Report of dolphin mortalities and pathology in Adelaide Dolphin Sanctuary 1987 - 2020

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Summary

This report describes necropsy and life history data for dolphins stranded or dead and floating in the Adelaide Dolphin Sanctuary (ADS) between June 1987 and December 2020. This includes a total of 93 records comprising two cetacean species, 74 (66 Indo Pacific bottlenose dolphin (*Tursiops aduncus*) and 8 common dolphin (*Delphinus delphis*)) were examined post-mortem at either the South Australian Museum (SAM), the University of Adelaide or the Australian Marine Wildlife Research and Rescue Organisation.

Objectives of the study were to determine the cause of death, if known; to assess the health of the animals prior to death (including skin lesions); to collect life history data (size, weight, age and reproductive status); and to provide data on pathogens that may cause disease in humans or domestic animals that have come into contact with marine mammals that have drifted ashore or stranded. A database framework has been established by the SAM, containing information on anthropogenic events, life history data and results of pathology investigations, and a system of categorising 'circumstance of death' established.

The circumstance of death for *Tursiops* was non-anthropogenic in 53.5% (n=35) of cases and anthropogenic (shooting, stabbing, and fishing gear entanglements) in17.5% (n=11), and Unknown circumstance made up reminder (30%). Non-anthropogenic cause included disease which was 40% (n= 26).

Regardless the circumstance of death, the most common disease was infection (32 out of 50), including pneumonia and systemic general infection. Pathogen involved in of infection were, viruses, bacteria, fungi and parasites. Pathologies were associated with 10 genera of bacteria and 3 genera of fungi, morbillivirus and toxoplasma and several helminths including lung nematodes and trematodes in the liver, and pancreas.

Seven types of gross skin lesions were confirmed and, in 60% of dolphins examined, they were associated with histopathology. Skeleton pathology was observed in 63% (n = 39) of

dolphins examined. Lytic lesions were most frequently observed, flowed by trauma/ fractures, degenerative and malformations.

Data collected over the last 30 years will provide a baseline for further research and management of the Sanctuary.

Introduction

Over the past several decades there has been a great deal of interest in the conservation of cetaceans worldwide. This has led to a gradual increase in research into their pathology of in the wild. However, as many countries are limited by laws concerning the killing of cetaceans, scientists have studied decomposing or stranded animals. As stranded animals are often diseased, necropsies can be performed to explain a variety of pathologies.

Long term studies of the pathology of stranded small cetaceans have been published from the USA (Stroud and Roffe 1979, Bossart et al. 2017, 2019), Hong Kong (Parsons and Jefferson 2000), Belgium and France (Jauniaux et al., 2002), New Zealand (Duignan 2003, Stockin et al., 2009), Holland (Camphuysen et al.,2007), Adriatic Sea, Italy (Mazzariol et al. 2007), Spain (Arbelo et al. 2013, Díaz-Delgado et al. 2018), Brazil (Domiciano et al. 2016). Van Bressem et al. (2009) reviewed infectious diseases worldwide. In Australia, there have been studies on cetaceans from Tasmania (McManus et al. 1984) and from South Australia (Kemper et al. 2005). In addition, a series of reports have been written by the SA Museum to the Natural Resource Management Board that include dolphin mortalities in the ADS.

Studies on cetacean strandings and related events in South Australia have included analyses of 660 events from 1881 to 2000 and have focused on 361 necropsy records from 1985 to 2000. The study showed that events involving common dolphins (*Delphinus delphis*) and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) were classified as 25% anthropogenic, 20% non-anthropogenic and 5% were deliberate killings, between 1985 and 2000. The latter included 5% shootings or stabbings, some within the current ADS area (Kemper et al. 2005).

The Adelaide Dolphin Sanctuary was established in 2005 with the aim of protecting the *Tursiops* population inhabiting the area. Because this area is within the metropolitan area, the dolphins are influenced by the urban environment. The establishment of this protected area may have reduced anthropogenic mortality e.g. vessel collision/ propellor wounds, shooting, and entanglement with fishing gear (Adamczak et al., 2018).

ADS dolphins are regularly observed by staff of Department of Environment and Water and by Whale and Dolphin Conservation and associated volunteers. In addition, they identify individuals by the characteristics of their dorsal fins and record and report on their body and skin condition when they appeared unusual.

In the last few years, it has been suggested that there is a high mortality rate of young pre-weaned dolphins in the ADS. During 2020 and 2021, there appeared to be an increase in dolphins rapidly becoming emaciated and dying.

The aim of this report is to describe and analyse necropsy reports and data collected by the South Australian Museum and others in order to inform the issues listed above and possible future research and conservation. In the report, we analyse threats to dolphins through investigation of past mortality events and put this into context with conservation and management.

Materials and Method

Summary of number of animals examined

A total of 93 strandings were analysed, 74 of which were retrieved (*Tursiops aduncus* =66, *Delphinus delphis* =8) and of those, 63 (*T. aduncus* = 58, *D. delphis* = 5) included underwent a full or part necropsy.

Records were obtained opportunistically from a variety of sources, including government and the community. Records (both collected and non-collected carcasses) were databased by the South Australian Museum (SAM). Test items and number of animals tested was summarised in Table 1.

Table 1. Topics examined and number of animals tested for each dolphin species

	Number of dolphins examined		
	Tursiops aduncus	*Delphinus delphis*	total
Mortality	86	7	93
Life history	66		66

Body condition	52		52
Circumstance of death	66	8	74
Gross pathology	65		65
Histopathology	51		51
Bacteriology	32		32
Parasitology	65		65
Virology	10		10
Skin lesions	60		60
Skeleton pathology	48		48

The data collected for each record were as detailed as possible so that both core data (e.g. location, dates observed or collected) and necropsy information allowed further analyses. Example datasheets and explanations for fields are found in Appendix 1.
32 (29 *T.aduncus*, 3 *D.delphis*) dolphins were examined fresh (i.e. not frozen) and these provided more comprehensive pathology data, other carcasses were frozen before post mortem examination.

A state of decomposition code was assigned to each dolphins examined according to Geraci and Lounsbury (2005).

Statistical analyse were not conducted because sample size was small.

Life history

A relative age category was assigned to all carcasses (Appendix 2). In the case of cetaceans, this used a combination of skeletal development (amount of vertebral epiphysis fusion examined in the prepared skeleton), body size, external features (to distinguish neonates from calves) and reproductive maturity. The last was based for the most part on gross appearance of the organs (e.g. mammary glands producing milk, foetus present, ovaries active, testes large), although in a few cases male maturity was assessed by histological examination of the testes.

Many of the *Tursiops aduncus* were aged (in years) by examining tooth structure (Growth Layer Groups) using decalcified, stained, thin-sections. In some cases, these estimates were compared with known-age information obtained during studies of live, free ranging ADS dolphins (Mike Bossley, pers. comm.).

Circumstance of death

The SAM has developed a set of criteria for assigning a circumstance of death category to each record or mortality for marine mammals (Appendix 3). They include evidence gathered at the time of observation in the field, at necropsy and from pathology investigations carried out post-necropsy. It is worth noting that the circumstance of death is not always the primary cause of death. For example, some dolphins had shotgun pellets in their carcasses but died days or perhaps weeks after they were shot. Instead, the primary cause of death was infection in an organ in which pellets had lodged.

Pathology

Prior to 2005, sporadic effort was made in collecting pathology data and tissues. This was because the necropsy programme did not always have a pathology researcher assisting with the study of marine mammal carcasses collected by the SAM, since 2005 Ikuko Tomo (IT) has routinely carried out necropsies on most of the marine mammal carcasses collected for the SAM since 2005.

External examination

Body condition was assigned to 52 *Tursiops aduncus* based on notes on the external appearance of the body were made at necropsy and photos at necropsy, in the case of carcasses collected prior to 2014. In some cases, additional information was sourced from photos of animals before they were made available to the Museum.

Four features were examined (Kemper et al. 2016) and recorded as degree of presence (or absence) of 1) a dorsal concavity behind the head, 2) a concavity along the lateral body (indicative of loss of epaxial muscle mass), 3) convexities along the lateral peduncle (indicating loss of tissue over the transverse processes of the vertebrae) and 4) convexities in the thorax (indicating loss of tissue over the ribs). Since number 4 was rarely observed, in this report it was excluded from the analysis. Each feature was rated 0 (absent), 1 (visible, but not extreme condition) and 2 (clearly visible/extreme condition). Each dolphin was assigned a condition category using the following methodology. In some cases, it was not possible to

evaluate all three features because the data were not available. Scores were totalled (maximum 6) and expressed as a proportion of the total available: 0 = robust, 0.17 and 0.33 = slightly emaciated and ≥0.5 = moderately/very emaciated. Neonates (less than about 2 months old) were excluded from the analysis because most of them were decomposed and an emaciation score may have been irrelevant at such a young age.

Before incision, carcasses were examined for lesions and marks, including on the skin, in the eyes, mouth, blowhole, genital slit and anus. If discharges such as mucous and/or pus were observed, swabs were taken for bacterial testing (if carcass had not been frozen). The skin, eyes and other orifices were examined for external parasites.

