammation secondary to muscle damage although a concurrent inflammatory event may also be present.

Glucose is moderately decreased. Note this was measured in a mildly aged plasma sample and some artefactual glucose reduction is likely to have occurred. If the decrease is real it may reflect decreased feeding/energy production. The glycerol is slightly elevated compared to most domestic livestock. Elevated glycerol indicates increased catabolism of adipose tissue for energy production and can indicate anophagia or malnutrition.

Vitamin A is markedly decreased although this parameter is typically low in neonatal animals as milk contains little vitamin A. Interestingly vitamin E, which is also low in milk, appears adequate. Similarly, a very low iron may reflect normal neonatal iron stores rather than deficiency or sequestration during inflammation.

Bacterial culture isolated a range of organisms in light to moderate growth from a blowholeswab. These are likely to be contaminants or normal flora; interpretation should be made in conjunction with respiratory histopathology. Culture of the lung swab did not isolate bacteria.

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Parasitology Results

Test Type: Toxoplasma Antibodies - Indirect Fluorescence Antibody Test

Spec No.	Spec ID	Spec Description	Antibodies
2	Whale	Clotted x 7	Negative

Serology Results

Spec No.	Spec ID	Spec Description	Brucella abortus/melitensis CFT
2	Whale	Clotted x 7	negative @ 1/4

Virology Results

Test Type: Avian Influenza virus antibody-detection ELISA

Spec No.	Spec ID	Result
2	Whale	Antibody NEGATIVE

Bacteriology Results

Spec No.	Spec ID	Spec Description	Erysipelothrix rhusiopathiae	Haemophilus sp.	Rhodococcus sp.	Unidentified GPR
4	Whale Blowhole	Blowhole/lung swab	neg	neg		
5	Whale Blowhole	Blowhole swab	neg	neg	light growth	light growth

Spec No.	Spec ID	Spec Description	Corynebacterium sp.	Phocoenobacter sp.	Routine culture	Vibrio sp.
4	Whale Blowhole	Blowhole/lung swab			no growth	
5	Whale Blowhole	Blowhole swab	light growth	moderate growth	Significant growth	light growth

Spec No.	Spec ID	Spec Description	Routine fish bacterial culture	Pasteurella-like organism	Pasteurella sp.
4	Whale Blowhole	Blowhole/lung swab	no growth		neg
5	Whale Blowhole	Blowhole swab	Significant growth	light growth	POSITIVE

Comment: No Brucella cultured. NB. NATA accreditation does not cover the scope of this test

Test Type: Culture for Mycoplasma sp.

Spec No.	Spec ID	Spec Description	Mycoplasma sp.
4	Whale Blowhole	Blowhole/lung swab	neg
5	Whale Blowhole	Blowhole swab	neg

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Case Number: AS-11-2101-F-V2 **Final Report**

Biochemistry Results

Specimen Type: BLOOD

Spec No.	Spec ID	Copper in plasma	Cholesterol in plasma or serum	Calcium in plasma or serum	Conjugated Bilirubin in plasma or serum	BHB in plasma or serum
		mg/L	mmol/L	mmol/L	umol/L	mmol/L
1	Whale	0.93	4.83	1.74	0	0.00
Reference range:			3.9 – 77.6	2.2 – 3.0		

Specimen Type: BLOOD

Spec No.	Spec ID	Albumin Globulin	CK in plasma or serum	Creatinine in plasma or serum	ALT in plasma or serum	GGT in plasma or serum
		Ratio	U/L	umol/L	U/L	U/L
1	Whale	1.10	41170	130	101	3
Reference range:			50 - 120	26.5 - 265.2	5 - 18	

Specimen Type: BLOOD

Spec No.	Spec ID	GLDH in plasma or serum	Glucose in plasma or serum	Glycerol in plasma or serum	GSHPx in red blood cells	Haptoglobin
		U/L	mmol/L	mmol/L	U/g Hb	mg/mL
1	Whale	21	3.71	0.28	172	4.39
Reference range:			5.2 – 6.4			

Specimen Type: BLOOD

Spec No.	Spec ID	Urea in plasma or serum	Iron in plasma or serum	Albumin in plasma or serum	Magnesium in plasma or serum	Vitamin A in plasma or serum
		mmol/L	umol/L	g/L	mmol/L	mg/L
1	Whale	22.4	3.9	22.2	1.72	0.03
Reference range:		17.9 – 32.1	19.7 – 71.6		0.8 – 1.9	

Specimen Type: BLOOD

Spec No.	Spec ID	Phosphorus (Pi) in plasma or serum	Total Protein in plasma or serum	Total Bilirubin in plasma or serum	Vitamin E in plasma or serum	Zinc in plasma
		mmol/L	g/L	umol/L	mg/L	mg/L
1	Whale	3.15	43.1	1	14.69	0.91
Reference range:		1.5 – 3.2	52 - 73	3.4 – 10.3		

Yours faithfully

Dr Shane Besier
VETERINARY PATHOLOGIST

942

VETPATH
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39 Epsom Avenue Ascot WA 6104
PO Box 18, Belmont WA 6984
Tel (08) 9259 3666
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A.Hrs 0418 916 436

Lab No : 103032
Client : WHALE
Date 18/05/2011
Collection Date
Species Other 6300

Carly Holyoake
Murdoch University Whale Health Project
42 West View Boulevard
MULLALOO WA 6027

Tests :
Out to Perth ref lab
referred test
CBC

Specimens :
Blood - B 8D E
Swab - 2J
Body Fluid - H
Smear 2Q

HAEMATOLOGY REPORT

Test		Result	Ref.	Test		Result	Ref.	Test		Result	Ref.
HB	g/L	40	-	WBC	x10 ^{9}/L	0.2	-	PT	secs		<
HCT	L/L	0.10	-	Neuts	%		-	PTT	secs		<
PCV	L/L	0.10	-	(Bands)	%		-	FIA			
RBC	x10 ^{12}/L	0.8	-	Lymphs	%		-	Clot			
MCHC	g/L	369	-	Mono	%		-	Lipaemia			
MCH	pg	50	-	Eosin	%		-	Haemolysis			
MCV	fL	135	-	Baso	%		-	Jaundice			
Retics (Corr)	%			Other	%			RBC Aggl			
T S Protein	g/L		-	Retics ABS	%		10^3/L				
Nuc RBC /100 WBC				Fibrinogen	g/l		-				
ESR 20min	mm			Platelets	x10 ^{9}/L	30	-				
ESR 30min	mm										

Comments WBC: MARKED LEUCOPENIA. INSUFFICIENT CELLS TO PERFORM MANUAL DIFFERENTIAL HOWEVER CELL TYPES DO APPEAR NORMAL.
RBC: ANISO 1+. POLY FEW.
PLTS: NUMBERS APPEAR VERY LOW ON SMEAR. ? COLLECTION METHOD.

The significance of the apparent anaemia, leukopaenia and thrombocytopenia is unclear. Possible artefact due to collection method and/or post-mortem changes.

The few neutrophils that are present do not have toxic changes.

Pathologist Dr Jon Meyer **Technologist** K D

Histopathological findings: H12-0188A-N

Thymus (A): No significant changes are noted.

Lung (A): There is multifocal to coalescing alveolar atelectasis. No significant changes are noted.

Liver (B): There is diffuse centrilobular (periacinar) sinusoidal hyperaemia; in some areas extending to become midzonal. It is, however, worst in the centrilobular areas, with dissociation and shrinkage of the hepatocytes in these areas. Occasional individual, randomly scattered hepatocytes exhibit 1-2 small microvesicular intracytoplasmic vacuoles. Rare, tiny intra-sinusoidal aggregates of cells resembling immature nucleated erythrocytes, often accompanied by a single large cell resembling a megakaryocyte are seen (extramedullary haematopoiesis).

Heart (B): No significant changes are noted.

Gastrointestinal tract, unknown site (B): No significant changes are noted. There is moderate mucosal autolysis making definitive determination of original sampling site difficult.
Aorta, LS and TS (C): No significant changes are noted.
Pulmonary artery, LS and TS (C): No significant changes are noted.
Mediastinal lymph node (C): No significant changes are noted.
Optic nerve within vascular rete (E): The optic nerve itself is unremarkable. Within the connective tissues of the vascular rete there is minimal to mild, patchy inflammatory infiltration. The inflammatory cell population is predominantly comprised by lymphocytes and large granular lymphocytes (the latter possessing round nuclei and intensely eosinophilic intracytoplasmic granules), lesser numbers of eosinophils and occasional histiocytes and neutrophils. No aetiologic agents are overtly visible in the sections examined and the vessels themselves are unremarkable.
Pancreas, adrenal (F): No significant changes are noted.
Heart (F): No significant changes are noted in the myocardium. Unfortunately the sample taken grossly did not include heart base adipose for assessment histologically.
Uterus (G): No significant changes are noted. The lumen is lined by pseudostratified columnar epithelium.
Main/glandular stomach and pyloric stomach (C2 and C3) (H; portion of pyloric stomach also in I): Both exhibit mild mucosal autolysis. The main/glandular stomach is unremarkable. The lamina propria of the pyloric stomach exhibits multifocal to coalescing aggregates of eosinophils (up to 25 per HPF) although no aetiologic agents are seen.
Forestomach (C1 stomach) (I): No significant changes are noted.
Kidney (J): No significant changes are noted. The glomeruli are foetal in morphology, being small with peripheral capillaries.
Small intestine (J): There is mild mucosal autolysis. The lamina propria exhibits multifocal to coalescing aggregates of eosinophils (up to 18 per HPF) although no aetiologic agents are seen.
Spleen (A): The splenic architecture appears normal. Occasional individual megakaryocytes (often accompanied by several immature nucleated eythrocytes; splenic extramedullary haematopoiesis) are seen scattered throughout the parenchyma. The white pulp appears unremarkable.
Colon (K): There is mild mucosal autolysis. No significant changes are noted.
Ovary (L): The ovary appears unremarkable however is foetal/neonatal in morphology, with numerous primordial follicles and little intervening stroma present. Rare follicles show slight enlargement (presumably under the influence of maternal hormones in utero).
Skin including blubber and underlying skeletal muscle (M): The epidermis and immediately subjacent dermal fibrous connective tissue are unremarkable. The reticular dermis transitions into the adipose connective tissue of the panniculus adiposus (blubber) throughout which numerous fibrous (collagen) bundles are interspersed. Deeper in the section the blubber transitions to skeletal muscle, which is attached to the adipose connective tissue by loose fibrovascular connective tissue. Numerous depots of adipose connective tissue are strewn amongst the skeletal muscle even deep within the section. In contrast to the blubber of 12/0135 (Gnangara/Quinn's Rocks) and 11/0292 (Bremer Bay) where immature mesenchymal precursors and ground substance predominates, the blubber of this individual appears relatively well developed; with the presence of mature adipocytes, each of which possess a prominent intracytoplasmic lipid vacuole. The lipid vacuoles are subjectively diffusely slightly small and there is increased

