A2. Dolphin mortalities in Gippsland Lakes (K. Charlton, Monash University, personal communication). This document has not been published.

Lesions on coastal bottlenose dolphins in the Gippsland Lakes, Victoria, Australia

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Background

Nine bottlenose dolphins have stranded in the Gippsland Lakes region since October 2006. Four of these dolphins stranded during a 10 day period in October-November 2007, two of which had severe lesions across their entire body. Researchers from the Dolphin Research Institute (DRI) and Monash University along with the Department of Sustainability and Environment (Vic. Government) and Melbourne Zoo staff are in collaboration to ascertain potential primary causes and the evident secondary fungal skin lesions. The deceased dolphins are a part of the genetically unique resident population, a potentially new species of coastal bottlenose dolphin as outlined in Charlton et al. (2006). Full morphometrics, photos, genetics, toxicology, histopathology and skin cultures were taken from these dolphins. Melbourne Zoo veterinarians, Michael Lynch, has processed the skin cultures and histopathology. Researchers, Alissa Monk will be processing the toxicology and Kate Charlton will be analysing the morphometrics, genetics and skull information.

Mitchell River Jones Bay dolphin (29th October 2007)

Mitchell River Jones Bay dolphin (29th October 2007)

Results from Paynesville dolphin

• Teeth wear indicates the animal was very old
• Histopathology
  • Extramedullary haemopoiesis seen in liver and spleen; possibly indicating bone marrow suppression
  • Multifocal glomerulopathy (renal disease) of mild to moderate severity; common in old animals
  • Heavy growth of Vibrio species liver and lung
  • Myotic dermatitis

Skin Culture

• Mixed growth of fungi from the skin lesion; suggests opportunistic fungal infection of immunosuppressed animal

Results from Paynesville dolphin

Paynesville dolphin (1st November 2007)

Lesions on living animals

Lesions on living animals

Surveys undertaken in November 2007 of the resident population found approximately 40% of the 40 animals had various skin lesions. These were not observed during surveys conducted in March and June 2007. The area surrounding the Gippsland Lakes is subject to intensive pastoralism and during December 2006 the region experienced severe fire and during June 2007 severe flooding. Current water temperature is approximately 28°C an average increase of 10°C since June 2007.

A3. Water quality sampling sites for the Swan and Canning Rivers [From: Swan River Trust]
A4. Tables and figures for the dolphin mortalities in the Bunbury area

**Figure Bunbury 1**: Map of study area showing the three transect areas: Back Beach (Area 1), Buffalo Beach (Area 3) and the Bunbury inner waters (Area 2).
### Table Bunbury 1: Number of completed transects/area/season March 2007 - November 2009

<table>
<thead>
<tr>
<th>Area</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUTUMN</td>
<td>WINTER</td>
<td>SPRING</td>
</tr>
<tr>
<td>Area 1</td>
<td>mar/apr/may</td>
<td>june/july/aug</td>
<td>sept/oct/nov</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
Table Bunbury 2: Sighting histories of the fourteen dolphins that consistently used Bunbury inner waters (Leschenault Inlet, Leschenault Estuary, Collie River & inner and outer harbours) from March 2007-Sept 2009.

<table>
<thead>
<tr>
<th>Dolphin ID</th>
<th>Status (Nov 2009)</th>
<th>Gender</th>
<th>Age class</th>
<th>Year of birth</th>
<th>Sighting frequency in inner waters</th>
<th>No. of sightings (Mar/07-Sept 09)</th>
<th>First seen</th>
<th>Last seen</th>
<th>Date found dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>Dead</td>
<td>Male</td>
<td>Juvenile</td>
<td>~2006</td>
<td>Consistent</td>
<td>31</td>
<td>26-Mar-07</td>
<td>26-Jul-08</td>
<td>18-Apr-08</td>
</tr>
<tr>
<td>Zippy</td>
<td>Dead</td>
<td>Male</td>
<td>Calf</td>
<td>2007</td>
<td>Consistent</td>
<td>41</td>
<td>4-Apr-07</td>
<td>8-Sep-09</td>
<td>6-Nov-09</td>
</tr>
<tr>
<td>Radar</td>
<td>Dead</td>
<td>Female</td>
<td>Juvenile</td>
<td>~2006</td>
<td>Consistent</td>
<td>37</td>
<td>26-Mar-07</td>
<td>21-Apr-08</td>
<td>12-Jan-09</td>
</tr>
<tr>
<td>Arrow</td>
<td>Dead</td>
<td>Female</td>
<td>Adult</td>
<td>~2006</td>
<td>Consistent</td>
<td>26</td>
<td>26-Mar-07</td>
<td>12-Apr-08</td>
<td>25-Aug-09</td>
</tr>
<tr>
<td>Turbo</td>
<td>Dead</td>
<td>Male</td>
<td>Juvenile</td>
<td>~2006</td>
<td>Consistent</td>
<td>27</td>
<td>4-Apr-07</td>
<td>30-Apr-09</td>
<td>17-May-09</td>
</tr>
<tr>
<td>UNID</td>
<td>Dead</td>
<td>Unknown</td>
<td>Juvenile?</td>
<td>NA</td>
<td>Unknown</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>5-Apr-08</td>
</tr>
<tr>
<td>Crinkle</td>
<td>Presumed dead</td>
<td>Unknown</td>
<td>Calf</td>
<td>2007</td>
<td>Consistent</td>
<td>24</td>
<td>14-May-07</td>
<td>15-Sep-08</td>
<td>NA</td>
</tr>
<tr>
<td>Zodiac</td>
<td>Presumed dead</td>
<td>Female</td>
<td>Adult</td>
<td>2007</td>
<td>Consistent</td>
<td>30</td>
<td>4-Apr-07</td>
<td>28-Aug-08</td>
<td>NA</td>
</tr>
<tr>
<td>Slasher</td>
<td>Presumed dead</td>
<td>Female</td>
<td>Adult</td>
<td>2007</td>
<td>Consistent</td>
<td>26</td>
<td>14-May-07</td>
<td>15-Apr-09</td>
<td>NA</td>
</tr>
<tr>
<td>Bistro-lookalike</td>
<td>Alive</td>
<td>Unknown</td>
<td>Juvenile</td>
<td>2007</td>
<td>Consistent</td>
<td>30</td>
<td>14-May-07</td>
<td>8-Sep-09</td>
<td>NA</td>
</tr>
<tr>
<td>Fragile</td>
<td>Alive</td>
<td>Female</td>
<td>Adult</td>
<td>2007</td>
<td>Consistent</td>
<td>39</td>
<td>4-Apr-07</td>
<td>8-Sep-09</td>
<td>NA</td>
</tr>
<tr>
<td>Mars</td>
<td>Alive</td>
<td>Female</td>
<td>Juvenile</td>
<td>2007</td>
<td>Consistent</td>
<td>45</td>
<td>26-Mar-07</td>
<td>19-Sep-09</td>
<td>NA</td>
</tr>
<tr>
<td>Shredder</td>
<td>Alive</td>
<td>Female</td>
<td>Adult</td>
<td>2007</td>
<td>Consistent</td>
<td>56</td>
<td>26-Mar-07</td>
<td>8-Sep-09</td>
<td>NA</td>
</tr>
<tr>
<td>Icecream</td>
<td>Alive</td>
<td>Unknown</td>
<td>Juvenile</td>
<td>2007</td>
<td>Consistent</td>
<td>26</td>
<td>22-Feb-08</td>
<td>8-Sep-09</td>
<td>NA</td>
</tr>
<tr>
<td>Sunny</td>
<td>Alive</td>
<td>Unknown</td>
<td>Juvenile</td>
<td>2007</td>
<td>Consistent</td>
<td>8</td>
<td>29-Jan-09</td>
<td>19-Sep-09</td>
<td>NA</td>
</tr>
</tbody>
</table>
Figure Bunbury 2:
(a) Sighting locations of now confirmed dead Bunbury inner water dolphins. Sighting records were obtained during boat-based line transect surveys (March 2007-Sept 2009).
Figure Bunbury 2:

(b) Sighting locations of now missing and presumed dead Bunbury inner water dolphins. Sighting records were obtained during boat-based line transect surveys (March 2007-Sept 2009).

(c) Sighting locations of alive Bunbury inner water dolphins (as of Nov 2009). Sighting records were obtained during boat-based line transect surveys (March 2007-Sept 2009).
Figure Bunbury 2: (c) [continued] Sighting locations of alive Bunbury inner water dolphins (as of Nov 2009). Sighting records were obtained during boat-based line transect surveys (March 2007-Sept 2009).
A5. Post-mortem reports from Murdoch University Veterinary School (MUVS), Perth Zoo, and Department of Agriculture
Date: 13-DEC-2007
Date Received: 19-NOV-2007
Submission Number: 08956

To: Dr Simone Vitali
Perth Zoological Gardens
Labouchere Road
South Perth
WA 6151

cc.

Owner: Simone Vitali - Labouchere Rd - South Perth
Project: Zoo testing services
Species: Zoo animal - Bottlenose Dolphin
Samples Received: 1 fixed Total: 11

Brief History: Dead dolphin with numerous target shaped skin lesions and a large haemorrhagic ulcerative area extending from the blowhole dorsally along the length of its body recovered from the Swan River near Bicton. Tissues submitted for histopathological examination.

Histopathological Examination:

Heart (slide 2) - No significant lesions.
Lung (slides 1 & 4) - No significant lesions.
Intestine (slides 5, 8 & 9) - No significant lesions.
Kidney (slide 3) - Mildly congested. Protein casts are present in the lower tubules.
Liver (slide 8) - Mild and widespread periacinar congestion. Associated with that are random zones of acute to sub acute hepatocyte necrosis. These have no zonal pattern and are of irregular size. They tend to be mid zonal but some are closely associated with central veins. Sinusoids are dilated and contain numerous macrophages and some plasma cells. Portal areas are typically encased in fibro-elastic tissue
Lymph nodes (slide 4) - The structures at the base of the mesentery are (or were) lymph nodes. Remnant lymphoid tissue is present but the node is now heavily indurated and fibrosed. One node in particular could not be section because it was heavily calcified.

Skin – Several sections were prepared and examined. Typically there are a range of changes evident. At the periphery of the “target” lesions there is perivascular protein leakage at the apices of the dermal papillae. With increasing frequency towards the centre of the lesion there are Civit bodies and necrosis of individual cells of the stratum germinativum. Mononuclear inflammatory cells appear amongst the proteinaceous exudate and around some superficial blood vessels. The shape of the epidermal rete ridges also change, becoming broader and more elaborate at their tips. Many cells in the intermediate layer (stratum intermedium) have become vacuolated and nearer the surface (stratum externum) the cells are oedematous and rounded instead of their normal horizontally flattened appearance with elongated nuclei. In the sections examined there is also a significant decrease in thickness of the intermediate and external layers.
The other skin lesion is much more acute. The epidermis is completely absent and replaced by a dermal layer of haemorrhagic debris. The lack of inflammatory cells and invading opportunist bacteria indicate the lesion is fairly acute.