Internal examination

We followed standard necropsy protocols, and collected histology samples if they were fresh enough to evaluate pathological changes (i.e. for carcasses assigned to Geraci code 2 and 3). Subdermal muscles and facia were examined for haemorrhaging and the position, size and severity were recorded. The subcutaneous tissue was also examined for any other unusual findings, including parasites. The posterior part of the brain and the most anterior part of the spinal cord were examined through the foramen magnum after the skull was separated from the spine. The South Australian Museum prioritised preserving the skulls its complete form, so whole brains were taken from two dolphins during 2013 and one in 2018.

Histopathology

The following tissues were routinely collected from fresh carcasses: kidney, liver, lung, heart , adrenals, mesenteric lymph node, spleen and spinal cord. Additional samples were taken when required, including the normal tissue adjacent to the lesion. Formalin fixed tissues were trimmed to a thickness of 1 cm and put into labelled histology cassette before transport to the histology laboratory (Forensic Science South Australia before 2010 and Gribbles Pathology after 2010) where they underwent routine preparation (6 µm thickness) and haematoxylin and eosin staining. If further investigation was required, special staining was performed (i.e. Periodic Acid Schiff (PAS), Gram and Ziehl-Nelson).

Parasitology

Parasites were removed and preserved in 10% formalin to allow morphological identification by the Helminthology Section, SAM. When multiple parasites of were located, these were

preserved in 70% alcohol to allow future genetic identification. In some cases parasites were found during histological examinations.

To confirm toxoplasma infection, 24 dolphins' blood serum antibody titres were measured under the collaboration of a student research project of Veterinary School, University of Adelaide under the supervision of Assoc. Prof. Ryan O'Handley. These data are the property of Ryan O'Handley and the University of Adelaide.

Bacteriology

Since autolysed carcasses often contain contaminating bacteria, samples were only taken from those that were relatively fresh (Geraci codes 2 and 3). Samples for bacterial testing were taken opportunistically with sterile transport swabs (Copan Innovation Ltd.) where there were lesions and any suspicious areas like the blowhole of cetaceans. The samples were taken before or during necropsy then sent to a commercial pathology laboratory (Gribbles Pathology) where culture and sensitivity tests were performed.

Virology

For morbillivirus testing, fresh-frozen, formalin preserved and paraffin-embedded tissue samples of lymphoid tissues (spleen and/or lymph nodes), lung and central nervous system tissues were sent to and tested at the Australia Animal Health Laboratory (AAHL), where the rt-PCR test and Immunohistochemistry (IHC) tests were performed. Five *Tursiops aduncus* were tested during Unusual Mortality Event (UME) in 2013, after the UME, two *Tursiops aduncus* that had lesions consistent with morbillivirus were tested.

Skin lesions

Skin lesions of 66 dolphins were observed at necropsy, some of which had follow-up histology. Of these, the lesions of 37 dolphins were considered suitable for evaluation. Carcasses were examined for skin lesions before incision and the lesions were identified as to whether they occurred pre- or post-mortem for the dolphins examined after 2005. The location, outline, size, depth and severity of any pre-mortem lesions were recorded on a diagram and images were taken. The lesions were categorized according to a skin lesion catalogue (Appendix 4) created in 2010 and updated each year.

Skeleton pathology

A total of 48 skeletons were examined. After gross necropsy examination was completed, carcasses were flensed and the skeletons macerated in warm water (30–35 º C) for 3–5 months, after which they were scrubbed in soapy water to remove any remaining tissue and the adipocere.

Skulls and skeletons were examined to determine the type of pathology and the location of each case on the skeleton. Representative photographs were taken. Pathologies were classified into four types following Tomo et al (2010),

1. Lytic lesions included osteomyelitis, spondylitis, osteolysis, periodontal lysis and crassicaudiasis. Osteomyelitis, spondylitis and osteolysis were observed in vertebrae, ribs, scapulae and bones of the flipper.
2. Degenerative lesions included hyperostosis, osteophytes and spondylosis. They were generally smooth in appearance. Hyperostosis and osteophytes were found in older dolphins at the periphery of vertebral bodies.
3. Fractures included both healed and unhealed lesions. Normally the healed fractures formed a callus that protruded from the surface of the bone. Some healed fractures were aligned.
4. Malformations were the result of congenital disruption of skeleton morphology.

Results

General

Fig. 1 summarises ADS dolphin mortalities between 1987 and 2020. Fig. 2 summarises the sex of ADS *Tursiops aduncus* during the same period.

Mortality of dolphins during this study period appeared to increase after 2000. Since 2009, when NRM commenced support for the project of monitoring mortality of cetaceans in Gulf St Vincent, the increase may also have been a result of more efforts to collect information on stranded animals. An Unusual Mortality Event occurred in 2013 and this may have elevated mortalities between 2011 and 2015 (Kemper et al. 2016).

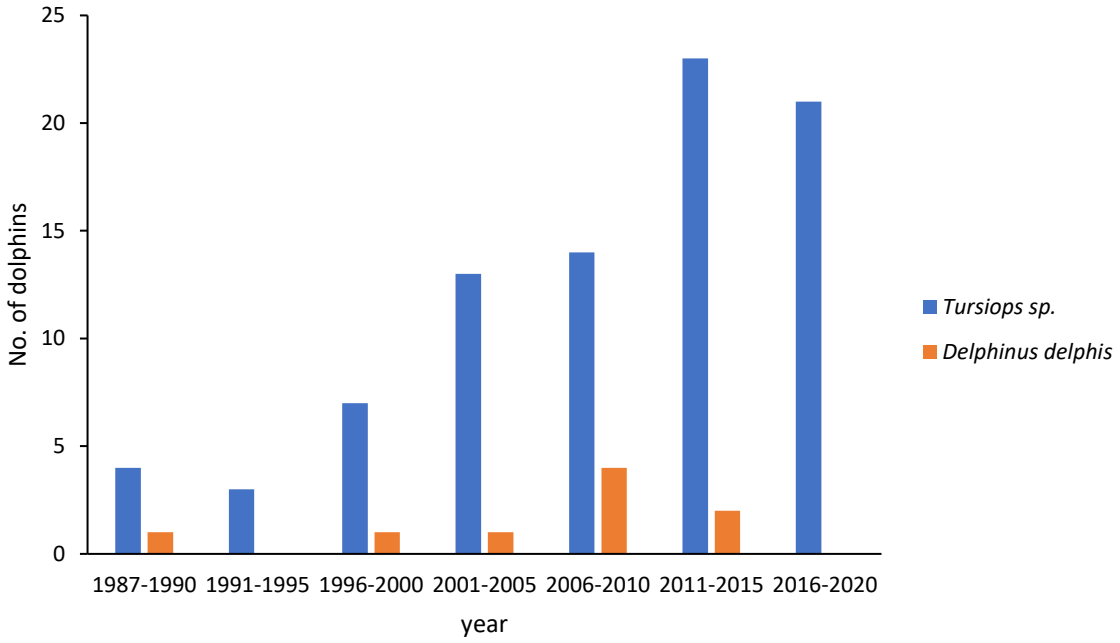


Fig. 1: Number of *Tursiops* sp. (mostly *T. aduncus*) and *Delphinus delphis* mortalities recorded in the ADS between 1987 and 2020 in 5- year intervals.

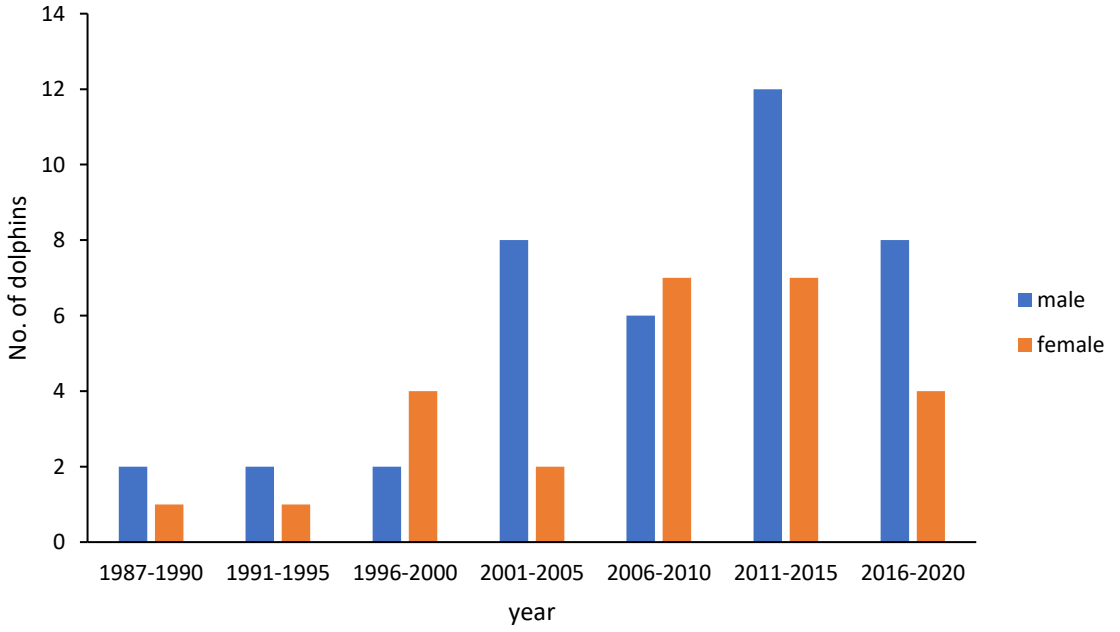


Fig. 2: Number of *Tursiops aduncus* of each sex recorded in ADS between 1987 and 2020 in 5- year intervals.