variability in the size of the lipid vacuoles between adipocytes, however the adipose connective tissue otherwise appears relatively well developed.
Bladder (M): No significant changes are noted.
Iris and cornea, retina (N): The retina has largely degenerated (inadequate fixation/preservation). The ciliary body and lens are unremarkable. There is a focally extensive area of ulceration of the nonkeratinised stratified squamous epithelium covering the anterior surface. Immediately subjacent to the ulcer is a focal area of stromal necrosis throughout which numerous degenerate neutrophils and eosinophils plus fibrin are intermingled. The superficial 1/3 of the stroma in this area and immediately on either side is expanded by oedema and also appears hypercellular; the latter being due to early granulation tissue formation as well as a focally extensive, florid eosinophilic infiltrate (occasional neutrophils are admixed). Gram Twort, Periodic Acid-Schiff and Silver stains failed to identify the presence of any bacteria and fungal organisms.

Final Diagnosis:

1. **Connective tissue of the optic vascular rete: minimal to mild, subacute, multifocal lymphocytic and eosinophilic inflammation.**
2. **Liver: marked, acute, diffuse centrilobular hyperaemia (congestion) with marginal multifocal microvesicular vacuolar hepatopathy.**
3. **Pyloric stomach (C3 stomach): mild to moderate, acute to subacute, multifocal to coalescing eosinophilic gastritis.**
4. **Small intestine: mild, acute to subacute, multifocal to coalescing eosinophilic enteritis.**
5. **Eye: moderate, acute to subacute, focal eosinophilic and ulcerative keratitis.**

Final Comment: Please also refer to the 'Gross Comments' section above. The presence of foetal glomeruli (present for a short time postpartum) in the kidney is consistent with the estimation of the individual's age (recent neonate).

This individual is the only one of the 3 neonates examined post-mortem by full necropsy that was considered to be in good body condition (and indeed the only one even including the other calves merely visually assessed without necropsy). Corollary to this, it had significantly higher blubber lipid content than found in the 2 age-matched individuals also examined (12/0135 Gnangara Pines/Quinn's Rocks and 11/0292 Bremer Bay; both of which exhibited severe, generalised adipose hypoplasia. Interestingly, 12/0136 was the only individual that exhibited histologically normal blubber characterised by the presence of relatively well-developed (albeit small) adipocytes, and it is likely that this individual more truly approaches the normal neonate in terms of its blubber lipid content and blubber histological appearance. The subjectively small size of the individual adipocytes and the variability in inter-adipocyte lipid vacuole size is likely to represent the fact that significant further development and accumulation of intracytoplasmic lipid occurs during the lactation phase.

Histological examination of the cornea grossly affected by a focal opacity confirmed an ulcerative and eosinophilic keratitis to be present; no aetiological agents were evident on routine and special histochemical stains. The cause remains unknown as the changes are aetiologically non-specific; physical trauma may simply have been the cause. Being a small lesion, limited to the outer one-third of the corneal stroma, and already showing an early reparative reponse with no aetiological agents overtly visible; it is likely to have healed without incident, had the animal lived.

During stranding, pressure causing compression of the thoracic and abdominal vasculature compromises blood flow leading to acute to subacute passive hepatic hyperaemia (liver congestion) and subsequent hepatocellular hypoxia (Jaber et al. 2004). Acute hepatocellular necrosis typical of shock is not seen in the centrilobular areas, although is commonly a sequela to prolonged hepatic hypoxia. This fact, as well as the fact that erythrophagocytosis and evidence of haeme breakdown is not seen, indicates the vascular changes are acute in this case. The vacuolar changes are marginal, multifocal and non-specific in aetiology, although may reflect an early degenerative change secondary to hepatocyte hypoxia. Haematopoiesis is a normal feature of mammalian foetal livers, and often persists in the neonate as EMH (i.e. normal finding).

The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete is unknown. *Toxoplasma gondii* has been reported as a cause of endophthalmitis in a number of mammalian species and histologic lesions can be seen not only in the retina and uvea, but also in the extraocular muscles; however it tends to be necrotising with granulomatous to lymphocyte rich inflammation (the infiltrate seen in this individual is more lymphocytic in its morphology than that seen in both 12/0135 and 11/292; however necrosis is not a feature). Retinal sections were unfortunately too degenerated for adequate assessment (inadequate preservation). Disseminated necrotising infection by *T. gondii* (particularly involving encephalitis) has been demonstrated in pinnipeds, sirenians and odontocetes (Dailey, M.D. 2001; Inskeep, W. et al 1990) however has not been documented in mysticetes. There is evidence, however, that mysticetes may be affected (presumably from contact with water contaminated by oocysts, given they are primarily plankton feeders, supplemented with small fish), with seropositivity documented in a humpback whale from British waters (Forman, D. et al 2009) and molecular (PCR) and immunohistochemical techniques documenting the presence of *T. gondii* in various tissues from a Fin whale (Mazzariol, S. et al 2012). Due to the state of decomposition the histology of these tissues was not described in the latter report, so it is unknown what lesions the apicomplexan was associated with, however this individual was also RT-PCR positive for Morbillivirus and was additionally reported to have high organochlorine (OC) pollutant levels in blubber; so it is postulated by the authors to have been immunocompromised. Immunohistochemistry (IHC) of this case is pending (block E); however *T. gondii* is considered unlikely to be a cause of the inflammation seen. Unfortunately the brain of this individual was not sampled, so it is not known if this individual had encephalitis that might increase the index of suspicion for *T. gondii*. Having said that, indirect fluorescence antibody testing (IFAT) for *T. gondii* antibodies was negative at AHL and is therefore highly unlikely. IHC on E (vascular rete of optic nerve) is pending, however the serology makes this unlikely and furthermore, the use of *T. gondii* monoclonal antibody is not currently validated for use in cetaceans. Larval helminthiasis (larval migrans) could also potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found.

The most likely cause of eosinophilic gastritis and enteritis is likely to be gastric infestation by nematodes (e.g. family Anisakidae), although no nematodes were found grossly or histologically to enable confirmation and identification.

Extramedullary haematopoiesis (EMH) is reported to be uncommon in cetaceans (Rommel et al. 2001), however it is not surprising to see EMH in a neonate, given erythropoiesis is a major function of the foetal mammalian spleen, and is known to persist in other neonatal mammals (e.g. horses and ruminants) for several weeks postpartum (Press et al. 2006).

In summary, it is not known what killed this animal, as no overt causes were identifiable both grossly and histologically. What has been documented are likely to have been incidental lesions, apart from the hepatic changes which are likely to reflect

hepatocellular damage due to stranding and the resultant circulatory changes from recumbency. Maternal abandonment remains a possibility.

Addendum 25th May, 2012:

Immunohistochemistry for *Toxoplasma gondii* was carried out at AHL (DAFWA) on sections from the optic vascular rete; definitive staining of organisms was not detected, hence testing did not indicate the presence of *Toxoplasma gondii* (personal communication with Dr Shane Besier, see also AHL case number AS-12-0808-F-V1 filed under the Bremer Bay neonate 11/292). The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete therefore remains unknown. Larval helminthiasis (larval migrans) could potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found.

Liver was sent to AHL (DAFWA) to measure the concentration of various nutrients/trace elements, the results are as follows:

- Vitamin A (mg/kg) ar – 84.2
- Selenium (mg/kg) – 1.8
- Copper (mg/kg dw – 469
- Vitamin E (mg/kg ar) – 66.4
- Zinc (mg/kg dw) – 115

However, the significance of these figures is unknown as normal reference ranges for Humpback Whales are not known. It is interesting to note that the figures for Vitamins A and E are significantly higher than those from the Bremer Bay neonate (11/292) and higher than those from the Quinns Rocks neonate (12/135), both of which were in poor body condition whereas this individual was in good body condition. These figures may represent variations in maternal vitamin status. Intake of fat soluble vitamins also occurs in milk and this source must be considered, even though the low GGT recorded in this neonate indicates that this animal received little or no colostrum (as the reference range for humpback neonates has not been established).

Yours Sincerely,

Nahiid Stephens BSc BVMS (Hons) MANZCVSc (Vet Path)
Associate Lecturer in Veterinary Pathology

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Checklist: (√ = no gross lesions; **H** = sample for histology; **C** = culture; **P** = photographed)									
	Eyes		Lungs		Stomach		Adrenals		Other organs
	Skin		Bronch LN		S. intestine		Testes		(list)
	Head LN		Heart		Caecum		Ovaries		
	Tongue		Liver		Colon		Meninges		
	Oesophagus		Gall bladder		Mesent LN		Brain		
	Thyroid		Spleen		Kidneys		Bone Marrow		
	Parathyroid		Pancreas		Bladder				
	Thymus		Forestomachs						
Frozen samples:□ Liver □ Fat □ Kidney □ Brain □ Other (list)									

Disease Process 1	**INA**	**Disease Process 2**	
System 1	**EYE**	**System 2**	
General Cause 1		**General Cause 2**	
Aetiology 1		**Aetiology 2**	
Common Name 1	**Ulcerative keratitis**	**Common Name 2**	

Dr. Nahiid Stephens

Appendix F: Post-mortem report for Quinns Rocks neonate

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Western Australia 6150
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Facsimile: (61-8) 9310 4144
Duty Pathologist: 04202 77743

MURDOCH UNIVERSITY
PERTH, WESTERN AUSTRALIA

ANATOMIC PATHOLOGY NECROPSY REPORT

Pathology No:

12/0135

Date In:
24/08/2011
Pathologist: Dr Nahiid Stephens (with assistance from Dr Shane Besier, AHL/DAFWA)

Owner's Details: **C/O Dr Carly Holyoake (Conservation Medicine)** Ph: **0407 335 262**	Consulting Veterinarian: ***Drs Carly Holyoake and Nahiid Stephens*** Date of Consult: **25/08/2011**
Patient's Details: Clinic Number: **146215** Name: **Anatomic Pathology (Humpback Whale) – Quinn's Rocks/Gnangara Pines neonate** D.O.B: **Unknown**	Species: ***Humpback Whale (Megaptera novaeangliae)*** Breed: ***N/A*** Gender: **Female** Current Age: **Neonate**

History: Found at 8.30am 24/08/2011 at Quinn's Rocks. Corner of Camilla Drive and Ocean Drive. May have originally beached alive (reported by public alive?). Weather around time of stranding - WSW 15-20 knots, swell 3m. PM performed 25/08/11 following post-mortem transport to Gnangara Pines.