**Morphological Diagnosis:** Dermatitis, multifocal, non purulent, chronic.
Epidermitis, necro-haemorrhagic, severe, acute, full depth, extensive
Hepatic necrosis, acute, widespread, non zonal

**Aetiological Diagnosis:** Uncertain.

**Comment:** A number of opinions on the aetiology of the target lesions have been offered. One of them is based on their histopathological similarity to inflammatory responses seen with contact allergies or toxins. The suggestion offered is that the dolphin has had close contact with stinging jellyfish or irritant micro-organisms. A number of documents also suggest the lesions are a consequence of the polluted environment it is exposed to.

I have sought information from a number of sources including Sea World at Surfers Paradise. I have yet to hear from Karrie Rose at Taronga Zoo. Two possible diagnoses have been considered, lobomycosis (fungal dermatitis) and dolphin pox. In my opinion the more likely of the two is dolphin pox, even though the described eosinophilic inclusion bodies are not evident.

Wendy Blanchard at Sea World also suggested erysipelas, but added that the cases she had seen had classic geometric lesions ultimately resembling a chessboard (nothing like this case).

The more acute skin lesion has a separate aetiology, almost certainly linked to sunburn.

The cause of the liver necrosis is uncertain. It may be linked to a state of hypoxia in the period prior to death or it may have an infectious cause. Similar liver lesions have been described in seals infected with Herpes virus.

Similar indurated, fibrotic and calcified lymph nodes are sometimes seen in older terrestrial mammals and probably represent a state of chronic exhaustion due to parasites and chronic inflammatory processes.

Yours faithfully

Dr Cleve Main
VETERINARY PATHOLOGIST

*Testing conducted at no charge*
# Anatomic Pathology Necropsy Report

**Pathology No:** 09/663  
**Date In:** 23/06/2009  
**Pathologist:** Dr Nahid Stephens/Dr Phil Nicholls  
**Consulting Veterinarian:** Dr Carly Palmer  
**Date of Consult:** 23/06/2009

### Owner’s Details:
- **Dr C Palmer**  
  01.29.41.0173.2.xxxxx.06232  
  Wildlife and Conservation Medicine  
  1.098 VCS Building  
  Ph: 0407335262

### Patient’s Details:
- **Clinic Number:** 119175  
  **Name:** Anatomic Pathology  
  **D.O.B.:** Unknown

### Genus: Tursiops (Delphinidae)

### Breed: Male

### Current Age: Juvenile (est. 2-3 years)

**History:** Swan river dolphin #1. Male calf recovered on Sunday June 7th from the Swan River near Fremantle – the dolphin had died approximately 5 days earlier.

**Submission:** A juvenile male dolphin in good body condition.

**Status:** Post natural death.

- Post mortem interval: > 2 weeks; stored frozen from approximately 5 days after death onwards.
- Post mortem decomposition: Advanced.
- Identifying features: Nil.

### Measurements:

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length</td>
<td>210cm</td>
</tr>
<tr>
<td>Snout-genital slit</td>
<td>122cm</td>
</tr>
<tr>
<td>Snout-origin flipper</td>
<td>51.5cm</td>
</tr>
<tr>
<td>Flipper width</td>
<td>12.5cm</td>
</tr>
<tr>
<td>Dorsal fin base</td>
<td>26cm</td>
</tr>
<tr>
<td>Maximum girth</td>
<td>114cm</td>
</tr>
<tr>
<td>Blubber depth (dorsal)</td>
<td>2.3cm</td>
</tr>
<tr>
<td>Blubber depth (ventral)</td>
<td>1.6cm</td>
</tr>
<tr>
<td>Snout-anus</td>
<td>145cm</td>
</tr>
<tr>
<td>Snout-origin dorsal fin</td>
<td>101cm</td>
</tr>
<tr>
<td>Flipper length</td>
<td>33cm</td>
</tr>
<tr>
<td>Dorsal fin height</td>
<td>19cm</td>
</tr>
<tr>
<td>Fluke width</td>
<td>45.5cm</td>
</tr>
<tr>
<td>Axillary girth</td>
<td>102.5cm</td>
</tr>
<tr>
<td>Weight</td>
<td>85kg</td>
</tr>
</tbody>
</table>

### Visible Lesions:

#### Significant External Findings:
- **External examination:** The eyes were macerated and the skin on the snout, melon, dorsal fin, parts of the peduncle and around the eyes was sloughing in places (autolysis). The ventrum was discoloured a red-brown (hyostasis). There were multiple excoriations on the sides and a roughly circular 3cm partial thickness defect in the skin on the dorsal peduncle which was surrounded by multiple superficial...
excoriations – none of these were associated with either haemorrhage or signs of healing and repair and thus it is likely they were sustained post-mortem.

2. **Skin and subcutis**: The blubber was diffusely discoloured with a green tinge and was gelatinous in consistency (autolysis).

**Significant Internal Findings**

3. **Respiratory system**: The lungs were slimy and especially friable and bilaterally mottled a dark red to purple; the surface was marred by multifocal gas bubbling (autolysis). On palpation there were 3-4 small areas of slightly firmer texture in the dorsal aspect of the mid right lobe and in the dorsal aspect of the left cranial lobe, on incision these appeared to be a darker brown compared to the surrounding red-purple parenchyma; however there were no true nodules per se.

4. **Cardiovascular system**: The heart appeared unremarkable and weighed 0.5kg. This was used to extrapolate the total body weight (85kg) using the formula $log W = (log H + 2.2)/0.984$ where $H$ = heart weight and $W$ = body weight, both in kg.

5. **Alimentary system**: The entire serosal surface of the gastrointestinal tract was mottled a red-brown to purple (autolysis) with mild intestinal gaseous distension and the organs were extremely friable. The gastrointestinal tract was empty apart from two types of filamentous parasites; one population formed by several individuals in the main stomach and the other by several individuals in the pyloric stomach.

**Gross Summary:**

1. **Body as a whole**: Advanced post-mortem autolysis, evidence of gastric parasitism.

**Gross Comment**: There were minimal necropsy findings and unfortunately it is likely that the limited histopathology undertaken will be hampered by advanced autolysis.

**Ancillary Tests**: Parasites from the main stomach submitted for parasitological identification were unfortunately fragmented and desiccated and only identifiable as Nematode sp. Parasites from the pyloric stomach were identified as *Corynosoma cetaceum*.

**Histopathological findings**: (H09-0625)

A. **Lung, adrenal, heart** – severe autolysis with multifocal mixed bacterial (coccii and filamentous rods) typical of saprophytic post-mortem invaders. No significant findings.

B. **Kidney, liver** – severe autolysis with bacteria as above. Multifocal scattered trematode eggs present in the hepatic parenchyma.

C. **Brain** – mild autolysis with multifocal ice crystal artefactual clefting, no significant findings.

D. **Fore, main and pyloric stomachs** – severe autolysis with no significant findings.

**Final Diagnosis**: Open/unknown.

**Final Comment**: There was advanced autolysis. Unfortunately there were no significant gross or histopathological findings indicative of probable cause of death and the only readily identifiable lesion was that of gastric and hepatic parasitism which to some extent is expected in wild cetaceans.

*Addendum 16/11/2009*: case and histopathology reviewed by Dr Padraig Duignan, University of Melbourne.

Yours Sincerely,

Nahid Stephens BSc BVMS (Hons)
Associate Lecturer in Veterinary Pathology

Philip K. Nicholls PhD FRCPath

<table>
<thead>
<tr>
<th><strong>Checklist</strong></th>
<th>(V = no gross lesions; H = sample for histology; C = culture; P = photographed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes</td>
<td>Lungs</td>
</tr>
<tr>
<td>Disease Process 1</td>
<td>Disease Process 2</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>System 1</td>
<td>System 2</td>
</tr>
<tr>
<td>General Cause 1</td>
<td>General Cause 2</td>
</tr>
<tr>
<td>Aetiology 1</td>
<td>Aetiology 2</td>
</tr>
<tr>
<td>Common Name 1</td>
<td>Common Name 2</td>
</tr>
</tbody>
</table>

Nahiid Stephens
ANATOMIC PATHOLOGY NECROPSY REPORT

Pathology No: 09/664
Date In: 23/06/2009
Pathologist: Dr Nahid Stephens/Dr Phil Nicholls

Consulting Veterinarian: Dr Carly Palmer
Date of Consult: 23/06/2009

Owner's Details:
Dr C Palmer
01.29.41.0173.2.xxxx.06232
Wildlife and Conservation Medicine
1.098 VCS Building
Ph: 0407335262 Fax To:

Patient's Details:
Clinic Number: 119175
Name: Anatomic Pathology
D.O.B: Unknown

Genus: Tursiops (Delphinidae)
Breed: 
Gender: Female
Current Age: Juvenile to sub-adult

History:
Swan River dolphin #2. Female juvenile. Recovered on Sunday 21 June and refrigerated. There was some monofilament fishing line wrapped around the right tail fluke which had started to incise and created a significant amount of scar tissue (this is the same dolphin observed with fishing line attached by SRT staff a couple of months ago). There were also some superficial prop marks (only through the outer skin layer) between the blowhole and dorsal fin; these may have occurred after the dolphin had died. She did not appear in particularly poor condition and appears to have died within the last couple of days judging by minimal body decomposition. There is an old shark scar LHS posterior to the eye.

Submission: A female juvenile to possibly sub-adult dolphin (age unknown) in good body condition.
Status: Post natural death.
Post mortem interval: At least 3 days.
Post mortem decomposition: Mild to moderate post mortem decomposition.
Identifying features: Old shark bite scar on the left side caudal to the eye, monofilament fishing line tangled around the right fluke with resultant chronic proliferative scar tissue.