The number of dolphins in each body condition category was summarised for 5-year periods, as well as 2021 (Fig.3). All time periods recorded moderately or very emaciated dolphins but the proportion appears to have been greater since 2005. The assumption is that the data after 2005 are the most reliable because observer effort was likely to have been consistent after the

creation of the ADS. One of the limitations of this study is that it does not take into account the dolphins that disappeared (died or emigrated) from the ADS.

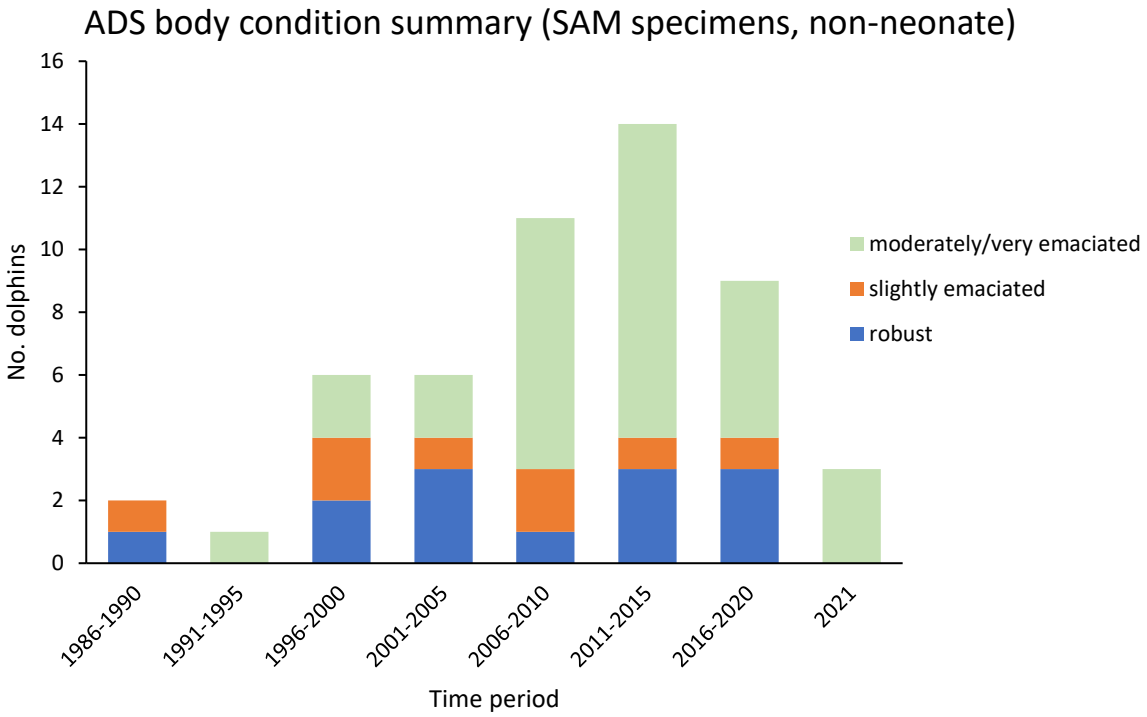


Fig. 3. Summary of *T. aduncus* evaluated for body condition from the ADS using specimens collected for SAM and excluding neonates.

Circumstance of death

A total of 74 dolphins were assigned to a circumstance of death category and these are summarised in Table 2. Fig. 4 shows prevalence of *Tursiops* circumstance of death.

Table 2: Circumstance of death of ADS dolphins.

	Tursiops aduncus	*Delphinus delphis*
Disease	26	4
Intentional Killing	3	
Known Entanglement	2	
Live Stranded	3	
Other Natural	6	
Other Unintentional	6	1
Unknown	20	3

Total	66	8

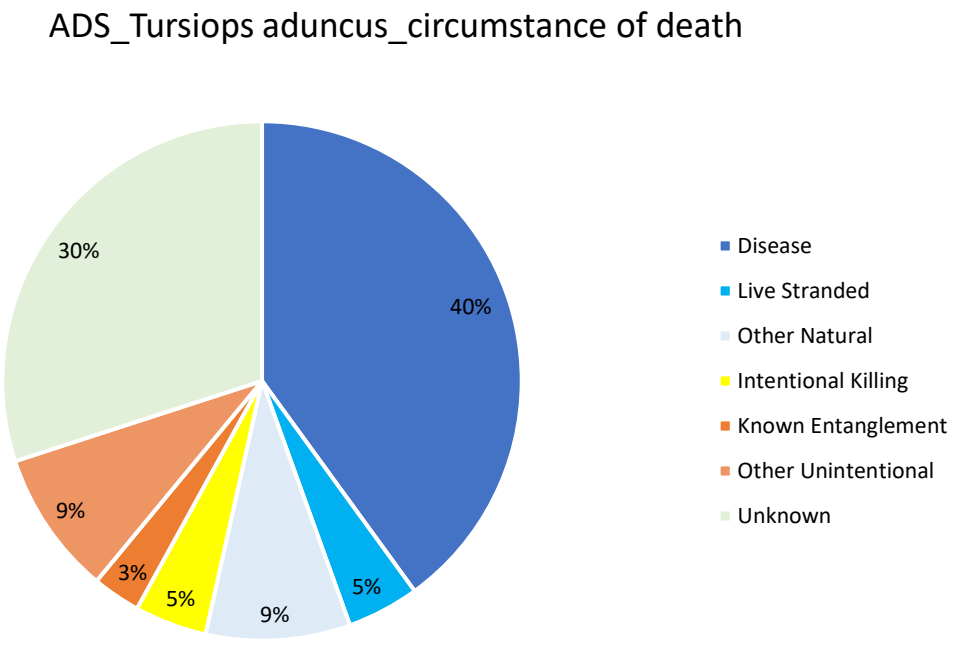


Fig. 4. Proportional representation of the circumstance of death of ADS for *Turisops aduncus* between 1987 and 2020

Turisops aduncus is the predominant species in the ADS. Therefore, analysis focused on the pathology of *Tursiops aduncus*.

Pathology

In total, necropsy records were obtained for 66 *Tursiops aduncus*. Of these 35 included a series of histology samples that were examined. Sixteen cases had histological findings for several organs. However, seven of the 66 dolphins had very limited records because of advanced decomposition or because they were collected prior to systemic pathology examination.

- *Disease as circumstance of death*

The distribution of the number of *Tursiops* that died as a result of disease (n = 26) during 5 year periods (Fig. 5) showed a peak in 2011–2015. The highest number was observed in 2013 (n = 5) during the Unusual Mortality Event associated with morbillivirus infection.

Throughout the study period, the most frequently recorded was infectious disease which includes pneumonia (n = 8) (Fig. 6), systemic infections (n = 7), other infections (n = 4).

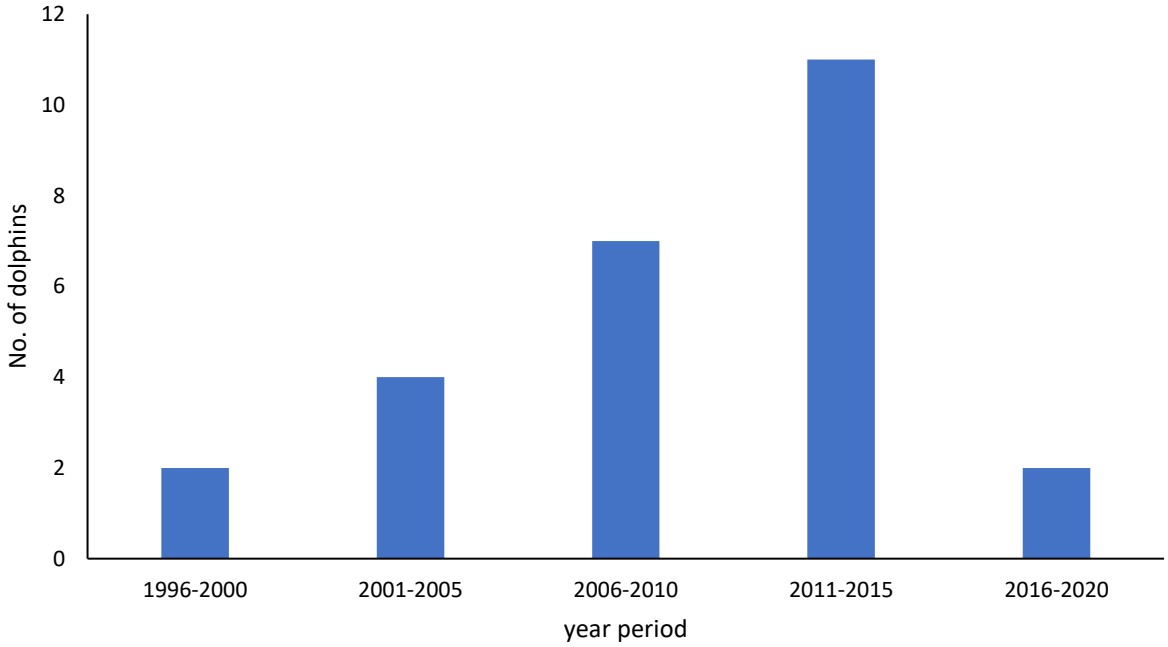


Fig. 5. Number of *Tursiops aduncus* with circumstance of death categorised as "disease", for every 5- year periods. No histology sampling prior to 1996.