Submission: A female neonatal Humpback Whale in poor body condition (see table below for condition scores), estimated to be <48 hours old.
Status: Post natural death and movement off public beach to Gnangara Pines.
Post mortem interval: Approximately 24-36 hours.
Post mortem decomposition: Carcase condition code at commencement of post-mortem examination – 2a to 2b.
Identifying features: Dark grey skin. No identifying wounds/entanglements.

Morphometrics:

Measurement	Centimetres
Total length (tip of upper jaw to deepest part of fluke notch)	390
Tip of upper jaw to centre of eye	84
Length of gape (tip of upper jaw to corner of mouth)	90
Tip of upper jaw to blowhole	70
Tip of upper jaw to anterior insertion of pectoral fin	127

Tip of upper jaw to tip of dorsal fin	250
Tip of upper jaw to centre of anus	300
Maximum girth (reaching by doubling the half of maximal girth measurement)	Girth 30cm proximal to umbilicus = 220, girth at umbilicus = 200
Length of pectoral fin (anterior to tip)	130
Width of pectoral fin (maximum)	35
Width of tail flukes (tip to tip)	127
Depth of notch between flukes	6
Height of dorsal fin (tip to base)	11
Blubber thickness at axilla	Dorsal = 3.4
	Lateral = 3.8
	Ventral = 3.2 (to top of throat groove) or 1.4 (to bottom of throat groove)
Blubber thickness at umbilicus	Dorsal = 4
	Lateral = 3.2
	Ventral = 3.4
Blubber thickness at anus	Dorsal = 8.9
	Lateral = 3.2
	Ventral = 3.7
Post-cranial condition score (1,2 or 3)	1
Scapular condition score (1 or 2)	1
Lateral flank condition score (1 or 2)	1

□ **External examination system**
See Below

□ **Skin and subcutis**
See below

□ **Body cavities**
See below

□ **Respiratory system**
See below

□ **Cardiovascular system**
See below

□ **Alimentary system**
See below

□ **Lymphoreticular system**
No visible lesions

□ **Urogenital system**
No visible lesions

□ **Endocrine system**
No visible lesions

□ **Musculoskeletal system**
See below

□ **Nervous system**
See below

Visible lesions:

Significant External Findings

5. External examination: The female calf was in poor body condition with reduced body mass, visually and palpably evidenced by a postcranial depression, protrusion/ prominence of the scapula, and bilateral depressions along the dorsolateral aspects of the flanks. Each of these sites was therefore awarded a condition score of 1 (see table above), using the scoring system developed by Bradford (2011). See photo below – the postcranial depression is particularly evident.

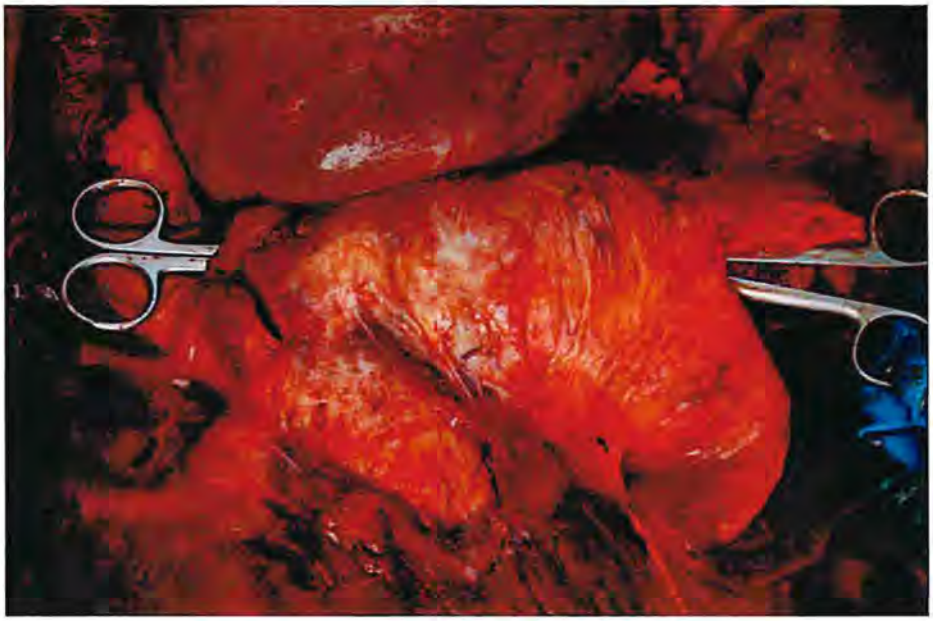
6. Skin and subcutis, musculoskeletal system: There was no external evidence of trauma nor were there any identifying scars/ barnacles; the skin was dark grey. Many linear to curvilinear superficial epidermal excoriations were present disseminated over the body, particularly the dorsum and lateral flanks (inter-individual interaction marks, excoriations sustained during stranding; see photo above). A few isolated cyamids ('whale lice') were present on the ventrum and around the mouth and eyes, see image below.

The blubber appeared diffusely sparse, pale pink and fibrous in consistency (i.e. prominent fibrovascular component with poor adipose stores). See table above for blubber thickness measurements.
The blubber/ subcutis in the occipital, cervical (particularly the dorsal and right ventrolateral aspects) and right mandibular regions exhibited multifocal to coalescing red discolouration ranging from pinpoint red spots (petecchial haemorrhage) to less well-defined patchy red areas ('paintbrush' ecchymoses) and was diffusely slightly gelatinous (oedema). The subjacent muscle in these areas also exhibited multifocal to coalescing poorly defined gelatinous red discolourations (acute haemorrhage). The fascia overlying the right mandibular ramus exhibited numerous pinpoint red spots (petecchial haemorrhage).

Significant Internal Findings

1. Body cavities: There was approximately 300ml of pale yellow, transparent, thin and non-clotting fluid (serous fluid) free within the thoracic cavity; slightly less than that was present in the abdominal cavity. Swabs were taken at both sites for bacteriology. There was scant adipose connective tissue within the body cavities and within the mesentery or surrounding organs (poor adipose stores).
2. Cardiovascular system: There was virtually no adipose connective tissue present at the heart base and that present was smooth, gelatinous and translucent yellow

(adipose hypoplasia versus serous adipose atrophy). A probe patent ductus arteriosus with a 12mm diameter was present. The image below shows the aorta and pulmonary artery with scissors in the patent ductus; note the marked lack of adipose connective tissue at the base of the great vessels.



The umbilicus appeared fresh and moist and was trailing strands of white to pale pink connective tissue (neonate). The external surface of the umbilicus appeared clean and free from exudate, however upon incising into it to expose the superficial portions of the vascular structures a focally extensive (80 x 30mm), yellow to tan area of friable to strand-like (fibrinonecrotic exudate) material was evident. Deep to this the internal vascular structures felt firm and each contained a dry, granular red-brown cast of their lumens which was adherent to the endothelial wall and was completely obscuring the lumen (physiological thrombosis secondary to umbilical involution).

3. Respiratory system: The lungs were diffusely atelectic however floated upon immersion. Small amounts of straw-coloured to slightly red-tinged fluid oozed out of the bronchi and distal trachea (terminally-aspirated water versus pulmonary oedema); copious amounts of stable white frothy fluid was present within the distal two-thirds of the trachea (surfactant mixed with terminally aspirated water versus pulmonary oedema).
4. Alimentary system: The entire alimentary tract was devoid of ingesta and there was no colostrum/ milk in the forestomach/ glandular / pyloric stomach. A small amount of viscous sticky to tarry green-brown faeces was present in the terminal colon/ rectum (meconium).
5. Nervous system: The leptomeningeal vasculature appeared diffusely distended and erythematous (hypostasis, hyperaemia).

Gross Summary:

6. **Body as a whole: severe generalised adipose hypoplasia (ddx: adipose atrophy).**
7. **Skin of ventral, peri-oral and peri-ocular regions: mild multifocal cyamid infestation.**
8. **Blubber, muscle and fascia in occipital, cervical and right mandibular regions: mild acute diffuse oedema with multifocal to coalescing petecchial and ecchymotic haemorrhage.**

9. Cardiovascular system: probe patent ductus arteriosus.

Gross Comment: The size of this animal was consistent with it being a neonatal calf. Given the fact that the umbilicus appeared fresh, the ductus arteriosus was probe-patent and there still appeared to be meconium within the terminal colon/ rectum; it is likely to have been born a relatively short time prior to stranding/ presentation (likely <48 hours prior). Furthermore, given the alimentary tract was devoid of milk/ ingesta, it does not appear to have fed (i.e. likely not to have received colostrum).

One would normally expect to see relatively well developed adipose connective tissue present within the blubber, even in a neonate. Assuming the calf was indeed <48 hours old, the most likely interpretation would be a lack of adequate failure in utero (i.e. adipose hypoplasia) rather than depletion of stores post-natally (i.e. adipose atrophy). Hopefully histological examination will help determine if this is indeed the case.