Measurements:
1. Total length: 222.5cm
2. Snout-anus: 158cm
3. Snout-genital slit: 157cm
4. Snout-origin dorsal fin: 102cm
5. Snout-origin flipper: 58cm
6. Flipper length: 36cm
7. Flipper width: 16.3cm
8. Dorsal fin height: 24cm
9. Dorsal fin base: 36cm
10. Fluke width: 57cm
11. Maximum girth: 120.5cm
12. Axillary girth: 114cm
13. Blubber depth: 2.7cm (dorsal), 1.5cm (lateral), 1.9cm (ventral)
14. Weight: 137.17kg (extrapolated from heart weight)

- External examination
  - Lymphoreticular system
    - No visible lesions
  - Urogenital system
    - See below
  - Endocrine system
    - No visible lesions
  - Musculoskeletal system
    - No visible lesions
  - Nervous system
    - No visible lesions
- Skin and subcutis
  - See below
- Body cavities
  - No visible lesions
- Respiratory system
  - See below
- Cardiovascular system
  - See below
- Alimentary system
  - See below
Visible lesions:

Significant External Findings

1. External examination: The skin on the snout, ventral tip of the mandible and lateral sides was sloughing in places (autolysis). There was a small abrasion above the right eye. There were 5 parallel curvilinear partial thickness defects (the largest down to blubber, the smallest not entirely through the epidermis) in the skin on the dorsal thorax between the blowhole and the dorsal fin; the caudal-most was 11cm rostral to the dorsal fin. These marks ranged in size between 2-8cm long and 0.8-1.9cm wide. None were associated with haemorrhage or evidence of healing and repair; hence it is likely they were incurred post-mortem and from the pattern they are likely to be boat propeller marks. There were numerous linear excoriations on the right caudal peduncle ranging from 0.2-1cm in length. There was a 2 x 2.5cm ovoid area of white skin discolouration (possible scar) at the right base of the dorsal fin. On the left side of the cranial thorax there were multiple semi-linear scars – dorsally (behind the blowhole and rostral to the propeller marks) there was one which was 22cm long; laterally there was one above the left flipper which was 12cm long. Midway below the dorsal fin and the ventrum there was a lateral curvilinear scar which was 23cm long. Between the left eye and the left flipper there were 7 punctuate to linear scars varying between 6-8mm in size; these were likely to be tooth marks from a previous shark bite. See image below:

Wrapped around the right fluke and cutting into it such that it was partially embedded into the fluke was a tangled mass of monofilament fishing line with some weed and various bits of vegetable matter attached to it. This had created a 5cm indentation into the mid right fluke leaving a triangular defect of tissue (the base of which was 6cm wide). Surrounding the embedded line on both sides of the right fluke was a focally extensive mass of proliferative fibrous scar tissue, suggesting some chronicity of the lesion; although there were multifocal areas which were associated with fresh haemorrhage where the line was most deeply embedded. See images below:
2. **Skin and subcutis:** See above under external examination. The blubber was of adequate thickness suggesting this individual was in overall good body condition.

**Significant Internal Findings**

3. **Respiratory system:** There was red-tinged froth in the trachea and mainstem bronchi. Both lungs were mottled red-purple. Throughout the lobes on both sides there were numerous pinpoint yellow to tan firm spots as well as several irregular large nodules ranging between 0.8-2cm in size – these were firmer than the surrounding parenchyma and were a cream-tan to grey-green colour with a honeycombed internal architecture. Some (but not all) were centred on airways. It was estimated that approximately 30% of the
pulmonary tissue was affected. A sample was collected in a sterile fashion and submitted for bacterial and fungal culture – see results below under ancillary tests. See image below:

4. **Cardiovascular system:** The heart appeared unremarkable and weighed 0.8kg. This was used to extrapolate the total body weight (137.17kg) using the formula \( \log W = (\log H + 2.2)/0.984 \) where \( H \) = heart weight and \( W \) = body weight, both in kg. There were chicken fat clots in both atria and ventricles.

5. **Alimentary system:** The stomachs and intestines were mottled a red-purple with multifocal green-grey patches; with multifocal areas of sub-serosal gas bubbling (autolysis). Several worms were found in the forestomach (likely nematodes). A single worm was found in the common bile duct (likely a trematode).

6. **Urogenital system:** There was a focal 2cm area of yellow-cream thick pasty material in the cranial aspect of the right kidney (caseous abscessation). The left kidney had a focal 0.5 x 3cm dorsal sub-capsular similar area and both kidneys had scattered renicles that had focal to multifocal white-yellow foci throughout them with similar pasty material (micro-abscesses) – it was estimated that approximately 30-40% of the renal tissue was affected.

**Gross Summary:**
1. **External examination:** Evidence of previous shark bite wounds on the left side and of severe, chronic, focally extensive proliferative fibrous scar formation on the right fluke due to fishing line entanglement with ongoing multifocal local trauma and haemorrhage due to persistent embedded line.
2. **Respiratory system:** Moderate, acute, multifocal suppurative bronchopneumonia.
3. **Urogenital system:** Moderate, acute, multifocal suppurative nephritis (possibly embolic in nature).

**Gross Comment:** Microbiology and histopathology is pending, see below.

**Ancillary Tests:**

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<th>01.29.41.0173.2.xxxxx.06232 119175 Nahiid Stephens 23/06/2009</th>
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<td>Wildlife and Conservation Medicine</td>
<td>1.098 VCS Building Phone: 0407335262 Fax:</td>
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09/664 Lung for culture
09/664 Kidney for culture

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*Charged to 01.26.21.0099.5.XXXX.00000 -- Pathology Charging Account*

**MICROBIOLOGY REPORT**

Privileged information: - not for unauthorised publication.

**Culture:**

A: +++ mixed growth of a beta haemolytic gram negative rod and Staphylococcus aureus  
INTERIM REPORT

B: +++ Staphylococcus aureus

Further report: The gram negative rod was put through the API20E ID system with a closest fit of Mannheimia haemolytica (66.5%) or Morganella morganii (26.3%).

Fungal culture in progress: No growth after 9 days incubation. INTERIM REPORT

SUPPLEMENTARY REPORT: Penicillium species culture: probable contaminant FINAL REPORT

Microbiologist: GA.
**Histopathological findings: (H09-0626)**

**A. Lung** – There is moderate to marked autolysis. The pulmonary parenchyma is effaced by multifocal ‘starburst’ colonies of septate branching fungal hyphae (PAS positive) present scattered throughout it (some appeared on the edge of bronchi, others appeared to be centred on blood vessels; however the degree of autolysis makes interpretation difficult). These colonies are surrounded by multifocal copious mixed inflammatory infiltrate which is predominantly neutrophilic and in places degenerate; there are also numerous macrophages within the alveoli. Multifocal bronchi/bronchioles contain similar inflammatory infiltrate. There are multifocal small areas of mineralisation. There appears to be multifocal to coalescing areas of surrounding coagulative necrosis. The remaining alveoli are ateletic, autolysed and they and the remaining bronchi/bronchioles are filled with pale eosinophilic material (oedema, autolysis). Neither syncitia nor viral inclusion bodies typical of morbillivirus are present.

**Kidney** – There is moderate to marked autolysis. The architecture of the renicles is effaced by multifocal to coalescing interstitial (although the degree of autolysis makes localisation difficult as glomeruli are not readily discernible) foci of coagulative necrosis and neutrophilic exudate (much of it degenerate) surrounding multifocal large colonies of coccoid bacteria. These are predominantly medullary, although there are some cortical areas of necrosis and suppurative exudate.

**B. Kidney** – As above. **Mediastinal lymph node** – There is moderate to marked autolysis. There appear to be numerous diffuse degenerate neutrophils scattered throughout the parenchyma. There is severe lymphoid depletion of the germinal centres.

**C. Ovary, adrenal, heart** – No significant findings, autolysis. **Spleen** – Autolysis. Severe lymphoid depletion of the white pulp.

**D. Adrenal** - No significant findings. **Mesenteric lymph node** – Severe lymphoid depletion of the germinal centres. **Fibrous scar tissue from right fluke** – Mild autolysis. The overlying epidermis is acanthotic and hyperplastic with the formation of long rete pegs. The underlying dermis consists of dense, well-organised collagenous fibrous connective tissue. There are multifocal foci of neutrophilic and histiocytic inflammation containing small scattered colonies of coccoid bacteria and multifocal multinucleate giant cells as typically seen in response to foreign bodies.

**E. Liver, brain** – No significant findings, autolysis.

**F. Gastrointestinal tract** – No significant findings, severe autolysis.

**Final Diagnosis:**

1. **Lung:** Moderate to severe, acute to subacute, multifocal necrotising and suppurrative bronchopneumonia with intraleional fungal organisms.
2. **Kidney:** Moderate to severe, acute, multifocal to coalescing necrotising and suppurrative interstitial nephritis with intraleional coccoid bacteria.
3. **Mediastinal lymph node:** Moderate to severe, acute, diffuse suppurrative lymphadenitis; severe, chronic, diffuse lymphoid depletion.
4. **Right fluke:** Moderate, chronic, multifocal pyogranulomatous dermatitis with intraleional coccoid bacteria.
5. **Spleen/medisenteric lymph node:** Severe, chronic, diffuse lymphoid depletion.

**Final Comment:** Given the evidence of multi-organ bacterial infection, it is possible that *Staphylococcus aureus* gained entry and was disseminated from the original and ongoing fluke lesion; and it is possible this individual died as a result of septicaemia. Normally a commensal of skin (and occasionally the nasopharynx), it can infect tissues once the normal barriers have been breached and result in septicaemia, pneumonia, meningitis and endocarditis to name but a few. There may have been preceding intermittent embolic showers of the bacterium which the individual was able to recover from.

It is likely the fungal bronchopneumonia (most likely *Aspergillus sp.*, although no fungal organisms were eventually cultured, which may be due to the fact that post mortem examination was delayed for at least 3 days in which time the carcass was refrigerated) is the result of an opportunistic infection in this individual which more than likely was already immunosuppressed as a result of an ongoing bacterial infection in the fluke. The significance of the gram negative rod cultured (most likely *Mannheimia haemolytica*) is unknown; this bacterium is known to cause a fibrinous to fibrinosuppurative bronchopneumonia with pleuritis in cattle and sheep. The bacteria is usually carried in the tonsils, oropharynx and nasal cavity of normal animals; and given the right circumstances when stressors impair normal defence mechanisms, it can colonise the lungs and establish a pulmonary infection (and rarely, septicaemia in young lambs < 8 weeks of age). Such stressors in cattle and sheep include weaning, handling, treatments such as de-worming and dipping, transport, fatigue, crowding, mixing of different populations, inclement weather, poor nutrition, pre-existing or concurrent viral or bacterial infections. It is not known whether this species occurs naturally as a commensal in dolphins; however it is possible it is a secondary invader causing opportunistic infection in this individual.

The severe lymphoid depletion seen in the spleen and the lymph nodes are indicative of chronic antigenic stimulation.
Addendum 16/11/2009: case and histopathology reviewed by Dr Padraig Duignan, University of Melbourne. He comments: "the lymph nodes and the spleen are severely [depleted] and equivalent to what I would expect in morbillivirus infected animals; however no inclusions were seen.” (Personal communication).