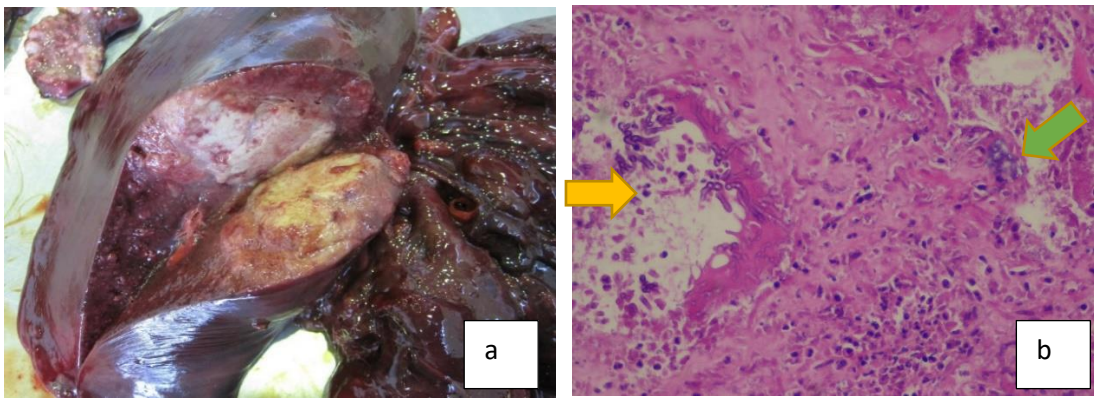


Fig. 6 a, b. *Tursiops aduncus* (M 26013,13.028), a, large nodular caseous necrosis on the lung (no detection of anti-acid bacteria) b, its lungs had focal infectious nodules with probable bacterial (green arrow) and fungal (yellow arrow) infection. It was collected within the first peak of the morbillivirus event in 2013.

- *Other circumstances as circumstance of death*

  Table 3 summarises the list of pathology of dolphins categorised as other than "disease" under the circumstance of death.

Some dolphins were diseased but were not categorised as such for circumstance of death. This was because the condition was not considered to be a major cause of death.

Table 3: Pathology of *Tursiops aduncus* in which the circumstance of death was categorised as other than disease.

Circumstance of death	Pathology	Year
Known entanglement	lymphadenitis, abscess the muscle, pneumonia	1987
Intentional killing	pneumonia	1998
Other unintentional	pneumonia, peritonitis	2000
Other natural	pneumonia. small abscesses on tongue.	1998
	pneumonias	2003
Live stranded	general systemic infection	2007
	general systemic infection (dermatitis, pneumonia)	2009
Unknown	pneumonia	2011
	pneumonia, otitis	2015

- *Histopathology*

Decomposition status was classified as Geraci code 2 (i.e. very fresh) in 55% (n=37)) *T. aduncus*. Histopathological specimens of many organs were in good enough condition to evaluate in 35 cases. In an additional 15 *T. aduncus* a less complete was assessable.

1. *Infectious disease*

Table 4 summarises the numbers of *T. aduncus* with different types of infectious disease during 5-year periods. This included all circumstances of death. A mild sign of infection was not included as significant infection. Of those dolphins found to be infected, 80% (n = 40) were in poor body condition (slightly, moderately/severely emaciated).

Table 4: Numbers of *T. aduncus* from the ADS examined that had moderate or severe infections.

	Pneumonia	Sepsis (general systemic infection)	Other significant infection	No significant infection	Total examined
1986-1990	1	0	0	1	2

1991-1995	0	0	1	0	1
1996-2000	3	2	0	1	6
2001-2005	1	1	3	5	10
2006-2010	3	4	0	5	12
2011-2015	6	3	1	4	14
2016-2020	1	1	1	2	5
Total	14	10	6	20	50

2. *Non-infectious disease*

During the study period, the pathologies observed, apart from infection, were as follows

- fatty liver associated with malnutrition (n=9),
- circulation disorder with cardiomyopathy (n= 3),
- yellow-coloured skin disease (n=1)

3. *Lymphoid depletion*

A total of 41 dolphins were sampled and examined for lymphoid tissues. Of these, 26 were appropriate for evaluation and detection of evidence of lymphocyte depletion. Table 5 summarises their level of severity of lymphoid depletion.

In the more than half of the cases, lymphoid depletion was not observed. No trend could be identified in the level of severity in the lymphoid nodes. A severe case observed in 2013 was clearly due to infection with morbillivirus, which targets the lymphoid organs to create necrosis in the lymphoid follicles (Fig. 7 a, b)

Table 5: Lymphoid depletion and its severity among *Tursiops aduncus* in the ADS.

	No. of dolphins
Mild	7
Moderate	1
Severe	1
No visible signs	17

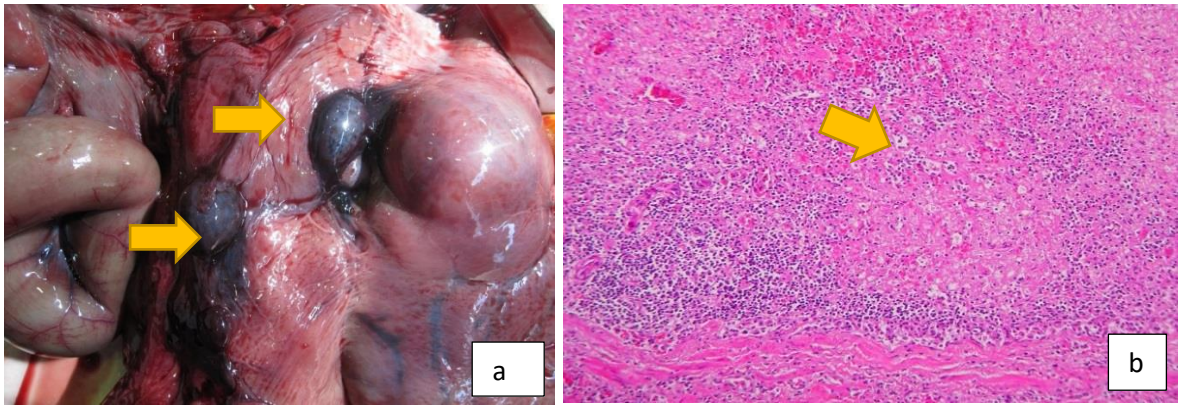


Fig 7 a, b. *Tursiops aduncus* (M 26206,13.020) with swollen gastric lymph nodes (a, arrows) with necrosis at the centre of lymph follicle (b, arrow). It was collected within the first peak of the morbillivirus event in 2013

- *Death of young dolphins*

SAM received 28 carcasses of young dolphins (neonates = 17, calves = 11) during 1987–2020 and an additional three dead neonates were reported but not collected. The number of mortalities in young animals appeared to have been gradually increasing since 2005 (Fig. 8).

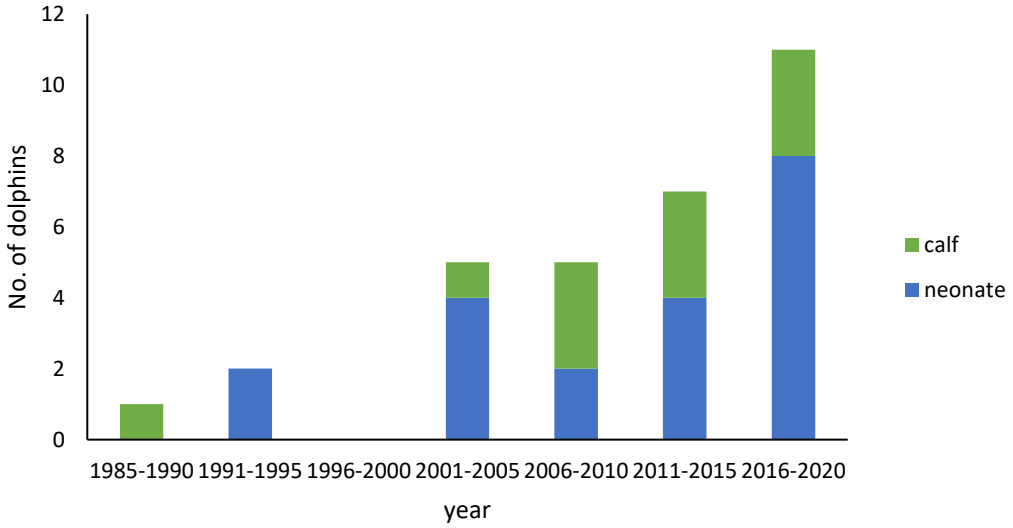


Fig. 8: Mortalities of *Tursiops* sp. neonates and calves during 5-year periods

Histology examination was conducted on 4 neonates and 7 calves. Disease was found in 3 neonates and 7 calves. All three neonates had pneumonia, whose etiology was parasites (necro-suppurative pneumonia), bacterial (necro-suppurative bronchopneumonia) and meconium aspiration (neutrophil infiltration in alveolar with meconium debris). The 6 calves also had general systemic infections (two were killed by trauma) and 1 fungal myocarditis.