The appearance of the lungs and the fact they floated on immersion is consistent with them having been inflated prior to death (i.e. the calf was not stillborn; rather it had breathed air following birth). The fluid present in the airways is likely to have been due to terminal aspiration of water whilst becoming stranded, with subsequent mixing with surfactant producing the froth seen in the trachea.

The significance of the fluid present within the thoracic and abdominal cavities is unknown, in appearance it resembled a transudate/ modified transudate; bacteriology is pending, however it may simply represent an incidental finding and/ or be within normal limits for what could be expected for an animal of this size.

The acute oedema and haemorrhage noted in the soft tissues of the head, neck and over the right mandible were pre-mortem changes, however are likely to have been sustained very shortly prior to death (i.e. likely terminal changes during the act of becoming stranded). The cyamid infestation was mild and incidental.

Ancillary Tests:

Abdominal and thoracic fluid swabs as well as a brain swab were submitted to AHL for bacteriology. Blood was submitted to AHL for biochemistry. Liver was submitted to AHL for trace elements/ nutrient analysis; see Appendix at end for grouped preliminary results on this. *Toxoplasma gondii* immunohistochemistry on histology blocks J and N (optic vascular rete and cerebrum). The results are on the next page(s) (some are outstanding/ongoing). See Appendix at end for grouped urine/ vitreous humor ketone results and blubber lipid analysis.

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Case Number: AS-11-2182-F-V2 **Final Report** **Page 1 of 3**

Date: 3-APR-2012 Your Ref: Not Supplied
Enquiries: Dr Shane Besier(Pathology Perth)

To: Dr Carly Holyoake

cc:

42 West View Blvd
Mullaloo
WA 6027

Owner:
Project: Animal disease diagnosis
Species: Cetacead - Humpback Whale
Samples Received: 1 animal - 3 blood; 3 swabs; 1 fluid
Date Collected: 25-AUG-2011 Date Received: 26-AUG-2011 Submission Number:

History
A neonatal Humpback whale calf beached and found dead at Quinns Rocks. External examination noted multiple lacerations to the dorsum. Necropsy performed approximately 36 hours post-mortem. Necropsy revealed no stomach content, meconium in the terminal colon, a probe patent ductus arteriosus, thrombosed umbilical arteries. Several litres of serous thoracic fluid and a small volume of serous abdominal fluid were noted. Swabs of the cavity fluids, fresh fluid and heart blood were submitted for testing.

Aetiological diagnoses
See comments

Comments
Note clinical biochemistry must be interpreted very cautiously given the long post-mortem interval. The low GGT suggests little colostral transfer has occurred but the total protein and globulin fractions contradict this to some extent. I suspect plasma protein is not reliable in clotted post-mortem blood.

Serum zinc was markedly elevated. Such marked elevation may be artefactual.

Two bacterial isolates were detected from the brain swab. The presence of two isolates is suggestive of contamination or post-mortem invasion. Histopathology of the brain may confirm the presence or absence of meningitis/encephalitis compatible with bacterial infection.

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Biochemistry Results

Specimen Type: BLOOD

Spec No.	Spec ID	Creatinine in plasma or serum	Albumin in plasma or serum	ALT in plasma or serum	Calcium in plasma or serum	Conjugated Bilirubin in plasma or serum
		umol/L	g/L	U/L	mmol/L	umol/L
1	HB	125	38.1	108	2.23	2
Reference range:						

Specimen Type: BLOOD

Spec No.	Spec ID	Albumin Globulin	CK in plasma or serum	Cholesterol in plasma or serum	GGT in plasma or serum	GLDH in plasma or serum
		Ratio	U/L	mmol/L	U/L	U/L
1	HB	1.10	5970	6.22	40	3357
Reference range:						

Specimen Type: BLOOD

Spec No.	Spec ID	Glycerol in plasma or serum	GSHPx in red blood cells	Haptoglobin	Iron in plasma or serum	Magnesium in plasma or serum
		mmol/L	U/g Hb	mg/mL	umol/L	mmol/L
1	HB	1.25	145	2.66	25.3	2.94
Reference range:						

Specimen Type: BLOOD

Spec No.	Spec ID	Phosphorus (Pi) in plasma or serum	Total Protein in plasma or serum	Total Bilirubin in plasma or serum	Urea in plasma or serum	BHB in plasma or serum
		mmol/L	g/L	umol/L	mmol/L	mmol/L
1	HB	11.76	72.5	6	29.6	0.12
Reference range:						

Specimen Type: BLOOD

Spec No.	Spec ID	Vitamin A in plasma or serum	Vitamin E in plasma or serum	Zinc in plasma
		mg/L	mg/L	mg/L
1	HB	0.05	13.42	8.82
Reference range:				

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Bacteriology Results

Spec No.	Spec ID	Spec Description	Plesiomonas shigelloides	Haemolytic E Coli	Routine culture
2	HB	Abdominal fluid swab			no growth
3	HB	Thoracic fluid swab			no growth
4	HB	Brain swab	light growth	moderate growth	Significant growth

Comment. E coli 0157 not isolated. Verotoxigenic E coli not detected. PCR negative for shiga like toxin genes SLT I & SLT II. PCR negative for virulence genes hlyA & eaeA

Bacteriology Comments
Abdominal fluid swab, Thoracic fluid swab and Brain swab were all negative for *Erysipelothrix*, *Campylobacter*, *Brucella*, *Haemophilus*, *Pasteurella* or *Vibrio* spp.

Yours faithfully

Dr Shane Besier
VETERINARY PATHOLOGIST

Histopathological findings: H12-0148A-Z

Liver (A): There is diffuse centrilobular (periacinar) sinusoidal hyperaemia; in some areas extending to become midzonal, and occasionally extending to the portal triads. It is, however, worst in the centrilobular areas, with dissociation and shrinkage of the hepatocytes in these areas. Occasional individual, randomly scattered hepatocytes exhibit 1-3 small microvesicular intracytoplasmic vacuoles.

Spleen (A): The red pulp appears diffusely diminished such that the reticuolendothelial network appears prominent; however the splenic architecture is otherwise normal. Occasional individual megakaryocytes (often accompanied by several immature nucleated eythrocytes; splenic extramedullary haematopoiesis) are seen scattered throughout the parenchyma. The white pulp appears unremarkable.

Pancreas (A): There is marked autolysis. No significant changes are noted.

Kidney (B): No significant changes are noted. The glomeruli are foetal in morphology, being small with peripheral capillaries.

Forestomach (C1 stomach) (B): No significant changes are noted.

Muscular artery (10mm diameter) (B): No significant changes are noted.

Main/glandular stomach and pyloric stomach (C2 and C3) (C; portion of pyloric stomach also in D): There is moderate to marked mucosal autolysis. No significant changes are noted.

Ureter (D): Much of the transitional epithelium has sloughed (autolysis). No significant changes are noted.

Small and large intestine (D): There is moderate to marked mucosal autolysis. No significant changes are noted.

Bladder (E): Much of the transitional epithelium has sloughed (autolysis). No significant changes are noted.

Mesenteric lymph node (E): There is mild autolysis. Some follicles possess germinal centres.

Thrombosed external umbilical artery (E): The lumen of the muscular artery lumen is entirely filled by an acute thrombus characterised by the presence of erythrocytes, throughout which are numerous disorganised layers of fibrin strands and admixed leukocytes. There is no lamellar organisation or recanalisation, nor is there any appreciable evidence of haeme breakdown by macrophages.

Tissue labelled as vagina (F): This tissue was labelled as vagina, however has pseudostratified columnar epithelium with apical cytoplasmic secretory product (goblet cell-like) (rather than stratified squamous as would be expected) which has sloughed in places. This fact may mean it is more consistent with terminal urethra, however there is no smooth muscle present as would be typical of the urethra, the submucosal tissues consisting of non-glandular fibrous connective tissue (consistent with being of vaginal origin). There is a mild, diffuse, eosinophilic inflammation of the superficial submucosa and rarely individual eosinophils can be seen exocytosing across the epithelium.

Ovary (F): The ovary appears unremarkable however is foetal/ neonatal in morphology, with numerous primordial follicles and little intervening stroma present. Rare follicles show slight enlargement (presumably under the influence of maternal hormones in utero).

Uterus and cervix (G):No significant changes are noted.

Skin overlying mammary gland (H): No significant changes are noted.

Skin at the edge of the umbilicus (H): The epidermis is focally absent at one end of the section; the immediately adjacent keratinocytes are necrotic and those the other side of that are spongiotic. The subjacent dermal connective tissue exhibits a focally extensive area of coagulative necrosis, throughout which are numerous thrombosed small vessels