Yours Sincerely,

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

Philip K. Nicholls PhD FRCPath

| Checklist: | (V = no gross lesions; H = sample for histology; C = culture; P = photographed) |
|---|---|---|---|---|---|---|
| Eyes | Lungs | Stomach | Adrenals | Other organs (list) |
| Skin | Bronch LN | S. intestine | Testes |
| 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 | |

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<th>Disease Process 2</th>
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<td>Necrotising and supplicative interstitial nephritis with intralesional coccoid bacteria</td>
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Nahiid Stephens
ANATOMIC PATHOLOGY NECROPSY REPORT

Pathology No: 09/637
Date In: 10/06/2009
Pathologist: Dr Nahid Stephens/
Dr Phil Nicholls

Consulting Veterinarian: Dr Carly Palmer (on behalf of the Swan River Trust)
Date of Consult: 08/06/2009

Owner’s Details:
Anatomic Pathology
Pathology Use Only - No Charges
Ph: 
Fax To:

Consulting Veterinarian: Dr Carly Palmer (on behalf of the Swan River Trust)
Date of Consult: 08/06/2009

Patient’s Details:
Clinic Number: 118886
Name: 09/637
D.O.B: unknown

Species: Exotic
Breed: Dolphin (Tursiops sp.)
Gender: Male
Current Age: 2-2.5 years

History:
Dr Carly Palmer submitted this individual on behalf of the Swan River Trust. It was found dead in the Swan River on Monday 8th June on the Coombe Reserve Beach (Mosman Park) by a council worker. According to those present at the site at the time it was found, it had few to no external marks. It was transported immediately to Murdoch and stored in the necropsy cool-room until today’s necropsy. I examined it on submission and felt it was at least 24 hours post-mortem.

Submission: A male dolphin (Truncatus sp.), approximately 2 to 2.5 years of age (ie: sub-adult).
Status: In good body condition.
Post mortem interval: At least 72 hours.
Post mortem decomposition: Advanced.

Identifying features/measurements:
1. Weight: 106.74kg (extrapolated from heart weight)
2. Total length: 210cm
3. Snout-anus: 147cm
4. Snout-genital slit: 130cm
5. Snout-origin dorsal fin: 95.5cm
6. Snout-origin flipper: 56cm
7. Flipper length: 35.5cm
8. Flipper width: 12.5cm
9. Dorsal fin height: 21.5cm
10. Dorsal fin base: 26.5cm
11. Fluke width: 49.5cm
12. Maximum girth: 126cm
13. Axillary girth: 122cm
14. Blubber depth: dorsal 2.2, lateral 1.2, ventral 1.6cm

External examination
Not examined/ No visible lesions/ See Below
Skin and subcutis
Not examined/ No visible lesions/ See below
Body cavities
Not examined/ No visible lesions/ See below
Respiratory system
Not examined/ No visible lesions/ See below
Cardiovascular system
Not examined/ No visible lesions/ See below
Alimentary system
Not examined/ No visible lesions/ See below

Lymphoreticular system
Not examined/ No visible lesions, See below
Urogenital system
Not examined/ No visible lesions, See below
Endocrine system
Not examined/ No visible lesions, See below
Musculoskeletal system
Not examined/ No visible lesions, See below
Nervous system
Not examined/ No visible lesions, See below

Visible lesions:

Significant External Findings
1. **External examination:** The individual was a young sub-adult male in good body condition. There was a fair amount of post-mortem abdominal bloating. The penis was partially extruded and the tip (20mm) was dessicated and black. The corneas were opaque and abraded with adherent sand and organic matter.

2. **Skin and subcutis:** There were numerous variably sized irregular excoriations over the entire body and extremities, especially the melon and lateral sides; these were fresh and not associated with any bruising nor any visible attempts at healing and repair (ie: likely from movement and transport). The skin was diffusely friable and there were multifocal areas of maceration and skin slippage over its entirety (autolysis). There was a 22mm circular full thickness ulcer on the ventral point of the mandible which was surrounded by a ring of haemorrhage (bruising) which was likely sustained shortly prior to death. The blubber layer was adequate and the individual appeared in good body condition. The musculature deep to the blubber was a dark red-purple and soft and friable (autolysis).

### Significant Internal Findings

1. **Body cavities:** There was a small amount of foul-smelling red-brown fluid in both the thoracic and abdominal cavities and the organs were friable and diffusely discoloured a dark red-purple (advanced autolysis). The parietal peritoneum dorsal to the kidneys was distended and ballooning outwards (autolysis).

2. **Cardiovascular system:** The heart appeared unremarkable and weighed 0.625kg. This was used to extrapolate the total body weight using the formula \( \log W = (\log H + 2.2)/0.984 \) where \( H \) = heart weight and \( W \) = body weight, both in kg.

3. **Alimentary system:** The rostral 15mm of the tongue was sloughed (autolysis). The entire gastrointestinal tract was moderately distended with gas and discoloured a dark red-purple (autolysis). The entire gastrointestinal tract was devoid of any ingesta. Low numbers of a 25-30 x 1mm pale cream worm were found in the stomach. On the mucosal surface of the squamous portion of the stomach 5 x 5-10mm papular thickenings with a 1-2mm central depression were found in a focal location; plus sparse multifocal 1-2mm umbilicated slightly nodular thickenings were also found. These irregularities were the same colour as the surrounding mucosa (glandular openings, foci of mucosal damage). A 220mm segment of jejunum was discoloured black and a 50mm length in the centre of this segment exhibited transmural thickening (13mm thick) mainly attributable to an increase in thickness of the tunica muscularis; the mesenteric artery supplying this intestinal segment was also discoloured black, however no gross thrombi were found in it nor the mesenteric vein (localised area of sulphide producing bacteria ie: autolysis, segmental intestinal infarction due to thromboembolic disease or secondary to intestinal accident ie: intussusception or torsion – although no definitive intussusception/torsion was located in the way the intestines were found, focal haemorrhage). See below.

![Image of internal organs](image_url)

The pancreas was not definitively located but it was felt that this may have been due to severe autolysis. Low numbers of a 25-30 x 1mm dark green segmented parasite were found in the bile duct, as well as a 10 x 4mm soft choleolith.
4. **Nervous system:** The brain appeared unremarkable externally, apart from being particularly friable and soft (autolysis). Upon sectioning it, a focal, poorly-defined 15-20mm cavitated, non-encapsulated area was found in the rostrodorsal outer cerebral cortex just caudal to the area of the olfactory bulb (abscess, autolysis). It appeared to be filled with a creamy (purulent) material, the tissue immediately surrounding it was extremely soft and friable.

![Image](09_637.jpg)

**Gross Summary:**
1. **Body as a whole:** the individual was in good body condition but was in an advanced state of autolysis (decomposition).
2. **Alimentary system:** focal, transmural thickening and black discoloration of a 220mm segment of jejunum (DDx: infarction, previous/reduced intussusception or torsion, haemorrhage).
3. **Nervous system:** focal cavitation of the cerebral cortex (DDx: bacterial/fungal abscessation, severe autolysis).

**Gross Comment:** The absence of any ingesta suggests the individual had been anorexic for 24-48 hours. Unfortunately the advanced autolysis made any interpretation of the gross findings limited.

**Ancillary Tests:**
Parasites collected were sent for identification. The parasites from the biliary duct were nematodes, they were in poor condition due to the advanced state of decomposition, however they were provisionally identified as *Campula sp.* Those found in the stomach were nematodes of *Anisakis sp.*

**Histopathological findings:** Note – the advanced state of decomposition meant that only limited histopathology was carried out and the severe autolysis of the tissues made interpretation extremely difficult.

**H09-0581A** Cerebral cortex – the grey and white matter and the meninges were disrupted by a multifocal to coalescing, severe neutrophilic infiltrate that appeared in places to be predominantly perivascular and in others disseminated throughout the parenchyma; as well as a circulating neutrophilia. Many neutrophils appeared degenerate. Associated with these areas were numerous septate and branching fungal hyphae (consistent with *Aspergillus sp.*) which were disseminated throughout both the grey and white matter as well as being visible in the wall of a large muscular artery and branching out into its lumen. In the grey matter worst affected by the inflammatory infiltrate there were multifocal eosinophilic, enlarged and angular neurons; consistent with neuronal necrosis. Multifocal thrombi were seen within the vasculature and there was diffuse hyperaemia and mild oedema as evidenced by widened Virchow-Robins spaces. Several large submeningeal bacterial colonies were seen, comprised of 2 bacteria – short gram negative coccobacilli (consistent with *Escherichia coli*) and filamentous gram positive coccobacilli (consistent with *Clostridia sp.*)

**H09-0581B** Spleen – several large bacterial colonies as described above were noted scattered throughout the section, as well as disseminated individual identical bacteria (saprophytic post mortem invaders). There was severe lymphoid depletion of the white pulp.

Stomach – no significant findings; the aforementioned area of papillary thickening appeared to be associated with a rugal fold and the 1mm pinpoint central depression appeared to be associated with a focal indented area of
hyperkeratosis, however there was no pathology present. Scattered bacteria were noted throughout the sections as described above.

(H09-0581C) Mesenteric lymph node chain – there was severe lymphoid depletion of the germinal centres. Tissue architecture was extremely poorly preserved, limiting interpretation, and there were numerous saprophytic post mortem bacteria present.

Common bile duct – there was marked thickening and fibrosis of the duct and contained within it were numerous trematode eggs and some yellow-brown pigment (bile). The tissue was poorly preserved; however there appeared to be a mild, multifocal to coalescing mixed inflammatory infiltrate throughout the duct/periductular tissues comprised of neutrophils, lymphocytes and the occasional histiocyte. Several thrombi were found. Scattered bacteria as noted above were seen throughout the sections.

(H09-0581D) Thickened jejunum – the submucosa was severely expanded by a focally extensive area of fibrin deposition and haemorrhage. There were multifocal thrombosed blood vessels (confirmed by Martius Scarlet Blue stain). Again, there were scattered bacteria as described above seen in the sections.

(H09-0581E) Mesenteric vessels associated with the abnormal jejunal segment – several thrombi were seen (again confirmed with MSB stain); again, there were several small bacterial colonies as well as scattered bacteria present as described above.

Final Diagnosis:

1. Severe, acute, focally extensive, suppurative and necrotising meningoencephalitis with intralesional fungal organisms and multifocal thrombus formation.
2. Severe, acute, focally extensive, submucosal jejunal haemorrhage with multifocal thrombus formation.
3. Severe, chronic, generalised lymphoid depletion of the spleen and mesenteric lymph node chain.

Final Comment: It is impossible to definitively say whether the jejunal pathology is linked to the changes seen in the brain; however given the fungal organisms (presumptive Aspergillus sp.) were seen clearly within the cerebral vasculature (indicative of haematogenous spread), it is possible that the jejunal submucosal haemorrhage occurred subsequent to intestinal infarction sustained due to haematogenous Aspergillus and thrombus formation. The 2 bacterial populations described in the sections are highly likely to be saprophytic (ie: post-mortem invaders) and are therefore indicative of severe autolysis. The real question that remains is why this individual, despite appearing to be in good overall body condition, contracted Aspergillosis. Aspergillus fumigatus is generally known to be an opportunistic pathogen and the greatest number of reported cases appears in dolphins that are infected with Morbillivirus (ie: are immunosuppressed). It characteristically causes pneumonia and encephalitis. No changes specifically attributable to Morbillivirus were seen in the cerebral cortex (ie: nonsuppurative meningoencephalitis with occasional eosinophilic intranuclear inclusions); however these are typically transient changes which can be masked by subsequent secondary opportunistic infection and resultant suppurative inflammation, hence it cannot be definitively ruled out. It could be argued that either this individual was suffering from prior immunosuppression (for reasons unknown) or it is possible that this individual had received excessive challenge to its system, for example – infection of skin wounds (eg: sustained due to contact with boats) and subsequent haematogenous spread. In any case, the exact reason why this individual became infected, and how it came to be so, remains unknown. The severe lymphoid depletion seen in the spleen/mesenteric lymph node chain is indicative of chronic antigenic stimulation.