Between 2016 and 2020, all recovered neonatal carcasses (n=8) were male. However, there were three unrecovered neonatal bodies during 2018 and 2019, so this could include females in this event.

Neonates stranded during 2016–2020 (n = 5) showed severe blunt traumatic injury (n = 1) and severe intra-abdominal bleeding with unknown etiology (n = 1) and the causes of death in another three were unknown due to advanced autolysis.

Bacteria and fungi

A total of 34 bacterial species from 20 genera, as well as 3 genera of fungi, were detected through culture and sensitivity tests of specimens taken from the carcasses of ADS dolphins. Table 6 lists those associated with pathology that could be related to infection, together with the origin of the specimen, year of collection and pathological diagnosis.

Bacteria that are not normally pathogenic can become pathogenic as opportunistic infectious bacteria when the dolphin's health is compromised.

Many of the bacteria associated with pathology were only recorded in a limited period, but *Clostridium* spp., *Enterococcus* sp., *Escherichia coli*, *Staphylococcus aureus* and *Vibrio* sp. were present during several years, although not with great frequency.

Table 6: Bacteria and fungi detected on *Tursiops aduncus* from the ADS with suspected infectious lesions

Bacterial type	**Organ**	**Years**	**Probable bacteria associated pathology**
Aeromonus spp.	Skin (abscess)	2015	dermatitis
	blowhole	2015	pneumonia and bronchiolitis
Clostridium perfringens	blowhole	2011, 2013	bronchopneumonia
Clostridium sp.	blowhole	2013	dermatitis
Corynebacterium renale	lymph nodes	1987	lymphadenitis, sinus catarrh
Corynebacterium ulcerans	many organs (no clear description)	1996	bronchopneumonia
Enterococcus sp.	blowhole	2010, 2011	bronchiolitis
	skin	2010	dermatitis

Escherichia coli	blowhole	2011	bronchiolitis
Listonella sp.	blowhole	2013	pneumonia
Proteus mirabilis	skin	2010	pyogenic dermatitis
	oral cavity	2011	hyper keratinisation
Pseudomonas aerouginosa	blowhole	2011	bronchiolitis
Shewanella (Pseudomonas) putrefaciens	lung	2007	pyogenic pneumonia
Staphylococcus aureus	abscess in lung	1999	pneumonia
	kidney	2009	nephritis
	larynx	2009	sepsis
Staphylococci coagulase negative	blowhole	2007	pneumonia
Vibrio alginolyticus	blowhole	2005	Pneumonia (parasitic)
	skin	2011	necrotic dermatitis
Vibrio harveyi	skin	2015	necrotic dermatitis
Vibrio parahaemolyticus	skin	2010	necrotic dermatitis
Vibrio rotiferianus	skin	2015	necrotic dermatitis
Vibrio sp.	blowhole	2015	bronchiolitis

Fungi

fungus	**organ**	**years**	**disease**
Candida albicans	blowhole	2009	Systemic inflammation
yeast	blowhole	2005	pneumonia (parasitic)
Aspergillus fumigatus	blowhole	2013	bronchopneumonia

Parasitology

- *Helminths*

During the study period lung nematodes and trematodes were recorded, including. lung nematodes in nine *T. aduncus*. Trematodes were found in the liver and pancreas of three animals and in one, trematodes caused a severe inflammatory reaction.

- *Toxoplasma*

Protozoan infection was confirmed by gross and histopathology examination with encephalitis (brain) on one *T. aduncus*. It was identified as *Toxoplasma gondii* by immunohistochemistry staining.

Blood serum was collected from 30 ADS dolphins, between 1993 and 2018. Figure 9 summarises the results of the tests for toxoplasma antibody.

In the ADS region, the number of dolphins having negative results was small. The presence of antibodies, as well as the level of titres, are not directly related to the clinical sign of the disease. However, nine dolphins were recorded with high antibody titres (>3200, 1996,1998 (n = 2), 2001, 2003, 2005, 2007, 2008, and 2011) and for seven of these individuals the circumstance of death was categorised as “disease”. Histopathological confirmation of Toxoplasma infection-related encephalitis was found in one case in 2013.

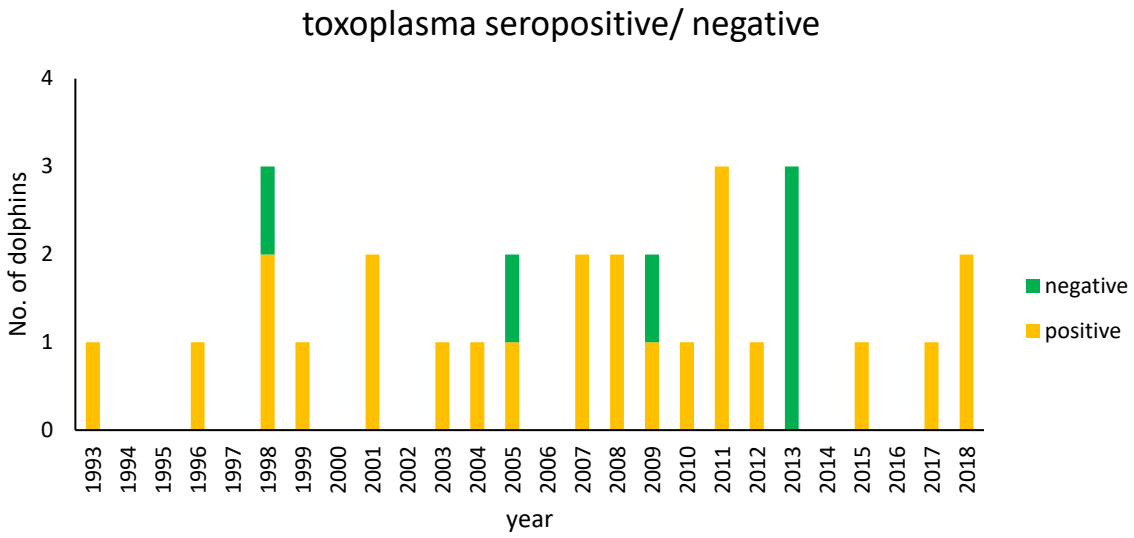


Fig.9. Results of *Tursiops aduncus* toxoplasma serology tests between 1993 and 2018.

Viruses

Dolphins were examined for morbillivirus using rt PCR tests and immunohistochemistry during the 2013 Unusual Mortality Event. Three dolphins were positive using both diagnostic tests, and 5 were positive for only the PCR test. More recently, suspected cases were tested for morbillivirus in 2018 (n = 1) and 2019 (n = 1), both of which were negative.

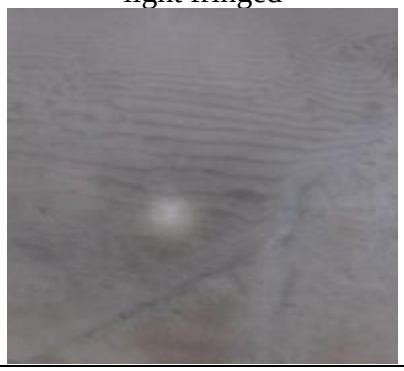
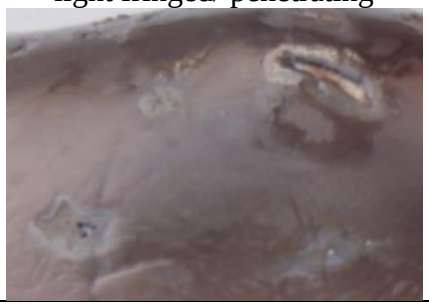
Skin lesions

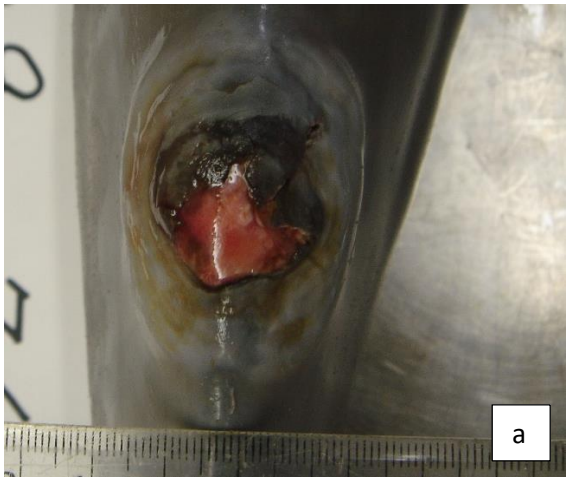
Ten dolphins that were not collected for post-mortem were found to have skin lesions by examining photographs and other information. A further 15 dolphins were found to have skin lesions at necropsy and histology examination (Table 7, Fig. 10a, b). Multiple skin lesions were observed on some animals. All types of lesions ranged from mild to severe and from acute to chronic stages. The circumstance of death for 60% of the dolphins (n = 15) exhibiting these skin lesions was “disease”. Two dolphins, which had been shot with a

shotgun survived for a while and showed skin lesions of excoriation/abrasion at the wound area created by the shotgun pellet.