and a florid mixed inflammatory response comprised of neutrophils, eosinophils and lesser numbers of lymphocytes and macrophages. Many of the cells (particularly the neutrophils) appear degenerate. This inflammatory infiltrate penetrates into the adjacent dermis which is also focally oedematous in this area; however the inflammatory infiltrate subsides gradually as one moves to the other edge of the section, although even on the contralateral side of the section reactive endothelial lining can be seen within blood vessels (plump endothelial cells) and rare small thrombosed vessels are seen. Routine H&E and Gram Twort stains demonstrate small colonies of gram negative coccobacilli present at the edges of the necrotic tissue, some of which have been phagocytosed.
Thrombosed internal umbilical artery (I): The muscular artery wall is diffusely expanded by oedema and a mild to moderate neutrophilic and eosinophilic inflammatory infiltrate (which occasionally appears degenerate), with occasional admixed lymphocytes and histiocytes. The arterial lumen is completely filled with disorganised fibrin strands strewn amongst a mass of degenerate neutrophils with lesser numbers of eosinophils and occasional lymphocytes, histiocytes and cells that resemble large granular lymphocytes. Occasional colonies of small coccobacilli are present amongst the inflammatory cell population and some phagocytes contain engulfed intracytoplasmic bacteria. Gram Twort histochemistry shows the bacteria to be gram negative coccobacilli (i.e. identical morphologically to those seen in H in the peri-umbilical skin).
Optic nerve within vascular rete (J and K): The optic nerve itself is unremarkable. Within the connective tissues of the vascular rete (most evident in the section contained in J) there is minimal to mild, patchy inflammatory infiltration. The inflammatory cell population is predominantly comprised by eosinophils with slightly lesser numbers of neutrophils, with occasional histiocytes and lymphocytes admixed. Rare inflammatory cells with round nuclei and intensely eosinophilic intracytoplasmic granules are also admixed; these are interpreted as large granular lymphocytes. No aetiologic agents are overtly visible in the sections examined and the vessels themselves are unremarkable.
Cervical spinal cord (K, L): No significant changes are noted.
Brainstem (L): No significant changes are noted.
Cerebellum (M): No significant changes are noted.
Cerebrum (N): Five tiny foci of gliosis are present in a single focal area of white matter; no aetiological agents are overtly visible and there is no associated necrosis or inflammation. The gray matter is unremarkable, as are the meninges.
Lung (O): No significant changes are noted. There is diffuse hyperaemia (ddx hypostasis) and atelectasis.
Trachea and oesophagus (P): No significant changes are noted.
Right ventricular myocardium and heart base adipose connective tissue (Q): No significant changes are noted in the myocardium and the pulmonary artery. The adipose connective tissue appears unusual histologically. Instead of normal adipocytes containing cytoplasmic fat vacuoles, there is instead very loose, paucicellular connective tissue comprised of small groups of poorly differentiated mesenchymal cell precursors accompanied by scant amounts of pale fibrillar eosinophilic material and a marked increase in interstitial space separating the individual precursor mesenchymal cells (ground substance). No well-formed adipose tissue with normal well-developed cytoplasmic vacuoles is seen (adipose hypoplasia).
Thyroid, tongue (R): No significant changes are noted.
Aorta, adrenal (S): No significant changes are noted.
Mediastinal lymph node (T): There is diffuse hyperaemia and the follicles are well developed with germinal centres.
Salivary gland, thymus (U): No significant changes are noted.

Right ventricle (V), left ventricle and heart base adipose connective tissue (W): The right and left ventricular myocardium is unremarkable. As in Q, The adipose connective tissue appears unusual histologically. Instead of normal adipocytes containing cytoplasmic fat vacuoles, there is instead very loose, paucicellular connective tissue comprised of small groups of poorly differentiated mesenchymal cell precursors accompanied by scant amounts of pale fibrillar eosinophilic material and a marked increase in interstitial space separating the individual precursor mesenchymal cells (ground substance). No well-formed adipose tissue with normal well-developed cytoplasmic vacuoles is seen (adipose hypoplasia).
Skin including blubber (X): The epidermis and the immediately subjacent dermal fibrous connective tissue are unremarkable. The blubber is unusual in its appearance as the normal expected adipose connective tissue component is non-existent. Instead of adipocytes separating collagenous bundles (the latter of which comprise the fibrous structurally supportive portion of the blubber), there is instead a scant amount of very loose, paucicellular connective tissue comprised of small groups of poorly differentiated mesenchymal cell precursors accompanied by scant amounts of pale fibrillar eosinophilic material and a marked increase in interstitial space separating the individual precursor mesenchymal cells (ground substance). No well-formed adipose tissue with normal well-developed cytoplasmic vacuoles is seen (adipose hypoplasia).
Optic nerve LS, optic disc (Y): The optic nerve itself is unremarkable. Within the connective tissues of the vascular rete adjacent the nerve (but not in direct apposition to it) there is a small focal area of minimal to mild inflammatory infiltration. The inflammatory cell population is predominantly comprised by eosinophils with slightly lesser numbers of neutrophils, with occasional histiocytes and lymphocytes plus rare large granular lymphocytes admixed. No aetiologic agents are overtly visible and the vessels themselves are unremarkable. These changes are identical to those described in J and K (although milder).
Retina, ciliary body and iris (Z): The retina has largely degenerated (inadequate fixation/preservation). The ciliary body and lens are unremarkable.

Final Diagnosis:

1. **Body as a whole: severe generalised adipose hypoplasia.**
2. **Superficial umbilicus and surrounding skin: severe, acute to subacute, focally extensive necrosuppurative omphalitis, omphalophlebitis and dermatitis.**
3. **Connective tissue of the optic vascular rete: minimal to mild, subacute, multifocal eosinophilic inflammation.**
4. **Cerebrum, white matter: minimal to mild, subacute to chronic, multifocal gliosis.**
5. **Submucosa of vagina: mild, acute, diffuse eosinophilic inflammation.**
6. **Liver: marked, acute, diffuse centrilobular hyperaemia (congestion) with marginal multifocal microvesicular vacuolar hepatopathy.**
7. **Skin of ventral, peri-oral and peri-ocular regions: mild multifocal cyamid infestation.**
8. **Blubber, muscle and fascia in occipital, cervical and right mandibular regions: mild acute diffuse oedema with multifocal to coalescing petecchial and ecchymotic haemorrhage.**
9. **Cardiovascular system: probe patent ductus arteriosus.**

Final Comment: Please also refer to the 'Gross Comments' section above. The presence of foetal glomeruli (present for a short time postpartum) in the kidney is consistent with the estimation of the individual's age (recent neonate). The acute nature of this umbilical artery thrombus identified grossly and histologically also correlates with this. Furthermore, the low GGT on biochemistry would suggest that little colostral transfer has occurred, correlating with the gross findings of the alimentary tract being devoid of ingesta.

The acute oedema and haemorrhage noted in the soft tissues of the head, neck and over the right mandible (interpreted to have been sustained pre-mortem a relatively short time prior to death) is likely to be responsible for the moderately elevated CK on biochemistry (i.e. indicative of muscle damage/ myonecrosis); this is a not unexpected result in a stranded animal that would have been recumbent for some time. To some extent the mildly elevated ALT may also be secondary to this (it can be elevated with significant myonecrosis); however this is a non-specific change (it is found elsewhere other than muscle, notably in the liver) and may well be associated with the hepatic changes instead (see further on below).

Histological examination of multiple sites has confirmed a generalised lack of adequate mature adipose connective tissue stores. Whilst the adipose connective tissue of many neonatal animals when born is relatively devoid of lipid, only filling quickly during the lactation period (Iverson, 2009), neonate baleen whales are said to be born with a blubber layer that is several centimetres thick (Iverson, 2009.). One would normally expect to see relatively well developed adipose connective tissue present within the blubber (and presumably also internally at other sites e.g. peri-renal, heart base etc), even in a neonate; however in this individual the connective tissue occupying the areas where one normally expects adipose is instead a loose, immature, poorly differentiated connective tissue characterised by the presence of immature precursor mesenchymal cells and ground substance. Due to the estimated age of this individual, this is likely to represent adipose hypoplasia. Given the severity of the change (particularly in comparison to the Peaceful Bay neonate [12/0136], the only one examined post-mortem that was considered to be in good body condition and which had a significantly higher blubber lipid content) and the likelihood that the neonate was no more than 48 hours old, it is possible that this adipose hypoplasia reflects insufficient nutrient supply in utero (i.e. poor nutritional status of the dam); as one would expect to see serous atrophy of formed mature adipocytes histologically if it were instead a case of atrophy due to excessive post-natal mobilisation.

The skin at the edge of the umbilicus is interesting. The inflammatory and necrotic changes seen here are more florid than one would expect with normal/physiologic umbilical involution in the neonate. Additionally, the bacteria seen are of a single morphologic type (gram negative coccobacilli), less consistent with simple bacterial contamination, and were furthermore observed phagocytosed in places.

Mirroring the above, the thrombosed internal umbilical artery shows similar changes. This section of tissue was taken from the umbilical stump immediately within the peritoneal cavity. Given the florid inflammation and gram negative coccobacilli present, it would appear that this individual had bacterial omphalitis. Unfortunately the umbilicus was not cultured. This is not a surprising result given the events that occur in normal postpartum umbilical involution, although the amount of inflammation is considered unusual. However, given the abdominal fluid swab failed to culture any bacterial growth at all, and given the liver and spleen did not demonstrate histological changes consistent with sepsis, the bacteria seen and the associated omphalitis,

omphalophlebitis and dermatitis are unlikely to be significant (i.e. it is unlikely that sepsis was a factor in this individual's death).

The development of germinal centres within the mesenteric lymph node is indicative of subacute to chronic antigenic stimulation, however is aetiologically non-specific. It is interesting to note their presence, in the context of the changes in H and I (necrosuppurative omphalitis, omphalophlebitis and dermatitis), however lymphocytolysis was not seen.

During stranding, pressure causing compression of the thoracic and abdominal vasculature compromises blood flow leading to acute to subacute passive hepatic hyperaemia (liver congestion) and subsequent hepatocellular hypoxia (Jaber et al. 2004) and hepatocellular injury. Acute hepatocellular necrosis typical of shock is not seen in the centrilobular areas, although is commonly a sequela to prolonged hepatic hypoxia. This fact, as well as the fact that erythrophagocytosis and evidence of haeme breakdown is not seen, indicates the vascular changes are acute in this case, reflecting changes occurring shortly prior to death. The vacuolar changes are marginal, multifocal and non-specific in aetiology, although may reflect an early degenerative change secondary to hepatocyte hypoxia. The hepatic changes are likely to be responsible for the markedly elevated GLDH and (at least in part, with some contribution possibly coming from dependent myonecrosis) mildly elevated ALT, both indicators of hepatocellular injury.

Extrapolating from the fact that the spleen of the *Tursiops truncatus* (bottlenose dolphin) has a thick capsule (fibrous externally and muscular internally) with the presence of thick smooth muscle trabeculae (extending from the muscular internal capsule) (Cowan et al. 1999), and assuming that Humpbacks are similar (as they appear to be both grossly and histologically), it is possible that splenic contraction can occur following catecholamine release under circumstances requiring blood volume redistribution (e.g. circulatory shock). Extramedullary haematopoiesis (EMH) is reported to be uncommon in cetaceans (Rommel et al. 2001), however it is not surprising to see EMH in a neonate, given erythropoiesis is a major function of the foetal mammalian spleen, and is known to persist in other neonatal mammals (e.g. horses and ruminants) for several weeks postpartum (Press et al. 2006).