As with most wild cetaceans, a parasite burden has been observed; which appears to be associated with a chronic, fibrosing inflammatory response seen in the common bile duct. This is considered an incidental finding. Various tissue and teeth samples were collected by Dr Carly Palmer for morphometric studies and routine testing.

Addendum 16/11/2009: case and histopathology reviewed by Dr Padraig Duignan, University of Melbourne.

Yours Sincerely,


Nahid Stephens BSc BVMS (Hons)
Associate Lecturer in Veterinary Pathology

Philip K. Nicholls PhD FRCPath
**Checklist:** (✓ = no gross lesions; H = sample for histology; C = culture; P = photographed)

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**Frozen samples:** Liver ☐ Fat ☐ Kidney ☐ Brain ☐ Other (list)

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**Aetiology 1**
- Suppurative and necrotising meningoencephalitis with intralesional fungal hyphae
- Aetiopathy 2
  - Submucosal jejunal haemorrhage with multifocal thrombus formation

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<thead>
<tr>
<th>Common Name 1</th>
<th>Cerebral Aspergillosis</th>
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<tr>
<td>Common Name 2</td>
<td>Jejunal infarction</td>
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CLINICAL HISTORY
An adult female dolphin (ID M090955) was observed staying around a particular area of the Swan River for a few days and was suspected to be ill. It was found dead on 17/9/2009.

GROSS PATHOLOGY FINDINGS
There were extensive areas with multifocal to coalescing skin lesions with greater abundance on the dorsal fin. Lesions range from localised areas of circular skin discolouration to raised vesicular lesions and other areas characterised by ulceration with discharge. The cut surface shows the lesions to be confined to the epidermis and superficial parts of the blubber.

The left axillary LN was enlarged and had a gelatinous appearance and contained haemorrhagic material. There were multifocal areas of ulceration and haemorrhage affecting the fundic and pyloric regions of the stomach and proximal duodenum. Small parasitic organisms resembling flukes were present in the pyloric region of the stomach. There is negligible gut fill. The right ovary was large with a large follicle present.

HISTOLOGY FINDINGS
Slide A1-A4: Skin.
Slide B1: lung, kidney.
Slide B2: pancreas, kidney, heart.
Slide B3: duodenum, stomach, adrenal.
Slide B4: heart, spleen, liver.
Slide B5: LL, LN, liver.
Slide B6: Intestine.
Slide C1: liver.
Slide C2: spleen.

Skin: multiple large abscesses occur at the junction between the epidermis and the dermis causing the overlying epithelium to protrude towards the surface. The epithelial surface is colonised by thick mats of
fungal hyphae. The hyphal elements were broad and variable in size and width, demonstrating nonparallel walls, irregular right-angled branching, and rare septations. Fungal hyphae are also seen in reduced numbers within the abscess as well as in the deep dermis. They stain poorly with PAS but are obvious using Gram and Giemsa staining method. Trapped within the matt are mixed bacterial colonies and cellular and acellular debris. Epithelial cells in affected areas show marked ballooning degeneration. The abscess comprises of large numbers of heterophils, erythrocytes, proteinaceous oedema fluid and fibrin. There is necrosis and evidence of bacteriophagia. Inflammatory cells extend laterally and into the deeper tissues between myofibres and corresponds to the presence of fungal hyphae.

**Lung:** there are occasional small foci of lymphocyte aggregates and focal areas of low protein fluid accumulation within some alveoli.

**Kidney:** intracytoplasmic protein globules are present within many proximal convoluted tubule epithelium. The Bowman’s capsule of glomeruli appear moderately thickened.

**Heart:** there is multifocal coalescing mature fibrous tissue that courses through, replacing and sometimes isolates bundles of cardiac myocytes. The majority of cardiac myocytes contain golden paranuclear cytoplasmic pigment.

**Intestine & stomach:** there is swelling of random individual myofibres within the muscularis propria.

**LN:** moderate cortical atrophy.

**Spleen:** there is evidence of some extramedullary haematopoiesis.

**Liver:** there are multiple areas with bile duct hyperplasia that dissects through the hepatic parenchyma and frequent nodular hyperplasia of hepatocytes.

**MORPHOLOGICAL DIAGNOSIS**

Skin: suppurative dermatitis, severe deep, multifocal, acute with intralesional fungal hyphae.

Heart: myocardial fibrosis, chronic, moderate, multifocal.

**AETIOLOGICAL DIAGNOSIS**

Mycotic dermatitis (Zygomycetes).

**COMMENTS**

There was discussion about whether this dolphin had suffered skin damage to predispose it to the fungal infection. There is a possibility that she might have swam through corrosive substances, low pH water, jellyfish, incurred thermal or other physical injury, creating portals for infection. Alternatively, the dolphin may have been immunocompromised.

The morphology of the fungus resembles Cunninghamella species which has been observed in mature male striped dolphin which died shortly after stranding. Culture and PCR for *Aphanomyces invadans* was negative. General mycology culture was also negative. Fungal culture is best conducted on fresh tissues rather than from frozen samples.
Bacteriology Results

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<th>Pseudomonas sp.</th>
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Test Type: PCR for Aphanomyces invadans identification (NATA accreditation does not cover the performance of this service.)

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Yours faithfully

Dr Richmond Loh
VETERINARY PATHOLOGIST

*Testing conducted at no charge*
ANATOMIC PATHOLOGY NECROPSY REPORT
FINAL

Pathology No: 09/1108
Date In: 26/10/2009
Pathologist: Dr Nahid Stephens/
Dr Mandy O'Hara

Owner's Details:
Anatomic Pathology
Pathology Use Only - No Charges
Ph: Fax To:

Consulting Veterinarian:
Date of Consult: 26/10/2009

Patient's Details:
Clinic Number: 122776
Name: 09/1108
D.O.B: Species: Tursiops (Delphinidae)

Breed: Gender: Female
Current Age: Unknown (adult)

History:
3 weeks ago a dolphin was sighted by Swan River Trust staff with skin lesions. On Sunday 25/10/2009 the dolphin (possibly the same individual) was seen in the Applecross region of the Swan River (near the Canning Hwy bridge) and was reported early in the afternoon to be swimming slowly and erratically, appearing distressed. By mid to late afternoon it had moved around Point Heathcote to Waylen Bay where it was being observed by the Marine Police, whom were subsequently met by a team in the Murdoch research dinghy. At this point it appeared very weak, it was swimming in the shallows leaning to the left with circling to the left, and stayed at the surface. It was noted to have severe, multifocal to coalescing skin lesions over its entire surface and some twigs/vegetation appeared to be protruding from just behind its right flipper. Within a short time (<20min) it beached itself in the shallows on its left side and was approached on foot, whereabouts it shortly (<5min) passed away (likely terminally from drowning given it was partly submerged) following agonal rigor. Following confirmation of death it was noted that the vegetation described previously was attached to a length of fishing line wrapped around the base of its right pectoral fin. It was transported in a Swan River Trust utility vehicle to Murdoch University and transferred to the necropsy cooler within 1.5 hours post mortem.

Submission: A female adult dolphin in poor/slim body condition.
Status: Post natural death and subsequent refrigeration.
Post mortem interval: 20-21 hours
Post mortem decomposition: Nil.
Identifying features: Dorsal fin photographed; multiple distinctive skin lesions (see external examination).

Measurements:
1. Total length: 244.5cm
2. Snout-anus: 172cm
3. Snout-genital slit: 154cm
4. Snout-origin dorsal fin: 108cm
5. Snout-origin flipper: 60cm
6. Flipper length: 40cm
7. Flipper width: 16cm
8. Dorsal fin height: 23cm
9. Dorsal fin base: 40cm
10. Fluke width: 63cm
11. Maximum girth: 121cm
12. Axillary girth: 117cm
13. Blubber depth: 3cm (dorsal), 1.5cm (lateral), 1.7cm (ventral)
14. Weight: 137.2kg (extrapolated from heart weight)

☐ External examination
☐ Skin and subcutis
☐ Body cavities

☐ Lymphoreticular system
☐ Urogenital system
☐ Endocrine system
Visible lesions:
Significant External Findings

1. Skin and subcutis: The entirety of the dolphin’s skin was covered in multitudinous, multifocal to coalescing irregularly-shaped, raised, umbilicated pale-grey to yellow-tinged plaques which frequently had a red, raw centre (central erosion/ulceration secondary to necrosis). The skin in these areas appeared swollen, oedematous and macerated, sloughing easily (see image A below: left side). Many of these lesions exuded a small amount of blood-tinged turbid fluid (serosanguineous exudate) with gentle pressure. The skin around both eyes had swollen significantly such that the eyes were obscured. It is estimated that approximately 65-70% of the skin surface was affected.

A single layer of pink fishing line was present wrapped around the right pectoral fin and was superficially embedded 1-2mm deep in the skin of the rostral aspect of the fin; this was easily removable. The line extended a short way caudally and was rubbing against the right ventrolateral chest; it had cut into a thickened plaque lesion and was found underneath a loose flap of skin. The distal end of the line was attached to a small branch and algal material; the proximal tip of the branch was also caught underneath the skin flap (see image B below: right ventrolateral aspect).

There was a 12 x 5cm ovoid area of abrasion on the ventral aspect of the lower jaw and a 30 x 1.5cm linear abrasion on the right lateral edge of the fluke; both of these were not present in the field and likely were sustained post-mortem during the transportation process.
2. **Musculoskeletal system:** The dolphin was in poor body condition; the dorsolateral epaxial muscles were moderately concave in profile.

3. **Alimentary system:** The mucosa covering the rostral aspect 15cm of both the bottom of the mouth and the dorsal palate was thickened and verrucous (ddx: hyperplasia, neoplasia). The mandibular rostral-most 1st, 2nd and 3rd teeth were bilaterally severely worn to half their normal height and their maxillary counterparts were missing. Several other teeth were also missing or were loose, see image (C) below.
1. **Body cavities:** Several localised multifocal to coalescing fibrous adhesions stretched between the left caudolateral aspect of the forestomach to the immediately adjacent peritoneal surface. There was a 2cm, circular brown-black serosal discoloration in this area and immediately beneath this there was a firm intramural nodule palpable (see alimentary system below).