Table 7: Characteristics and positions of significant skin lesions observed on ADS *Turisops* that were examined at necropsy

Type of skin lesion	**Years**	**Body region**	**Histopathology**
excoriation/ abrasion 	2009	peduncle	necro suppurative dermatitis
	2011	mandible and ventral body	
	2015	tail flukes and genital slit region	
	2015	peduncle	
	2017	peduncle	
penetrating, amputation 	2010	peduncle	necro suppurative dermatitis
penetrating 	2011	between umbilicus and genital slit	necro suppurative dermatitis
dark fringed and penetrating 	2011	trunk near dorsal fin	Focal superficial stratum corneum defect, very mild dermatitis
	2013	ventral trunk	

light fringed 	2014	dorsal trunk near dorsal fin	Focal superficial stratum corneum degeneration, very mild dermatitis
	2015	trunk near flipper	
	2015	tail flukes and near genital slit and lateral peduncle	
light fringed/ penetrating 	2014	head	necro suppurative dermatitis
	2018	head	
	2018	from head to neck	
Blisters 	2014	dorsal fin	not examined
	2015	dorsal fin	



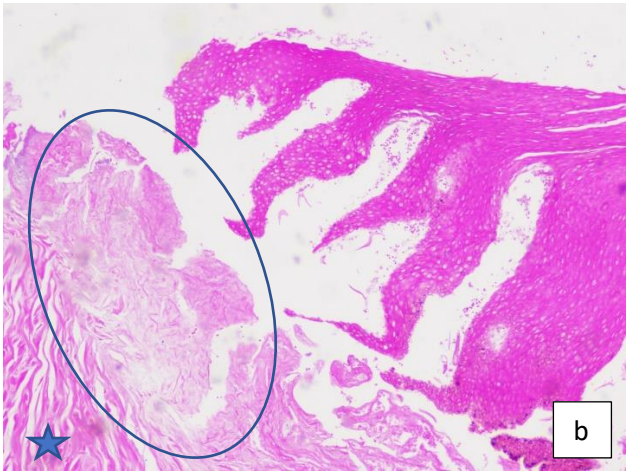


Fig. 10 a, b: *Tursiops aduncus* (M 24903, Chelsie) Skin lesion on the dorsal posterior body. a, Excoriations/ Abrasions on dorsal peduncle. b, detail of skin lesion showing incomplete epidermis,the upper layers of the dermis are necrotic (circle). Underneath, reorganised connective tissue layer(star).

Skeleton pathology

Of 61 dolphin skeletons that were examined, 63% (n = 39) showed skeleton pathology. Lytic lesions were the most common (n = 28), followed by trauma and fractures (n = 25), degeneration (n = 14) and malformations (n = 5). 22 dolphins had multiple skeleton pathologies. Of the neonate skeletons (n = 13), one case with a wound caused by a propeller showed a fracture, but the rest showed no skeleton pathology. Subadult dolphins (n = 2) showed no skeleton pathology.

Fig. 11 shows the prevalence of three types (lytic lesion, degenerative lesion and trauma/fracture) of skeleton pathology. Prevalence was calculated as the number of dolphins with pathology against the number of dolphins examined. As seen in previous studies in South Australia (Tomo et al. 2018), the prevalence of *T. aduncus* in ADS with skeleton pathology increased with relative age, and dolphins with multiple skeleton lesions also increased. Similarly, when the type of pathology was compared with age category, lytic lesions and degenerative lesions increased. The correlation between fractures and age did not appear to increase. This is in contrast to the results for *Turisops* spp. stranded in Gulf St Vincent whereby fractures increased with age (Tomo et al. 2018).

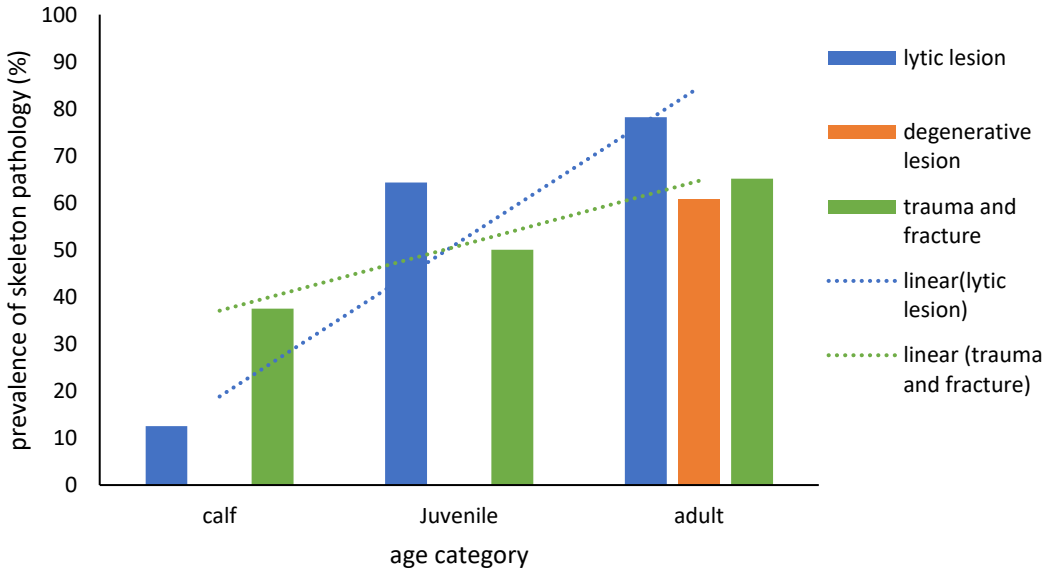


Fig.11. Prevalence of three types of skeleton pathology in *Tursiops aduncus* with age in the ADS.

Discussion

General

It is important to closely monitor cetacean mortalities and trends in the different world areas. Summaries of cetacean pathology are available from many world regions (Stroud and Roffe 1979, Parsons and Jefferson 2000, Jauniaux et al., 2002, Duignan 2003, Camphuysen et al.,2007, Mazzariol et al. 2007, Arbelo et al. 2013, Díaz-Delgado et al. 2018, Domiciano et al. 2016, Bossart et al. 2017, 2019).

In Australia, studies have been conducted on mortality in Tasmanian cetaceans (McManus et al., 1984) and South Australian cetaceans (Kemper et al., 2005) with good size of sample. ADS dolphins in a further study, a detailed analysis of the causes of death of ADS dolphins was carried out for a total of 57 events 53 animals from 1987 to 2013 (Adamczak et al. 2018).

The number of dolphin deaths in the ADS appeared to increase between 2011 and 2020. This may be related to the increase in the number of deaths due to morbillivirus infection associated UME in 2013 and the rise in neonatal and calf deaths seen in 2018 and 2019.

We find the general assessment of body condition to be useful in distinguishing between animals that died of chronic health problems and those that died of acute causes. (Read and Murray 2000).

Circumstance of death

- Anthropogenic causes

Studies of dolphin mortalities reported from around the world show that anthropogenic causes of death include vessel collision, entanglement in fishing gear, gunshot wounds and foreign bodies in the digestive tract (Arbelo et al. 2013, Diaz-Delgado et al. 2018, Bossert et al. 2003).

In SA, there as a relatively high mortality of cetaceans classified as anthropogenic (Kemper et al. 2005). In the ADS study, fishing gear entanglement was mostly recreational fishhooks and lines (Byard et al. 2020) and not the net entanglements referred to in Kemper et al. (2005). Large-scale fishing is not permitted in the ADS.

During the present study of ADS dolphins, there were 12 anthropogenic deaths (11 *T. aduncus*, 1 *D.delphis*), both intentional and unintentional. The main anthropogenic cause in

the ADS was vessel collisions, with a total of six. Since the ADS was established in 2005, shootings and fishing gear entanglements were not observed between then and 2013 (Adamczak et al. 2018). Two shotgun shootings were observed in 2013 and 2014 and one fishing gear entanglement was observed in 2019. Dolphins are not always killed instantly by shooting, and their wounds may be difficult to identify if time has passed, since the superficial lesions could have healed (Read and Murray 2000). In all three cases in ADS (2 cases in 1998, on case in 2014), pellets were found in the carcass, with two cases of systemic infection, and one case of severe autolysis for which no diagnosis was available. Both two dolphins with infections showed signs of emaciation, which suggests that they were suffering chronic conditions.