The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete is unknown. *Toxoplasma gondii* has been reported as a cause of endophthalmitis in a number of mammalian species and histologic lesions can be seen not only in the retina and uvea, but also in the extraocular muscles; however it tends to be necrotising with granulomatous to lymphocyte rich inflammation (rather than eosinophilic). Retinal sections were unfortunately too degenerated (inadequate preservation) for examination. Disseminated necrotising infection by *T. gondii* (particularly involving encephalitis) has been demonstrated in pinnipeds, sirenians and odontocetes (Dailey, M.D. 2001; Inskeep, W. et al 1990) however has not been documented in mysticetes. There is evidence, however, that mysticetes may be affected (presumably from contact with water contaminated by oocysts, given they are primarily plankton feeders, supplemented with small fish), with seropositivity documented in a humpback whale from British waters (Forman, D. et al 2009) and molecular (PCR) and immunohistochemical techniques documenting the presence of *T. gondii* in various tissues from a Fin whale (Mazzariol, S. et al 2012). Due to the state of decomposition the histology of these tissues was not described in the latter report, so it is unknown what lesions the apicomplexan was associated with, however this individual was also RT-PCR positive for Morbillivirus and was additionally reported to have high organochlorine (OC) pollutant levels in blubber; so it is postulated by the authors to have been immunocompromised. Immunohistochemistry (IHC) of this case is pending (optic

vascular rete and cerebrum - blocks J and N); however *T. gondii* is considered unlikely to be a cause of the eosinophilic inflammation seen, particularly as encephalitis is not a feature of this case. The gliosis seen in the cerebrum was minimal and likely incidental. Furthermore, the use of *T. gondii* monoclonal antibody is not currently validated for use in cetaceans. Larval helminthiasis (larval migrans) could also potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found.

The bacteriology results for the brain swab are interesting, however are unlikely to be clinically relevant given the brain and meninges appeared grossly (apart from vascular changes suggestive of hyperaemia or post-mortem hypostasis/ blood pooling) and histologically (apart from minimal cerebral gliosis unlikely to be related to the bacteriology results) unremarkable. That is to say, there was no evidence of significant brain/ meningeal inflammation that could be due to the presence of the bacteria found on brain swab. Given 2 isolates (haemolytic *Escherichia coli* and *Plesiomonas shigelloides*) were detected; it is more than likely that they represent contaminants or post-mortem invasion. Even so, the presence of *Plesiomonas shigelloides* is an interesting result for the fact that it has never to our knowledge been isolated from this species before. *Plesiomonas shigelloides* is an environmental bacteria, however it can be an opportunistic pathogen; it is more common in freshwater than marine species and is generally isolated from fish (Dr Shane Besier, personal communication). Typing on the *E. coli* isolate shows that it lacks most of the common virulence factors (Vero-toxin, Shiga toxin, hlyA and eaeA) and it's not a serotype O157 (Dr Shane Besier, personal communication).

The appearance of the epithelial lining of the vagina is not typical of the mature reproductive epithelium that is expected at this site. It is possible that the epithelial morphology of the vaginal mucosa represents a normal variation in appearance due to life stage (i.e. young age and sexual immaturity), particularly if it were sampled from the vestibule area. The significance of the eosinophilic infiltrate seen in the superficial vaginal submucosa is not known. Certainly there are no erosive/ulcerative areas to be seen, and the vagina was grossly normal, so it is unknown what might be inciting this, albeit mild, response.

In summary, this neonate was in exceptionally poor body condition. It is likely that lack of adequate blubber adipose stores adversely impacted this individual by (a) being inadequate for insulation and thermoregulation, (b) being inadequate for buoyancy, and (c) being inadequate as an alternative energy source. Hypothermia and lack of buoyancy leading to being unable to keep pace with the dam/ mother is likely to have led to this individual becoming separated from her, and subsequently becoming weak and eventually stranding (assuming the mother did not die herself first, leaving the calf orphaned). The real question is how a calf could come to be born with such inadequate and poorly developed blubber adipose stores in the first place; it is possibly a reflection of insufficient nutrient supply in utero, and may be associated with poor nutritional status of the dam/ mother.

Addendum 25th May, 2012:

Immunohistochemistry for *Toxoplasma gondii* was carried out at AHL (DAFWA) on sections from the optic vascular rete and cerebrum; definitive staining of organisms was not detected, hence testing did not indicate the presence of *Toxoplasma gondii* (personal communication with Dr Shane Besier, see also AHL case number AS-12-0808-F-V1 filed under the Bremer Bay neonate 11/292). The significance and aetiology of the inflammatory changes seen within the connective tissue of the vascular rete therefore

remains unknown. Larval helminthiasis (larval migrans) could potentially explain the presence of eosinophilic inflammation of the vascular rete connective tissue, however no such agents were found.

Liver was sent to AHL (DAFWA) to measure the concentration of various nutrients/trace elements, the results are as follows:

- Vitamin A (mg/kg) ar - 30.2
- Selenium (mg/kg) - 1.68
- Copper (mg/kg dw - 274
- Vitamin E (mg/kg ar) - 35.8
- Zinc (mg/kg dw) - 339

However, the significance of these figures is unknown as normal reference ranges for Humpback Whales are not known.

Yours Sincerely,

Nahiid Stephens BSc BVMS (Hons) MANZCVSc (Vet Path)
Associate Lecturer in Veterinary Pathology

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Checklist: (√ = no gross lesions; **H** = sample for histology; **C** = culture; **P** = photographed)									
	Eyes		Lungs		Stomach		Adrenals		Other organs
	Skin		Bronch LN		S. intestine		Testes		(list)
	Head LN		Heart		Caecum		Ovaries		
	Tongue		Liver		Colon		Meninges		
	Oesophagus		Gall bladder		Mesent LN		Brain		
	Thyroid		Spleen		Kidneys		Bone Marrow		
	Parathyroid		Pancreas		Bladder				
	Thymus		Forestomachs						
Frozen samples:□ Liver □ Fat □ Kidney □ Brain □ Other (list)									

Disease Process 1	**DYS**	**Disease Process 2**	
System 1	**SYS**	**System 2**	
General Cause 1		**General Cause 2**	
Aetiology 1		**Aetiology 2**	
Common Name 1	**Severe generalised adipose hypoplasia**	**Common Name 2**	

Dr. Nahiid Stephens

Appendix G-1: Interim report: Trace element and nutrient analyses of liver; biochemistry results for vitreous humour and urine; lipid analysis, fatty acid analysis

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Case Number: AS-12-0399-F-V1 **Final Report** **Page 1 of 9**

Date: 20-APR-2012 Your Ref: Not Supplied
Enquiries: Mr Gerard Smith(Biochemistry Perth)

To: Dr Carly Holyoake
School of Vet.& Biomedical Sciences
South Street
Murdoch
WA 6150

cc.

Owner:
Project: Research Services
Species: Other species - Whales
Samples Received: 12 animals - 12 whale blubbers; 5 fresh and 2 urine added 1_3_12
Date Collected: Not Supplied Date Received: 9-FEB-2012 Submission Number:

History
Blubber, urine, eye and liver received for biochemical testing from Humpback whales.

Comments
Please find results appended below.

Fat analysis was performed on three strata of fat from each sample received; a deep, central and superficial blubber layer.

BHB was moderately elevated in urine of whale 5 and also had a "moderate" positive reaction on dipstick testing (Multistix 10SG; Seimens). Note the clinical chemical method (AU400) is not validated for urine testing. Unfortunately there was no clear correlation between BHB in urine and vitreous humour. At subsampling for vitreous humour it was noted the consistency of the humour was extremely thick; it is possible sampling was not representative. In further cases perhaps aqueous humour could be considered for testing.

Vitamin A and E in liver were significantly lower in the Bremer Bay calf than in the calves from Quinns Rocks and Peaceful Bay. These may represent variations in maternal vitamin status. Intake of fat soluble vitamins also occurs in milk and this source must be considered although the calf with the highest hepatic vitamin levels (Quinns Rocks calf) did not have other evidence of having received colostrum.

Retrospectively thawed fat samples representative of each strata sampled for lipid analysis was formalin fixed and cut for histopathology. Sections were subjectively assessed for fat volume (probably more correctly "non-collagen volume" per sample) and adipocytes differentiation/fat quality. A middle strata sample from whale 1 was unable to be assessed due to sample quality/fixation/processing problems. Note this was a highly subjective assessment. Poorly differentiated adipose tissue contained (presumed) undifferentiated mesenchyme and cells contained small lipid vacuoles or vacuoles or highly

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variable size. Well differentiated adipocytes contained single large vacuoles with a minimum of mesenchymal tissue.

Sample number	Sample location	Fat/non-collagen content (% est)	Comment
1	Deep	50	Well differentiated, large vacuoles
3	Superficial	80	As above
4	Deep	10	Poorly differentiated, variable size
5	Middle	30	Adipocyte and vacuole size varies
6	Superficial	10	Poorly differentiated
7	Deep	20	Well differentiated
8	Middle	50	As above
9	Superficial	30	As above
10	Deep	40	Well differentiated
11	Middle	40	As above
12	Superficial	40	As above
13	Deep	60	Poorly differentiated, variable size
14	Middle	50	Variable adipocyte size, poor diff
15	Superficial	50	Moderate differentiation
16	Deep	30	Poor differentiation
17	Middle	50	As above
18	Superficial	20	As above
19	Deep	50	Well differentiated
20	Middle	70	As above
21	Superficial	70	As above
22	Deep	20	Few adipocytes
23	Middle	40	As above
24	Superficial	40	Poorly differentiated

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Case Number: AS-12-0399-F-V1 **Final Report**

Biochemistry Results

Specimen Type: BLOOD

Spec No.	Spec ID	BHB in plasma or serum
		mmol/L
25	2	0.01
29	5	0.56
Reference range:		

Specimen Type: TISSUE

Spec No.	Spec ID	Vitamin A in liver	Selenium in liver	Copper in liver	Vitamin E in liver	Zinc in liver
		mg/kg ar	mg/kg	mg/kg dw	mg/kg ar	mg/kg dw
32	Bremer Bay	1.6	2.25	30	2.3	186
33	Peaceful Bay	84.2	1.80	469	66.4	115
34	Quinns Rocks	30.2	1.68	274	35.8	339
Reference range:						

Specimen Type: VIT_HUMOR

Spec No.	Spec ID	Creatinine in Vitreous Humor	Calcium in Vitreous Humor	Urea in Vitreous Humor	BHB in Vitreous Humor	Magnesium in Vitreous Humor
		umol/L	mmol/L	mmol/L	mmol/L	mmol/L
26	3	150	0.92	18.8	0.02	1.08
27	4	302	0.89	13.9	0.05	1.20
28	5	66	0.92	23.1	0.03	1.15
30	6	92	2.05	9.4	0.01	6.23
31	8	157	3.99	15.2	0.03	10.45
Reference range:						

Specimen Type: VIT_HUMOR

Spec No.	Spec ID	Inorganic Phosphate in Vitreous Humor
		mmol/L
26	3	2.95
27	4	2.34
28	5	3.42
30	6	2.19
31	8	5.30
Reference range:		

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Test Type: Fatty acids methyl esters as mg/100g of the sample as received.