2. **Alimentary system:** A fish hook was lodged 28cm down the oesophagus in the ventral mucosal surface and had created a 1.5 x 0.3cm mucosal defect, the edges of which were everted and thickened; its tip had partially lodged in the inner tunica muscularis, however had not perforated transmurally. A 40cm length of fishing line was attached to the hook, the proximal end of which was visible in the caudal oropharynx. The mucosa of the distal half of the oesophagus had multifocal white raised plaque-like thickened areas on its surface (hyperplasia/hyperkeratosis). The forestomach, main stomach and pyloric stomach were empty; apart from a small amount of silt (likely ingested terminally). The forestomach contained a serrated fish tooth measuring 2.5cm long which had perforated the left caudolateral mucosal surface and was lodged in the tunica muscularis enclosed in a 3cm x 0.5cm fibrous tract. Immediately overlying this area there was a 2cm, circular brown-black area of serosal discoloration; and as previously noted above (body cavities) this region of the forestomach wall was joined to the adjacent peritoneal surface by multifocal to coalescing localised fibrous adhesions. See image (D) below – note some of the adhesions had been broken down in the process of opening the abdominal cavity. Several nematodes were found in the main stomach.

3. **Lymphphoreticular system:** The left sublumbar lymph node and the mesenteric lymph nodes appeared gelatinous in consistency (oedema) and slightly enlarged; and the left sublumbar lymph node had a central focus of opaque yellow viscous liquid (purulent exudate).

4. **Respiratory system:** The trachea, mainstem bronchi and larger intrapulmonary airways were filled with copious amounts of white frothy fluid (ddx: oedema, drowning). The left lung lobe was diffusely pale pink and was heavy and incompletely collapsed (ddx: oedema, drowning). The right lung lobe was diffusely dark red/purple and was heavy and incompletely collapsed (ddx: oedema, drowning, hypostasis – the individual had been placed in right lateral recumbency post mortem). Both lobes oozed white to haemorrhagic froth on cut surface (ddx: oedema, drowning). The cranioventral tip of the left lung lobe had 3-4 small 0.5cm intrapulmonary nodules palpable within the parenchyma; on cut surface these ‘nodules’ consisted of foci of purulent exudate amongst the parenchyma.

5. **Urogenital system:** Both ovaries contained surface and intraovarian cystic structures and both had multifocal raised, roughened pale white-cream areas on the capsular surface (capsular fibrosis from previous ovulatory episodes). The right ovary had a superficial focal nodule which was a pale tan-yellow on cut surface, likely a corpus luteum (signifying recent ovulation). The right uterine horn was 2-3 times larger

![Image of the abdominal cavity](image-url)
than the left and contained a small amount of opaque yellow liquid (ddx: purulent exudate, glandular secretions).

6. Cardiovascular system: The heart weighed 0.8kg. This was used to extrapolate the total body weight (137.2kg) using the formula $\log W = (\log H + 2.2)/0.984$ where $H =$ heart weight and $W =$ body weight, both in kg. The endocardial blood vessels appeared thick, white and more prominent than normal (ddx: fibrosis, atherosclerosis, mineralization, arteriosclerosis, normal anatomic variation secondary to age); there was minimal to no subepicardial fat (adipose atrophy consistent with poor body condition). At the apex of the left ventricle (immediately lateral of the coronary vasculature), there was a 1cm, focal, roughly circular area of white to cream discoulouration of the epicardial surface (ddx: fibrosis, oedema, cellular infiltrate).

**Gross Summary:**

1. **Skin:**
   a. Severe, subacute to chronic, multifocal to coalescing, irregular plaque-like, centrally necrotising dermatitis (ddx: viral ± bacterial/fungal pathogens).
   b. Acute fishing line entanglement involving the right pectoral fin.

2. **Alimentary system:**
   a. Mild, acute, focally extensive ulcerative oesophagitis secondary to fish hook deglutition and penetration of the mucosa/submucosa and partial penetration of the tunica muscularis.
   b. Mild, chronic, multifocal distal oesophageal mucosal hyperplasia and hyperkeratosis.
   c. Mild, chronic, focally extensive granulomatous and fibrosing gastritis (forestomach) secondary to ingestion of a fish tooth and full-thickness perforation of the wall resulting in localised peritonitis and subsequent fibrous adhesions.
   d. Mild, chronic, multifocal dental attrition of the rostral teeth.
   e. Mild gastric nematode burden.

3. **Lymphoreticular system:**
   a. Mild, acute, multifocal lymphadenomegaly (oedema)
   b. Mild, acute, focally extensive suppurrative lymphadenitis of the left sublumbar node.

4. **Respiratory system:**
   a. Moderate to marked, acute, diffuse pulmonary oedema (ddx: secondary to drowning as a terminal event, autolysis).
   b. Mild, acute to subacute, multifocal suppurative bronchopneumonia localised in the left cranial pulmonary lobe.

5. **Musculoskeletal system:** Moderate, chronic, generalised skeletal muscular atrophy (ddx: starvation, anorexia, maldigestion-malabsorption).

6. **Urogenital system:** Moderate, chronic, focally extensive right uterine horn hypertrophy (ddx: pyometron) with intraluminal fluid (ddx: purulent exudate, glandular secretion).

7. **Cardiovascular system:**
   a. Moderate, chronic, diffuse thickening of the epicardial vasculature (ddx: fibrosis, atherosclerosis, mineralization, arteriosclerosis, normal anatomic variation secondary to age).
   b. Mild, chronic, focal myocardial fibrosis of the left ventricular apex (ddx: oedema, cellular infiltrate).

**Gross Comment:** Given the dental attrition and evidence of previous follicular ovarian activity; this individual is a mature female, likely reasonably aged. The disparity in size between the left and right uterine horns may even suggest that she may have been gravid in the right horn in the past. Given the witness reports from those present at her time of death (including Dr N. Stephens) and the post mortem findings of pulmonary oedema, it is likely the individual drowned terminally.

Post-mortem examination confirmed the severity of the skin lesions noted in the field. Certainly the severity and extent of these skin lesions would have caused a debilitating loss of fluid and protein through the skin; additionally they would have been a source of considerable pain. At this stage their aetiology remains unclear. A recent comprehensive review indicates that there are a number of pathogens that may be associated with epidermal/dermal lesions, including: viruses (Caliciviridae, Herpesviridae, Papillomaviridae and Poxviridae), bacteria (Aeromonas spp., Dermatophilus spp., Erysipelothrix rhusiopathiae, Mycobacterium marinum, Pseudomonas spp., Staphylococcus delphini, Streptococcus iniae and Vibrio spp.), fungi (Candida albicans, Fusarium spp., Trichophyton spp. and Lacazia loboi) and ciliated protozoans (Kyaroikeus cetarius)\(^1\). Viruses tend to be primary pathogens, whereas most bacterial and fungal organisms are opportunistic pathogens, causing secondary infection following a break-down in normal host defences\(^2\) such as may occur with penetrating wounds or following immunosuppression. Various authors have suggested (as reviewed in the same paper) that the incidence of skin lesions (as well as their high prevalence in certain populations) is linked to environmental degradation; although it is likely that water temperature and salinity are also important factors in aetiopathogenesis\(^3\). In essence, skin lesions often reflect the overall health status of the individual cetacean\(^4\).
Collaborative work in Victoria in the past three years by researchers based at Monash University, Melbourne Zoo, the Dolphin Research Institute and the Department of Sustainability and Environment have found similar skin lesions in several dolphins that died following strandings in the Gippsland Lakes region. Their preliminary research (as yet unpublished) identified the presence of dolphin pox-virus (orthopox) in some of the lesions with multiple secondary opportunistic mixed bacterial and fungal infections that were unable to be further characterised (personal communication, Kate Charlton-Robb, School of Biological Sciences, Monash University). In addition, the affected individuals came from a population known to have high mercury loads (sufficient to cause death); hence it is likely that, as outlined above, the skin lesions were a manifestation of immunosuppression in individuals known to be living in extreme fluctuating environmental conditions ((personal communication, Kate Charlton-Robb, School of Biological Sciences, Monash University).

More recently and locally, recent fish surveys of Black Bream in the Canning River have found a number of fish affected by Epizootic Ulcerative Syndrome (Red Spot, Mycotic Granulomatosis) caused by an oomycete fungal pathogen *Aphanomyces invadans* (personal communication, Simon Allen, School of Biological Sciences, Murdoch University). This fungus is potentially zoonotic. There have been reports in the literature of similar epidemic dermal ulcerative syndromes in dolphins off the east coast of the United States in 1987-1988 following a preceding ulcer epidemic in estuarine fish populations in the same region; in the latter a toxic dinoflagellate (order *Dinamoebales*) was responsible, and studies showed that a major component of the toxin is hydrophobic, thus having the potential for adipose storage in sublethally affected fish and hence transmission up the food chain to the higher predators such as dolphins and potentially, man. Further investigation into the local dolphin and fish populations, as well as their local environment for possible pathogens, contaminants and causes of immunosuppression is warranted. Various tissue samples have been taken for testing of heavy metals and organochlorines and cytochrome P450 IHC; skin and blubber samples have been taken for DNA studies and teeth have been taken for aging. The serrated fish tooth found in the forestomach has also been submitted for prey species identification. Sections of skin lesions have been stored in 5% glutaraldehyde for future electron microscopy studies, if warranted. A final report will be issued once the results of ancillary testing and histopathology are available.

Ancillary Tests:
1. Parasitology: the nematodes from the stomach belong to the family Anisakidae.
2. Bacteriology in-house:

### CLINICAL PATHOLOGY MICROBIOLOGY REPORT

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Charged to 01.29.41.0173.2.xxxxx.06232 Wildlife and Conservation

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Culture: INTERIM REPORT

A: +++ pure growth of *Pseudomonas aeruginosa*

B: ++ mixed growth of *Vibrio alginolyticus* and *Shewanella putrefaciens*.

C: As for B

D: As for B
3. Other: Sections of skin have also been sent to the Animal Health Laboratories for bacterial/fungal cultures plus PCR for *Aphanomyces invadans*. Please see the full AHL report (AS-09-3397-F-V1) at the end (to be emailed separately to stakeholders). In summary:

a. **Bacteriology (skin):** Significant growth on routine fish bacterial culture. Moderate growth of *Listonella anguillarum* (non-haemolytic) and *Vibrio vulnificus*.

b. **Mykology (skin):** Smear – fungal growth very similar to hyphae seen in skin wet prep (wide irregular hyphae with no septate or spore heads). Cultured *Saprolegnia* sp. and *Candida* sp.

c. **PCR for *Aphanomyces invadans* (skin):** Negative.