- Non- anthropogenic causes

In general, stranded small cetacean mortalities in this category include disease, live stranding neonatal death, mother-calf separation, malnutrition, and predation (Arbelo et al. 2013, Díaz-Delgado et al. 2018, Kemper et al. 2005). During the ADS study, the most frequently observed non-anthropogenic circumstance was disease. However, many mortalities were classified as 'unknown'.

Pathology

Studies of cetacean mortalities reported from around the world show that infectious disease, especially pneumonia, was the most common pathology among stranded small cetaceans (Mazzariol et al. 2007, Arbelo et al. 2013, Domiciano et al. 2016). The etiology of the pneumonia varies, and it was sometimes related to geographic factors. As is the case in other parts of the world, ADS dolphins were most frequently found to have pneumonia at the time of death. The causes of pneumonia can be bacterial, fungal or parasitic. A helminthic pneumonia epidemic was seen in SA dolphins during 2005 and 2006 when many young common and bottlenose dolphins were recorded in SA (*Tursiops* spp.) (Tomo et al. 2010). Three species of lung nematodes, *Halocercus lagenorhynchi, Stenurus ovatus* and *Pharurus alatus,* were identified in SA in *Tursiops* spp. in that study. During this study, all seven dolphins infected with lung nematodes were younger than their juveniles in their age category, and five of them had significant suppurative pneumonia or systemic infection.

Suppurative broncho-pneumonia with morbillivirus infection has been reported in dolphins lungs (Di Guardo et al. 2013, Stephens et al. 2014). Morbilliviruses can also cause immunosuppression and can lead to opportunistic bacterial infections in dolphins (Duignan et al.1996, Raga et al. 2008, Stephens et al. 2014).

The acute or subacute suppurative bronchial pneumonia seen in *Tursiops aduncus* during the UME was associated with a morbillivirus infection, as well as systematic infection (Kemper et al. 2016). The same pattern was observed in the ADS dolphin study.

Although evidence of significant infectious disease declined from 2016, mild infection was observed in the lung, kidney, air sinuses and lymph nodes. Lymphatic organ damage can lead to systemic immune compromise, and it has been reported that exposure to environmental pollution and toxins can also lead to reduced immunity and endocrine disruption (Sonne et al. 2020). Lymphoid depletion was often reported in dolphins with morbillivirus infection in the lymph organs (Raga et al. 2008). A case of lymphoid depletion in a morbillivirus-infected case was found in an ADS dolphin.

- *Neonatal deaths in recent years (2018 – 2019)*

  Cetaceans' neonatal/perinatal pathology may include miscarriages, dystocia, early separation or neglect of mother and offspring; Possible causes are infections, lack of passive transfer immunity, prematurity, congenital malformations and infanticide (by mature males) (Arbelo et al. 2013, Groch et al. 2018). The causes of mortality in young dolphins in the ADS are currently unclear, because few of the carcasses examined were in a fresh condition.

Skin lesions

Skin lesions of cetaceans have been studied in a number of world regions (Wilson et al. 1997, 1999, Bearzi et al. 2009, Maldini et al. 2015). Various bacterial, viral, fungal and algae organisms have been reported as pathogens that can be related to skin lesions (Blanchard et al. 2001, Rehtanz et al. 2006, Van Bressem et al. 2008, Melero et al. 2011). In recent years, the fungal infection lobomycosis, which can cause zoonosis, has been reported in *Tursiops* sp. from the USA, Brazil and Europe (Van Bressem et al. 2015)

Detailed skin lesion investigations have been conducted on the ADS dolphins since 2010 and these showed various types, stages and degrees of lesion. There have also been cases where histopathological examination has shown the presence of bacterial involvement in skin

lesions, which are often seen as part of a systemic infection. One case has shown a grossly similar lesion to that lobomycosis or lobomycosis like lesion, but this was not tested by tissue culture or morphologically.

Often skin lesions of dolphins are a good indication of their overall body condition; for example, tattoo-like skin lesions have been reported that they may be an indicator of their general body condition (Van Bressem et al. 2003). ADS dolphins with chronic skin lesions often had chronic disease and were in poor health.

Observations of the wounds of ADS dolphins showed features that were consistent with the bite mark of pufferfish or leatherjackets and these species are known to inhabit the Port River (pers com. Ralph Foster). A penetrating wound on the skin exposes it to various micro-organisms in the water, causing inflammation. Even the micro-organisms on the teeth of the fish causing the bite wound can be pathogenic. As the natural healing of wounds depends on the individual's immune system, wounds often become chronic when the individual is not in good health.

There were also skin lesions where pinhole-sized holes were observed in the centre of the lesions. Although the ethology is not clear in ADS dolphins, Van Bressem et al. (1993) stated that a dark mark with a pinhole may develop into a tattoo like skin lesion.

Skeleton pathology

Full skeletal pathology of cetaceans has been reported in few publications worldwide (De Smet 1977, Kompanje 1995, Van Bressem et al. 2007, Tomo et al. 2010, Fettuccia et al. 2013).

A study of whole skeletons of *Tursiops aduncus* in the SAM found that 74% of the animals had lesions (Tomo et al. 2018). Among the types of skeletal lesions, fractures had the highest prevalence of four types. Fractures were often associated with anthropogenic circumstances, e.g., boat strikes. Since the establishment of the ADS, a decrease in anthropogenic mortality has been observed (Adamczak et al. 2020) and this may be associated with the decreased number of fractures and decrease in anthropogenic mortality in ADS since 2005. Because prior to 2005, fractures were found in 40% of individuals examined, regardless of age, and this was almost constant; after the establishment of the ADS, a positive correlation between dolphin age and the prevalence of lesions has been observed.

Five dolphins showed malformation of their skeleton but none of the pathologies was significant.

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Appendix 1: datasheet for whale and dolphin specimens at the South Australian Museum

WHALE & DOLPHIN STRANDINGS/SPECIMEN DATA SHEET

SOUTH AUSTRALIAN MUSEUM
North Terrace
ADELAIDE SA 5000

Ph: 8207 7458
Fax: 8207 7222
Mobile: 0434 906 744/ 0421 754 848

Came in _____ Date dissected _____ Reg No M _____ Accession/field No _____

Species _____ Identity confirmed by _____ Year _____ Rel__

Date:-First seen _____ by _____

Reported _____ by _____

Collected _____ by _____

Country/**State** _____ Latitude _____ Longitude _____ Precision of geocode __

Grid Reference _____ Datum _____

Nearest named place _____

Direction & distance from nearest place _____

_____

Special geographic unit (name of bay, gulf, peninsula etc) _____

_____

Map reference _____

X-ray _____ Autopsy report _____ **Photos** _____

Media coverage _____ Sketches _____

STRANDING INFORMATION

Number of animals stranded Total: _____ Males: _____ Females: _____

Seen alive? Yes/No _____ Date of death _____ Date & time of stranding _____

Circumstances of stranding _____

_____

Weather around time of stranding _____

_____

COLLECTION INFORMATION

Age of material Subfossil _____ Skeletal remains _____ Intact _____

Specimen type Skull _____ Mandibles _____ Baleen _____ Teeth _____

Postcranial _____

_____

Wet _____

_____

Preservation Frozen _____ Formalin _____ Alcohol _____ Spirit _____ Other _____

Habitat (eg beach type, rocky shore, estuary) _____

Remarks _____

_____

_____

_____

SPECIMEN DATA

Sex _____ **Age** _____

Colour alive above _____ below _____

dead above _____ below _____

Throat grooves (number & longitudinal extent) _____

Teeth Upper left _____ Upper right _____

Lower left _____ Lower right _____

Baleen (colour,shape) _____ Collected Yes/No

Condition of animals - injuries _____

_____

scars _____

_____

State of decomposition _____

_____ **Geraci Code** _____

External parasites (number, location) _____ Collected? Yes/No

Dissection remarks & internal parasites - nasal passages _____ mouth _____

stomach _____ liver _____

lungs _____ intestines _____

abdominal cavity _____ blubber _____

other _____ Collected? Yes/No _____

Stomach contents _____ Collected? Yes/No _____

Reproduction penis _____ testes _____

mammary glands _____ milk present/absent _____

uterus _____ ovaries _____

Tissues genetics (frozen) Blood ____ L___ K ____M____ S_____ (buffer) L ___ K_____M ____ S ____

heavy metals (frozen) liver _____ kidney _____ blubber _____ muscle _____

pathology (preservation) lung _____ liver _____ kidney _____ pancreas _____

other _____

Measurements (mm - straight lines except for girth)

1 **Total length** _____
(tip of upper jaw to deepest part of fluke notch)