Spec No.	Spec ID	Spec Description	C8:0	C10:0	C11:0	C12:0
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	0.0	54.4	0.0	87.3
2	1 Mid	whale blubber	0.0	30.1	0.0	106.2
3	1 Lower	whale blubber	0.0	39.6	0.0	107.9
4	2 Top	whale blubber	5.6	223.6	0.0	186.8
5	2 Mid	whale blubber	0.0	948.6	0.0	243.9
6	2 Lower	whale blubber	0.0	833.1	0.0	274.9
7	3 Top	whale blubber	0.0	1046.1	0.0	132.4
8	3 Mid	whale blubber	0.0	909.3	0.0	49.9
9	3 Lower	whale blubber	0.0	715.4	0.0	193.1
10	4 Top	whale blubber	0.0	619.8	0.0	238.5
11	4 Mid	whale blubber	0.0	493.1	0.0	197.0
12	4 Lower	whale blubber	0.0	1050.0	0.0	195.1
13	5 Top	whale blubber	0.0	318.8	0.0	130.8
14	5 Mid	whale blubber	0.0	1227.0	0.0	349.5
15	5 Lower	whale blubber	0.0	338.1	0.0	524.5
16	6 Top	whale blubber	0.0	725.4	0.0	165.7
17	6 Mid	whale blubber	0.0	1525.0	0.0	212.6
18	6 Lower	whale blubber	0.0	853.0	0.0	510.2
19	7 Top	whale blubber	0.0	77.3	0.0	15.0
20	7 Mid	whale blubber	0.0	92.8	0.0	10.7
21	7 Lower	whale blubber	0.0	90.0	0.0	11.5
22	8 Top	whale blubber	0.0	375.0	0.0	57.8
23	8 Mid	whale blubber	0.0	579.5	0.0	42.8
24	8 Lower	whale blubber	0.0	549.8	0.0	129.5

Spec No.	Spec ID	Spec Description	C13:0	C14:0	C14:1n5	C15:0
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	30.6	7938.7	1583.1	344.8
2	1 Mid	whale blubber	38.7	9650.4	826.7	347.1
3	1 Lower	whale blubber	36.9	10228.4	730.5	326.7
4	2 Top	whale blubber	50.3	5289.6	2313.3	232.9
5	2 Mid	whale blubber	97.3	6006.0	1903.5	194.5
6	2 Lower	whale blubber	105.6	5769.0	1503.9	153.6
7	3 Top	whale blubber	53.7	2939.1	2033.8	153.9
8	3 Mid	whale blubber	21.2	1629.3	430.1	81.0
9	3 Lower	whale blubber	35.9	689.6	115.1	18.1
10	4 Top	whale blubber	57.6	4112.9	2641.0	213.3
11	4 Mid	whale blubber	31.0	1692.4	625.7	53.9
12	4 Lower	whale blubber	54.2	3433.8	1224.5	135.7
13	5 Top	whale blubber	61.5	5636.8	125.8	226.6
14	5 Mid	whale blubber	24.7	2392.8	718.6	114.7
15	5 Lower	whale blubber	0.0	1558.4	229.0	102.6
16	6 Top	whale blubber	40.1	3461.1	22.7	191.6
17	6 Mid	whale blubber	40.7	1602.1	772.6	330.1

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Spec No.	Spec ID	Spec Description	C13:0	C14:0	C14:1n5	C15:0
18	6 Lower	whale blubber	0.0	948.0	280.8	70.4
19	7 Top	whale blubber	57.4	7118.8	144.4	288.6
20	7 Mid	whale blubber	26.0	7108.1	148.9	289.6
21	7 Lower	whale blubber	32.2	7445.7	147.8	297.8
22	8 Top	whale blubber	18.0	5373.8	81.2	250.9
23	8 Mid	whale blubber	42.9	3579.1	30.7	108.9
24	8 Lower	whale blubber	27.7	1598.1	555.1	51.2

Spec No.	Spec ID	Spec Description	C15:1	C16:0	C16:1n7	C17:0
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	0.0	10501.2	18504.1	163.0
2	1 Mid	whale blubber	0.0	13842.9	14278.3	162.3
3	1 Lower	whale blubber	0.0	15157.8	13237.5	165.1
4	2 Top	whale blubber	0.0	11457.7	28941.4	60.1
5	2 Mid	whale blubber	0.0	17328.0	1587.2	97.6
6	2 Lower	whale blubber	0.0	22618.0	1627.9	122.9
7	3 Top	whale blubber	0.0	9701.7	2229.0	98.7
8	3 Mid	whale blubber	0.0	5983.0	5371.1	229.5
9	3 Lower	whale blubber	0.0	2683.0	1734.0	176.1
10	4 Top	whale blubber	0.0	10375.3	2432.0	85.0
11	4 Mid	whale blubber	0.0	4863.3	4659.0	98.8
12	4 Lower	whale blubber	0.0	13108.2	1067.0	271.2
13	5 Top	whale blubber	0.0	10871.7	1777.7	191.7
14	5 Mid	whale blubber	0.0	6869.2	7213.0	175.6
15	5 Lower	whale blubber	0.0	6375.3	3943.4	308.6
16	6 Top	whale blubber	0.0	10780.0	2558.0	84.7
17	6 Mid	whale blubber	0.0	7255.8	8189.4	538.6
18	6 Lower	whale blubber	0.0	6267.4	4557.7	109.2
19	7 Top	whale blubber	0.0	11424.7	18253.5	53.0
20	7 Mid	whale blubber	0.0	10741.1	18346.7	48.9
21	7 Lower	whale blubber	0.0	12444.2	16702.5	70.3
22	8 Top	whale blubber	0.0	11716.8	30679.6	16.5
23	8 Mid	whale blubber	0.0	10321.8	11602.0	69.8
24	8 Lower	whale blubber	0.0	5712.7	5286.6	55.7

Spec No.	Spec ID	Spec Description	C17:1	C18:0	C18:1n9 cis & C81:1trans 9	C18:2n6 cis & C18:2 trans 9 12
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	0.0	1466.7	17940.0	1838.2
2	1 Mid	whale blubber	0.0	1728.0	16094.2	1677.8
3	1 Lower	whale blubber	0.0	1991.4	16747.8	2138.3
4	2 Top	whale blubber	0.0	1047.3	12201.4	632.3
5	2 Mid	whale blubber	0.0	2175.8	13179.7	431.5
6	2 Lower	whale blubber	0.0	2950.1	11245.5	1346.7
7	3 Top	whale blubber	0.0	1994.5	13918.7	919.9
8	3 Mid	whale blubber	0.0	2960.1	7905.0	335.3

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Spec No.	Spec ID	Spec Description	C17:1	C18:0	C18:1n9 cis & C81:1trans 9	C18:2n6 cis & C18:2 trans 9 12
9	3 Lower	whale blubber	0.0	1615.2	3058.9	160.8
10	4 Top	whale blubber	0.0	1394.8	16851.0	1052.8
11	4 Mid	whale blubber	0.0	1072.9	5827.0	412.0
12	4 Lower	whale blubber	0.0	3113.9	12540.3	1200.1
13	5 Top	whale blubber	0.0	2616.8	9591.8	1518.1
14	5 Mid	whale blubber	0.0	3991.2	11945.6	1129.3
15	5 Lower	whale blubber	0.0	5480.2	10031.5	1190.0
16	6 Top	whale blubber	0.0	1657.8	16516.0	960.5
17	6 Mid	whale blubber	0.0	2666.2	9797.5	698.7
18	6 Lower	whale blubber	0.0	4930.7	7575.5	566.6
19	7 Top	whale blubber	0.0	1964.3	6293.3	1832.6
20	7 Mid	whale blubber	0.0	1611.4	6172.5	1920.0
21	7 Lower	whale blubber	0.0	2050.0	6351.4	1585.4
22	8 Top	whale blubber	0.0	1327.5	8801.4	868.9
23	8 Mid	whale blubber	0.0	1222.1	7527.0	829.9
24	8 Lower	whale blubber	0.0	1531.6	4714.3	437.9

Spec No.	Spec ID	Spec Description	C18:3n6	C18:3n3	C18:4n3	C20:0
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	103.9	499.5	139.9	0.0
2	1 Mid	whale blubber	118.2	447.4	133.7	0.0
3	1 Lower	whale blubber	112.0	409.4	118.1	0.0
4	2 Top	whale blubber	46.1	111.9	45.8	0.0
5	2 Mid	whale blubber	46.8	72.6	25.0	0.0
6	2 Lower	whale blubber	29.5	65.8	0.0	0.0
7	3 Top	whale blubber	43.4	72.5	0.0	0.0
8	3 Mid	whale blubber	103.9	136.6	0.0	0.0
9	3 Lower	whale blubber	45.2	50.0	0.0	0.0
10	4 Top	whale blubber	50.2	111.2	0.0	0.0
11	4 Mid	whale blubber	14.3	0.0	0.0	0.0
12	4 Lower	whale blubber	54.2	137.0	158.0	0.0
13	5 Top	whale blubber	67.5	425.1	0.0	0.0
14	5 Mid	whale blubber	140.7	77.7	0.0	0.0
15	5 Lower	whale blubber	0.0	93.9	0.0	0.0
16	6 Top	whale blubber	45.6	69.3	45.2	0.0
17	6 Mid	whale blubber	150.2	122.1	0.0	0.0
18	6 Lower	whale blubber	35.4	159.2	0.0	0.0
19	7 Top	whale blubber	93.3	78.4	83.3	0.0
20	7 Mid	whale blubber	99.1	80.1	125.9	0.0
21	7 Lower	whale blubber	110.8	91.8	133.7	0.0
22	8 Top	whale blubber	62.3	80.9	90.9	0.0
23	8 Mid	whale blubber	24.3	70.4	77.0	0.0
24	8 Lower	whale blubber	39.1	47.0	68.5	0.0