**Histopathological findings: (H09-1129A-S)**

A. **Skin:** The epidermis is markedly and diffusely thickened due to severe hydropic degeneration of the stratum intermedium and stratum externum with relative sparing of the deeper stratum germinativum and stratum spinosum. The superficial half to two-thirds of the epidermis exhibits extensive coagulative necrosis. The epidermis is disrupted by multifocal to coalescing pustular/vesicular aggregates of degenerate leukocytes, predominantly neutrophils; this inflammatory infiltrate appears to extend from the dermis (particularly the dermal papillae, where they appear aggregated around the vasculature and demonstrate extravasation) and small aggregates can also be seen in the superficial hypodermis. There is dermal oedema and focal haemorrhage. Several arterioles are seen in the superficial dermis with very subtle hazy and hypereosinophilic change to the appearance of the tunica media, possibly suggestive of fibrinoid necrosis. Numerous non-pigmented mycelial fungi exhibiting budding are highlighted by PAAS (and less so by PAS) staining predominantly in the superficial necrotic epidermis; they appear non-septate and branching and are quite large (up to 36-45μm in width/diameter when compared with adjacent neutrophils) and irregular in shape, some hyphae appearing almost beaded. In some areas they extend to the dermo-epidermal junction and there are several hyphae present multifocally in the superficial dermis. Occasional aggregates of fusiform spores are also seen (ddx: yeast forms – dimorphic fungus versus separate yeast organism). Gram Twort staining confirms the presence of multitudinous gram negative short bacilli (both *Vibrio alginolyticus* and *Shewanella putrefaciens* are gram negative bacilli) within the epidermis; occasional single to small aggregates of free and phagocytosed bacilli are also present in the superficial dermis.

B. **Skin:** As above. A transition zone from relatively normal to affected skin is present, demonstrating the focally extensive thickening of the epidermis in the affected area, largely due to hydropic degeneration of the layers described above.

C. **Skin:** As above in A.

D. **Lung:** Two sections are present, changes described are representative of both (although one section is much more markedly affected; it is estimated that in one section 5% of the tissue is affected as described, and in the other 45% is affected). There are multifocal to coalescing areas of basophilic hypercellularity comprised of large numbers of degenerate leukocytes (predominantly neutrophils; with lesser numbers of macrophages, lymphocytes and rare plasma cells in a more marginal zone) superimposed on areas of necrosis effacing the normal pulmonary architecture. Towards the periphery of these regions they appear to be centred on bronchi/bronchioles; with multifocal to coalescing airways containing a mixed (but predominantly neutrophilic) inflammatory exudate. Remnant alveoli are atelectic and contain eosinophilic material (oedema, autolysis) as well as numerous neutrophils and lesser numbers of alveolar macrophages; there is extensive hyalinisation of the alveolar septa. Occasional small peripheral foci of mineralisation are seen. Rare early thrombi are present in the vasculature. Occasional free/phagocytosed gram negative bacilli are seen on Gram Twort stained sections. Many alveoli on the less affected section contain eosinophilic, proteinaceous material (oedema, autolysis).

E. **Liver:** There are multifocal, randomly oriented, small areas of necrosis and micro-abscessation scattered throughout the parenchyma. All perportal areas exhibit a marginal to mild lymphoplasmacytic inflammatory infiltrate, occasional neutrophils area also rarely present. A PAAS stain was negative for any fungal organisms.

F. **Heart – coronary vessels:** There is very little subepicardial fat; the coronary vessels appear normal with no signs of athero/arterio-sclerosis or mineralisation.

G. **Heart – left ventricle area of pallor:** There is a focally extensive area of epicardial thickening due to oedema (with multiple ectatic lymphatics present), hyperaemia and a marginal increase in lymphocytes; the underlying myocardium is unremarkable.

H. **Kidney:** There is diffuse distension of Bowman’s space by a lightly eosinophilic material; however there is no significant tubular proteinuria; this is considered to be artefactual and due to autolysis.

I. **Pancreas:** No significant changes; autolysis.
Lung: As described in (D).

J. Right ovary: A 14mm diameter corpus luteum is present (ie: recent ovulation). The ovary appears active with multiple primordial and primary follicles and rare secondary follicles.

K. Adrenal: There is marginal cortical hyperplasia.

Lymph nodes (2); Sections of the left sublumbar lymph node and a mesenteric node. One node exhibits severe lymphoid depletion of the germinal centres with diffuse mild to moderate oedema. The other node appears relatively normal with many follicles exhibiting an increase in the number of plasma cells present. The medullary sinuses are moderately distended with numerous neutrophils and lesser numbers of macrophages and some haemosiderophages (indicative of the nodes draining an area of inflammation and haemorrhage).

L. Adrenal/lymph nodes (2): As described in (K); again, one node exhibits severe lymphoid depletion of the germinal centres whereas the other does not.

M. Left ovary: The ovary appears active with multiple primordial and primary follicles and rare secondary follicles.

Lymph node: No significant changes.

N. Foreestomach – tissue around embedded tooth: The tunica muscularis is disrupted and effaced by a focally extensive area of well-vascularised immature granulation tissue with admixed small multifocal areas of necrosis. There is a mild to moderate neutrophilic, histiocytic and lymphocytic inflammatory infiltrate scattered throughout the granulation tissue. The overlying mucosal epithelium is unremarkable (ie: the tooth had burrowed a tract below it in this section).

O. Oesophagus around the lesion associated with the hook: There is a focally extensive area of mucosal ulceration present; immediately ventral to this there is a sero-cellular exudate overlying the ulcer bed, the predominant cell type is neutrophilic, although occasional histiocytes and lymphocytes are seen. The ulcer bed is supported by a focally extensive area of well-vascularised immature granulation tissue. There are multifocal to coalescing areas of granulation tissue, coagulative necrosis and predominantly neutrophilic inflammation within the submucosa. The tunica muscularis was intact on these sections; however it’s involvement on other sections (as suspected grossly) cannot be ruled out.

P. Main stomach, glandular stomach, duodenum: No significant changes; autolysis.

Q. Ileum, colon, rectum: No significant changes; autolysis.

R. Left uterine horn: There is a mild to moderate, diffuse neutrophilic inflammatory infiltrate within the superficial endometrium; the endometrium is diffusely hyperaemic.

Cerebrum: No significant changes.

S. Right uterine horn: As described in (R).

Cerebrum: No significant changes.

Final Diagnosis:

1. Skin:
   a. Severe, acute to subacute, multifocal to coalescing, pustulo-vesicular, necrosuppurative dermatitis with epidermal hydropic degeneration and the presence of intralesional fungal organisms and gram negative bacilli (*Vibrio alginolyticus* and *Shewanella putrefaciens* cultured).
   b. Acute fishing line entanglement involving the right flipper.

2. Alimentary system:
   a. Mild, subacute, focally extensive, ulcerative, necrosuppurative oesophagitis secondary to fish hook deglutition/lodgement.
   b. Mild, subacute, focally extensive, neutrophilic, histiocytic and lymphocytic, necrotising gastritis (forestomach) with granulation tissue formation within the tunica mucosa secondary to ingestion of a fish tooth and full-thickness perforation resulting in localised peritonitis and subsequent fibrous adhesions.
   c. Mild, chronic, multifocal dental attrition of the rostral teeth.
   d. Mild gastric nematode burden (family Anisakidae).

3. Respiratory system: Mild, subacute, multifocal suppurrative bronchopneumonia localised in the left cranial pulmonary lobe with the presence of intralesional gram negative bacilli (*Pseudomonas aeruginosa* cultured).

4. Liver:
   a. Mild, acute, multifocal random hepatocellular necrosis and microabscessation.
   b. Marginal to mild, chronic, diffuse, lymphoplasmacytic periportal hepatitis.


6. Urogenital system: Mild to moderate, acute, diffuse endometritis (*Vibrio alginolyticus* and *Shewanella putrefaciens* cultured from the right uterine horn).

7. Lymphoreticular system:
a. Mild to moderate, acute, multifocal lymphadenomegaly due to oedema and drainage of regional inflammation (*Vibrio alginolyticus* and *Shewanella putrefaciens* cultured from the left sublumbar lymph node).

b. Moderate, chronic, diffuse lymphoid follicular depletion and plasmacytosis.

8. **Cardiovascular system:** Mild, acute, focal, epicardial oedema of the left ventricular apex.

**Preliminary Comment 4/11/2009:** Please refer to the Gross Comments section above. In addition to this – it is thought that the fungal organisms seen within the skin are unlikely to be consistent with *Aphanomyces invadans*, which is generally much more invasive and also typically causes a granulomatous inflammatory response. It is more likely that the fungal organisms are representative of an opportunistic saprophyte that has gained entry via abraded skin and a breach in host defences. *Vibrio alginolyticus* and *Shewanella putrefaciens* are also secondary opportunistic marine bacteria. No viral inclusions were seen; however a primary viral infection (eg: Orthopox) cannot be ruled out. Unfortunately the possibilities for a primary inciting disease/event remain wide and varied and include: Orthopox viral infection, systemic immunosuppression secondary to pollutants/contaminants/heavy metals and skin trauma from a low pH environment, sunburn, chemical burn and physical trauma (eg: boating injuries). The level of bronchopneumonia seen in this individual is very mild compared to previous cases; *Pseudomonas aeruginosa* is also an opportunistic pathogen.

It is likely this individual was bacteraemic (+/- endotoxaemic) which may account for many of the other changes.

The areas of hepatocellular necrosis and microabscessation are likely to be associated with opportunistic bacterial pathogens having gained access via the portal circulation, possibly secondary to the breach in the foregut wall caused by the tooth foreign body; although a PAAS stain for fungal organisms is pending (follow-up: nothing significant found on PAAS stain).

Sections of skin have also been sent to the Animal Health Laboratories for bacterial/fungal cultures plus PCR for *Aphanomyces invadans*. Results are pending.

**Final Comment 26/11/2009:** Please note that the AHL report has confirmed PCR for *Aphanomyces invadans* was negative. AHL has cultured various opportunistic bacterial, fungal and yeast organisms; as suspected from histopathological examination – all are marine environment opportunists and have likely gained entry secondary to some form of primary skin damage. Dr Padraig Duignan has reviewed the case/slides and comments: "the hydropic change in the epidermis seen histologically is characteristic of that seen in dolphins going from full strength saline to fresh water; or can occur if the stratum externum is damaged, allowing the deeper cells to be affected by the environmental water – skin affected in this manner will form a substrate for commensal fungal, bacterial and yeast organisms as noted/cultured". It is possible that haematogenous dissemination of bacteria in the blood perhaps caused infarction of superficial dermal vessels in what was the centre of these skin lesions with the hydropic changes occurring around these areas. No herpesvirus inclusions were seen; the slides are to be reviewed for poxvirus inclusions. No morbillivirus inclusions/syncytia were seen in the lung." (Note: this is an insensitive means of identification and does not rule out prior infection).