Spec No.	Spec ID	Spec Description	C20:1n9	C20:2	C21:0	C20:3n6

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Spec No.	Spec ID	Spec Description	C20:1n9	C20:2	C21:0	C20:3n6
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	923.7	150.8	0.0	307.0
2	1 Mid	whale blubber	1285.7	87.4	0.0	293.9
3	1 Lower	whale blubber	1366.9	134.2	0.0	304.4
4	2 Top	whale blubber	496.3	56.7	0.0	495.6
5	2 Mid	whale blubber	425.9	819.6	0.0	998.5
6	2 Lower	whale blubber	348.6	803.4	0.0	823.6
7	3 Top	whale blubber	447.2	925.9	0.0	791.5
8	3 Mid	whale blubber	389.1	788.5	0.0	788.5
9	3 Lower	whale blubber	176.1	194.0	0.0	318.0
10	4 Top	whale blubber	460.8	1028.3	0.0	665.5
11	4 Mid	whale blubber	171.0	296.7	0.0	364.1
12	4 Lower	whale blubber	532.2	1071.8	0.0	867.2
13	5 Top	whale blubber	387.1	364.8	0.0	314.1
14	5 Mid	whale blubber	290.4	297.7	0.0	280.3
15	5 Lower	whale blubber	282.3	322.7	0.0	347.3
16	6 Top	whale blubber	419.2	993.4	0.0	609.3
17	6 Mid	whale blubber	285.3	351.1	0.0	695.8
18	6 Lower	whale blubber	142.1	214.1	0.0	0.0
19	7 Top	whale blubber	171.9	94.9	0.0	282.0
20	7 Mid	whale blubber	150.4	36.5	0.0	282.6
21	7 Lower	whale blubber	144.6	29.2	0.0	273.9
22	8 Top	whale blubber	359.0	644.8	0.0	124.2
23	8 Mid	whale blubber	280.4	424.4	0.0	42.8
24	8 Lower	whale blubber	163.6	310.6	0.0	306.2

Spec No.	Spec ID	Spec Description	C20:4n6	C20:3n3	C22:0	C20:5n3
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	591.1	42.4	0.0	3853.2
2	1 Mid	whale blubber	483.6	44.6	0.0	4163.7
3	1 Lower	whale blubber	492.6	39.1	0.0	3831.0
4	2 Top	whale blubber	567.9	0.0	0.0	1590.6
5	2 Mid	whale blubber	797.5	0.0	0.0	862.8
6	2 Lower	whale blubber	799.7	0.0	0.0	941.3
7	3 Top	whale blubber	1182.0	0.0	0.0	1090.3
8	3 Mid	whale blubber	1690.0	0.0	0.0	1250.0
9	3 Lower	whale blubber	972.8	0.0	0.0	504.6
10	4 Top	whale blubber	897.3	0.0	0.0	752.5
11	4 Mid	whale blubber	504.4	0.0	0.0	293.1
12	4 Lower	whale blubber	1257.2	0.0	0.0	585.9
13	5 Top	whale blubber	1284.8	0.0	0.0	2565.4
14	5 Mid	whale blubber	2275.0	0.0	0.0	2415.2
15	5 Lower	whale blubber	3451.4	0.0	0.0	2517.0
16	6 Top	whale blubber	825.9	0.0	0.0	829.8
17	6 Mid	whale blubber	1261.7	0.0	0.0	808.5
18	6 Lower	whale blubber	3063.2	0.0	0.0	1050.8

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Spec No.	Spec ID	Spec Description	C20:4n6	C20:3n3	C22:0	C20:5n3
19	7 Top	whale blubber	690.8	0.0	0.0	5776.4
20	7 Mid	whale blubber	683.1	0.0	0.0	5697.4
21	7 Lower	whale blubber	645.6	0.0	0.0	5450.9
22	8 Top	whale blubber	725.8	0.0	0.0	2742.0
23	8 Mid	whale blubber	494.6	0.0	0.0	842.4
24	8 Lower	whale blubber	892.7	0.0	0.0	887.0

Spec No.	Spec ID	Spec Description	C22:1n9	C22:2	C23:0	C22:4n6
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	164.5	0.0	0.0	170.6
2	1 Mid	whale blubber	214.5	0.0	0.0	112.6
3	1 Lower	whale blubber	226.4	0.0	0.0	120.9
4	2 Top	whale blubber	124.5	0.0	0.0	222.6
5	2 Mid	whale blubber	0.0	0.0	0.0	264.2
6	2 Lower	whale blubber	0.0	0.0	0.0	218.7
7	3 Top	whale blubber	0.0	0.0	0.0	368.6
8	3 Mid	whale blubber	87.9	0.0	0.0	1182.3
9	3 Lower	whale blubber	0.0	0.0	0.0	142.2
10	4 Top	whale blubber	96.3	0.0	0.0	400.1
11	4 Mid	whale blubber	0.0	0.0	0.0	127.6
12	4 Lower	whale blubber	18.0	0.0	0.0	353.5
13	5 Top	whale blubber	0.0	0.0	0.0	340.0
14	5 Mid	whale blubber	148.6	0.0	0.0	405.0
15	5 Lower	whale blubber	182.7	0.0	0.0	462.3
16	6 Top	whale blubber	84.5	0.0	0.0	267.3
17	6 Mid	whale blubber	0.0	0.0	0.0	210.8
18	6 Lower	whale blubber	0.0	0.0	0.0	403.6
19	7 Top	whale blubber	27.0	0.0	0.0	206.1
20	7 Mid	whale blubber	23.2	0.0	0.0	183.9
21	7 Lower	whale blubber	0.0	0.0	0.0	177.6
22	8 Top	whale blubber	34.2	0.0	0.0	355.6
23	8 Mid	whale blubber	0.0	0.0	0.0	224.5
24	8 Lower	whale blubber	52.4	0.0	0.0	233.2

Spec No.	Spec ID	Spec Description	C24:0	C22:5n3	C24:1	C22:6n3
			mg/100g	mg/100g	mg/100g	mg/100g
1	1 Top	whale blubber	0.0	5090.2	0.0	4962.7
2	1 Mid	whale blubber	0.0	3735.6	0.0	4553.1
3	1 Lower	whale blubber	0.0	3848.8	0.0	4564.3
4	2 Top	whale blubber	0.0	4825.9	0.0	2761.6
5	2 Mid	whale blubber	0.0	5059.6	0.0	2090.0
6	2 Lower	whale blubber	0.0	4262.6	0.0	2243.8
7	3 Top	whale blubber	0.0	5372.1	0.0	1367.7
8	3 Mid	whale blubber	0.0	2527.2	0.0	1145.5
9	3 Lower	whale blubber	0.0	1010.2	0.0	471.5

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Spec No.	Spec ID	Spec Description	C24:0	C22:5n3	C24:1	C22:6n3
10	4 Top	whale blubber	0.0	7537.9	0.0	1684.2
11	4 Mid	whale blubber	0.0	1692.5	0.0	543.4
12	4 Lower	whale blubber	0.0	3909.1	0.0	1264.2
13	5 Top	whale blubber	0.0	6721.7	0.0	2574.3
14	5 Mid	whale blubber	0.0	4602.9	0.0	1564.4
15	5 Lower	whale blubber	0.0	3873.3	0.0	1110.0
16	6 Top	whale blubber	0.0	4877.4	0.0	2359.9
17	6 Mid	whale blubber	0.0	2908.9	0.0	1018.5
18	6 Lower	whale blubber	0.0	2483.7	0.0	970.1
19	7 Top	whale blubber	0.0	6083.2	0.0	5957.2
20	7 Mid	whale blubber	0.0	5744.7	0.0	6021.1
21	7 Lower	whale blubber	0.0	5572.8	0.0	5558.6
22	8 Top	whale blubber	0.0	6652.8	0.0	3416.4
23	8 Mid	whale blubber	0.0	4332.7	0.0	1483.3
24	8 Lower	whale blubber	0.0	2195.2	0.0	1003.0

Yours faithfully

Mr Gerard Smith
RESEARCH OFFICER

Appendix G-2: Sample identification

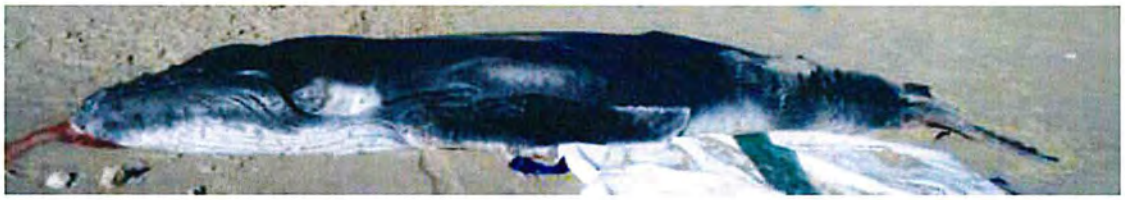
Specimen ID	Date	Location
1	10.9.10	Albany
2	19.5.11	Bremer Bay
3	12.7.11	Exmouth
4	19.7.11	Exmouth
5	24.8.11	Quinns Rocks
6	5.8.11	North Geraldton
7	17.8.11	Peaceful Bay, Walpole
8	25.8.11	Jurien

Appendix H: Photos used to assess body condition

May 19 Bremer Bay

July 12 Exmouth

July 19 Exmouth



August 5 North of Geraldton

August 6 Cape Arid

August 17 Peaceful Bay

August 24 Quinns Rock