Addendum: on further review Dr Duignan identified the presence of multifocal keratinocytes containing single/multiple, eosinophilic, variably sized, circular, intracytoplasmic viral inclusion bodies suggestive of poxvirus infection. Electron microscopy of glutaraldehyde samples is pending in an attempt to confirm the presence of viral particles ultrastructurally. The presence of skin lesions associated with poxvirus infection is unusual in an adult animal (as they generally have adequate protective immunity and any lesions are generally self-limiting) as a first point; secondly, the lesions were extremely severe and extensive. In is unknown exactly how confounding variables such as potential immunosuppression (due to various factors), osmoregulatory damage due to low salinity environments, and secondary opportunistic infection have influenced the precise aetiopathogenesis of these lesions (although we know there is secondary infection and osmoregulatory damage is likely as suggested by Dr Duignan); or whether the virus itself may be of unusual virulence. Given this case's similarity to the 17th September mortality examined at the zoo/animal health laboratories; it would be worth reviewing the histopathology from that case to check for inclusions.

Yours Sincerely,

Nahiid Stephens BSc BVMS (Hons)
Associate Lecturer in Veterinary Pathology
Dr Mandy O'Hara, BSc Hons, BVMS, MACVSc, Diplomate ACVP
Senior Lecturer in Pathology

<table>
<thead>
<tr>
<th>Checklist: (Y = no gross lesions; H = sample for histology; C = culture; P = photographed)</th>
</tr>
</thead>
</table>
| Eyes | Skin | Lungs | Bronch LN | Heart | Tongue | Larynx | Oesophagus | Heart | Head LN | Heart | Gall bladder | Spleen | Stomach | S. intestine | Stomach | Head LN | Heart | Gall bladder | Spleen | Stomach | S. intestine | Stomach | 4:21I#$%!NDF/!
| Adrenals | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) | Other organs (list) |
| Frozen samples: Liver | Fat | Kidney | Brain | Other (list) | Liver | Fat | Kidney | Brain | Other (list) | Liver | Fat | Kidney | Brain | Other (list) | Liver | Fat | Kidney | Brain | Other (list) | Liver | Fat | Kidney | Brain | Other (list) | Liver | Fat | Kidney | Brain | Other (list) | Liver | Fat | Kidney | Brain | Other (list) |

<table>
<thead>
<tr>
<th>Disease Process 1</th>
<th>INC</th>
<th>Disease Process 2</th>
<th>INA</th>
</tr>
</thead>
<tbody>
<tr>
<td>System 1</td>
<td>SKI</td>
<td>System 2</td>
<td>LIV</td>
</tr>
<tr>
<td>General Cause 1</td>
<td>General Cause 2</td>
<td>Aetiology 1</td>
<td>Aetiology 2</td>
</tr>
<tr>
<td>Common Name 1</td>
<td>Pustule-vesicular, necrosuppurative dermatitis with intralesional fungal organisms and gram negative bacilli</td>
<td>Common Name 2</td>
<td>Multifocal, random hepatocellular necrosis and microabscessation</td>
</tr>
</tbody>
</table>

Nahid Stephens


2 As above.

3 As above.

4 As above.


6 Personal communication, Dr Padraig Duignan, University of Melbourne, 16/11/2009.
Date: 25-NOV-2009  
Enquiries: Dr Richmond Loh (Pathology Perth)

To:  
Clinical Pathology Labs  
Murdoch Uni Vet Hospital  
Murdoch  
WA 6150

Owner:  
Project: Animal disease diagnosis  
Species: Unspecified - Dolphin  
Samples Received: 1 x fresh  
Date Collected: 27-OCT-2009  Date Received: 28-OCT-2009  Submission Number: 18936

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**CLINICAL HISTORY**

A paraphrased account of emails from Simon Allen (Research Fellow, Murdoch University Cetacean Research Unit) and from Roxane Shadbolt (Swan River Trust) outlining the history of this dolphin that died on Sunday 25th October 2009 and of dolphin deaths in the recent past:

This is the 6th Swan River dolphin to die since April - representing ~25% of the dolphins that use the river regularly - and the second or third to do so in an emaciated state with severe skin lesions.
Other dolphins that had died:

**5 June 2009**
Deceased dolphin calf reported, 5 June, recovered 7 June near Chidley Point, no obvious injury or disease, delivered to Murdoch University for necropsy, “minimal findings”

**8 June 2009**
Deceased dolphin located in Mosman Bay, juvenile male, no obvious injury or disease, delivered to Murdoch for necropsy by Town of Mosman Park, results refers to Apergillus fungal infection, likely immuno suppressed.

**21 June 2009**
Deceased dolphin located near Belmont Ski area, female, fluke entangled with fishing line and ulcerated/abscessed/scarred, delivered to Murdoch University for necropsy, conclusion – septicaemia, abscesses throughout internal organs, probably linked to injury from fishing line and shark bite scars.

**17 September 2009**
Deceased dolphin recovered from foreshore between Claisebrook Cove and Windan Bridge, believe this dolphin was the subject of sightings of a sick dolphin 14 September, covered in skin lesions, delivered to Perth Zoo for necropsy and samples submitted to the Animal Health Laboratory (Department of Agriculture & Food), thought to be of Swan River population. This dolphin (AS-09-2912) was diagnosed with a fungal dermatitis (Zygomycetes probably *Cunninghamella* spp.).

**9 October 2009**
Deceased dolphin located in Freshwater Bay, male, very decomposed and in consultation with DEC and Hugh Finn, was not delivered for necropsy, teeth and blubber samples taken and collected by Hugh Finn.

**25 October 2009**
Deceased dolphin located near Applecross jetty, covered in skin lesions similar to 17 Sept dolphin, delivered for necropsy and awaiting results.
There were reports of a massive die-off of bottlenose dolphins off the east coast of the US in the late 1980’s following observations of an outbreak of fish with ulcerative lesion. “Epidemic skin ulcers have had a major impact on marine mammal populations along the eastern coast of the US. A massive die-off of Atlantic bottlenose dolphins (Tursiops truncatus) occurred from about May 1987 to March 1988. The dolphins had blisters caused by swelling and vacuolation of the epidermal epithelium. The dermis was not involved until later stages, when various opportunistic bacteria produced large necrotic ulcers and internal infections (Geraci, 1989). An ulcer epidemic in the estuarine fish populations along the US Atlantic coast preceded the dolphin epidemic (Ahrenholz et al., 1986; Dykstra et al., 1989; Levine et al., 1990). Survivors of the fish ulcer epidemic, such as the planktivorous Atlantic menhaden that migrate seasonally to the near-coastal sea, could have been prey for the coastal dolphin population. A major component of the toxic dinoflagellate's toxin is hydrophobic (Noga, Baden, Robinette & Fan, unpub. data) and thus the potential exists for storage in fatty tissues and subsequent transmission to dolphins via sublethally exposed fish. Possible tropic transfer of the dinoflagellate's toxins up the food chain should be examined.”

The Centre for Fish and Fisheries Research have recently found black bream affected with ulceration. However, these were diagnosed with epizootic ulcerative syndrome (EUS - also known as ‘red spot disease’ and ‘mycotic granulomatoses’), caused by Aphanomyces invadans. It can result in mass mortalities in many fish species and occurs in periods of low temperatures and after periods of heavy rainfall.
CYTOLOGY FINDINGS
Three slides of smears prepared from superficial skin scrapes and stained with Diff Quik. Large numbers of bacterial rods, cellular debris, sand and low numbers of structures resembling fungal hyphae.

HISTOLOGY FINDINGS
Skin: single section though a large abscesses. There is marked orthokeratotic hyperkeratosis with marked ballooning degeneration of the superficial layers of keratinocytes. The epithelial surface is colonised by mats of fungal hyphae interspersed with mixed bacteria and debris. The hyphal elements were variable in size and width, demonstrating non-parallel walls, irregular right-angled branching, and rare septations. Fungal hyphae are also seen in reduced numbers within the abscess as well as in the deep dermis. They stain poorly with PAS but are obvious using Gomori’s staining method. The abscess comprises of large numbers of heterophils, erythrocytes, proteinaceous oedema fluid and fibrin.

MORPHOLOGICAL DIAGNOSIS
Skin: suppurative dermatitis, severe deep, multifocal, acute with intralesional fungal hyphae and bacteria.

AETIOLOGICAL DIAGNOSIS
Mycotic dermatitis (see Mycology Results).

COMMENTS
PCR results for *Aphanomyces invadans* was negative. Periods of low temperatures and of heavy rainfall are epidemiological factors that increase the likelihood of EUS, however, fungal invasion is usually secondary to skin damage from low water pH (run off from acid-sulphate soils) and depressed host immune system (low water temperature). *Aphanomyces invadans* tends to invade damaged skin from the external environment rather than from ingestion and subsequent systemic spread.

There was discussion about whether this dolphin had suffered skin damage to predispose it to the fungal infection. There is a possibility that this dolphin might have swam through corrosive substances, low pH water, jellyfish, incurred thermal or other physical injury, creating portals for infection. Alternatively, the dolphin may have been immunocompromised.

*There was a previous case of dolphin with skin lesions that came through the AHL (AS-07-3710) that was necropsied by the Perth Zoo on 18 November 2007 (Perth zoo ID: M181164), target shaped skin lesions and a large haemorrhagic ulcerative area extending from the blow-hole dorsally along the length of its body recovered from the Swan River near Bicton. The histomorphological diagnosis in that case was dermatitis, multifocal, non purulent, chronic and epidermitis, necro-haemorrhagic, severe, acute, full depth, extensive. No definitive aetiological diagnosis could be made, however, contact allergies, toxins, lobomycosis (fungal dermatitis) and dolphin pox were discussed as potential causes.*
Bacteriology Results

<table>
<thead>
<tr>
<th>Spec ID</th>
<th>Spec Description</th>
<th>Routine fish bacterial culture</th>
<th>Listonella anguillarum</th>
<th>non-haemolytic Listonella anguillarum</th>
<th>Vibrio vulnificus</th>
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</thead>
<tbody>
<tr>
<td>09-1108 Skin</td>
<td>Moderate growth</td>
<td>Moderate growth</td>
<td>Moderate growth</td>
<td>Moderate growth</td>
<td></td>
</tr>
</tbody>
</table>

Mycology Results

Smear examination: Fungal growth very similar to hyphae seen in skin wet prep. Wide irregular hyphae with no septae or spore heads.

<table>
<thead>
<tr>
<th>Spec ID</th>
<th>Spec Description</th>
<th>Fungal sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>09-1108 Skin</td>
<td>Saprolegnia sp. &amp; candida</td>
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</tr>
</tbody>
</table>

Test Type: PCR for Aphanomyces invadans identification (NATA accreditation does not cover the performance of this service.)

<table>
<thead>
<tr>
<th>Spec ID</th>
<th>Spec Description</th>
<th>Aphanomyces invadans</th>
</tr>
</thead>
<tbody>
<tr>
<td>09-1108 Skin</td>
<td>negative</td>
<td></td>
</tr>
</tbody>
</table>

Yours faithfully

Dr Richmond Loh
VETERINARY PATHOLOGIST

Please note that only the fee for PCR testing is being charged.