2 Tip of upper jaw to centre of eye _____

3 Length of gape _____
(tip of upper jaw to corner of mouth)

4 Tip of upper jaw to blowhole _____

5 Tip of upper jaw to anterior insertion of flipper ___

6 Tip of upper jaw to tip of dorsal fin _____

thickness - ventral _____ dorsal

Weight (kg) _____

7 Tip of upper jaw to centre of anus _____

8 Maximum girth (+ distance from upper jaw) _____

9 Length of flipper (anterior to tip) _____

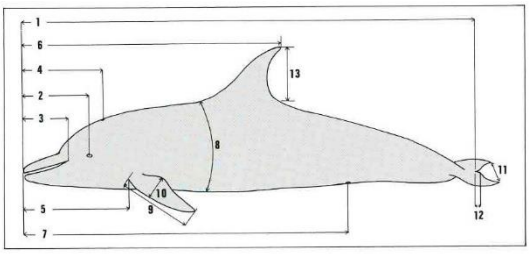
10 Width of flipper (maximum) _____

11 Width of tail flukes (tip to tip) _____

12 Depth of notch between flukes _____

13 Height of dorsal fin (tip to base) _____

14 Blubber



Appendix 2: **Relative age categories for cetaceans developed by the South Australian Museum.**

Neonate: signs of being newborn, including neonatal folds, rostral hairs, umbilicus present or scar unhealed, dorsal fin folded in some cases

Calf: young animal still suckling from mother but not neonate, tongue with papillae on margin, teeth not completely erupted. *Tursiops* body length <150 cm, *Delphinus* body length <130 cm.

Juvenile: not sexually or physically mature. *Tursiops* body length >150 cm, *Delphinus* body length >130 cm.

Subadult: sexually mature, not physically mature

Adult: both sexually and physically mature

Appendix 3: **Definition of circumstance of death categories, used at the South Australian Museum.**

Intentional Killing, e.g., shot, speared, stabbed

Known Entanglement, e.g. aquaculture-tuna, snapper net, salmon nets, mussel line, fisheries longline, pilchard purse-seine, shark net, other

Probable Entanglement, e.g. dorsal fin or flukes cut off, net marks, food in oesophagus, blood in abdomen

Other Unintentional, e.g. boat strike, propeller wounds, acoustic damage, skull fracture/damage, sudden death

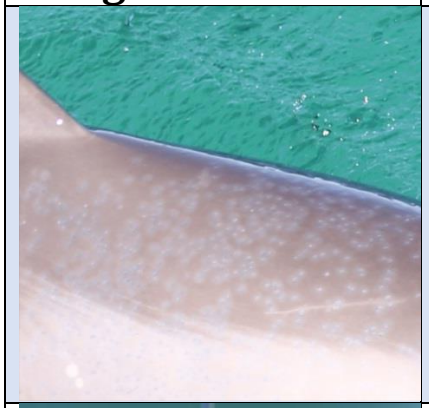
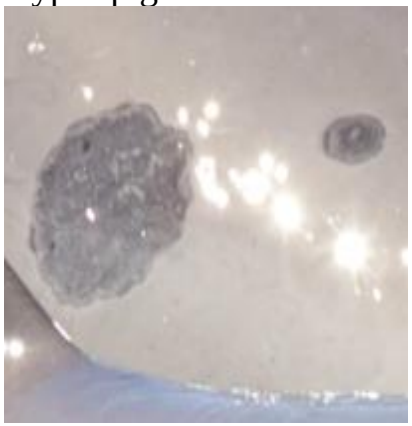
Live Stranded, e.g. put back to sea and died, euthanaised, died after stranding

Disease, e.g. significant parasites, heart disease, infection

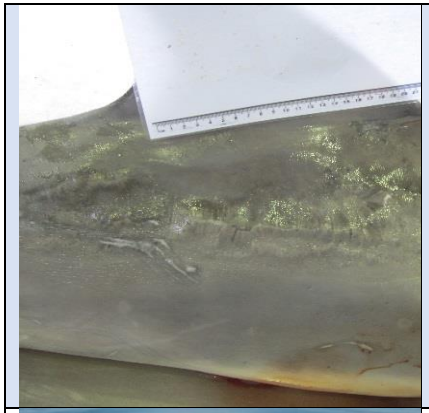
Other Natural, eg. choked on shark, starved, neonatal death, predator (killer whale, shark)

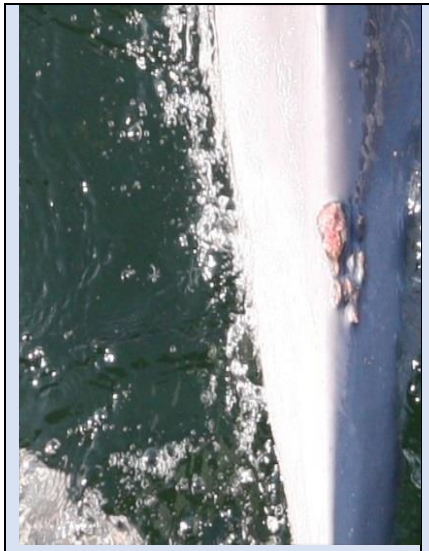
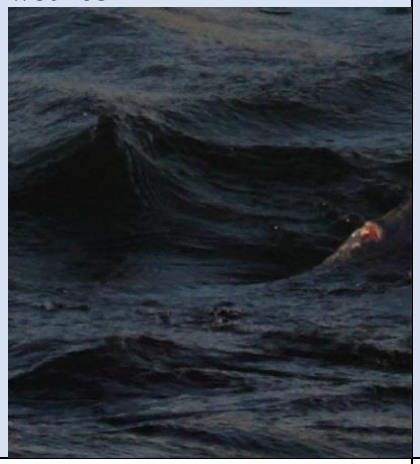
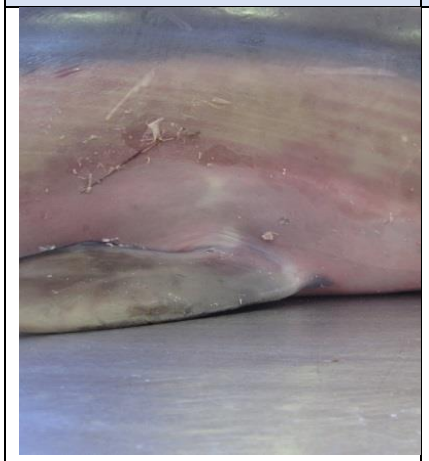
Unknown, e.g. no information, too decomposed, incomplete specimen

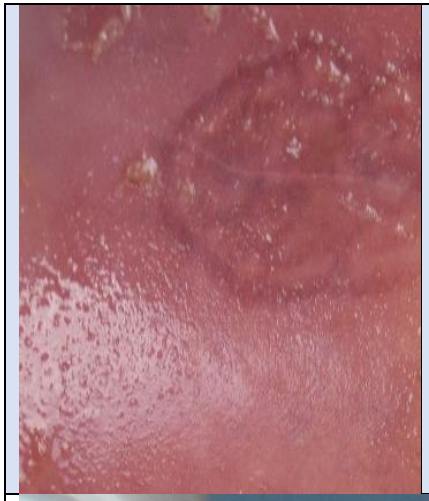
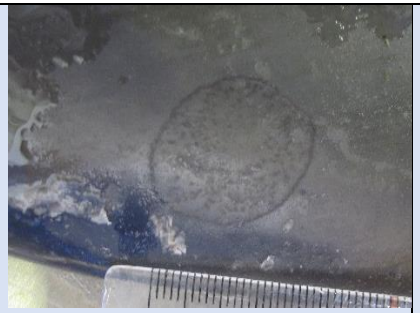
Appendix 4: Skin lesion catalogue of SA dolphins used in the South Australian Museum

Image	Category	Description	Other comments
	Light fringed	Circular, ovoid shape with light border,	 They may have pinholes in the centre of circle
	Hyper-pigmented concentric rings 	Circular or multiple layered saw-like lines with hyper-pigmented	Port River, sighted on Sep 2014

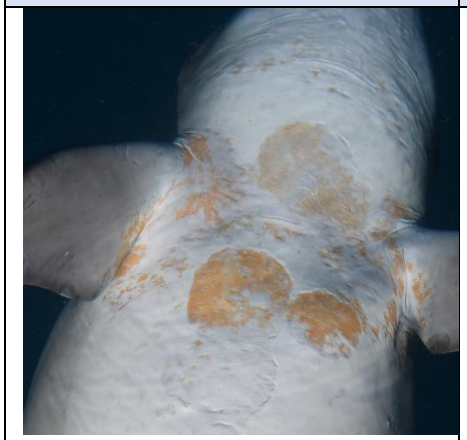
	Hypo-pigmented concentric rings	Circular or multiple layered saw-like lines with hypo-pigmentation	
	Ulcers and/or erosions	Skin surface is sunken. Ulcer: epidermis layer has completely disappeared Erosion: the dermis is not visible	Wave (Port River dolphin, photographed April 2010)
	Scars	Skin healed.	Wave (Port River dolphin, photographed May 2011)

			
	Excoriations and Abrasions 	Scratches, tooth rakes with deep thick linear marks, sometimes with deep penetrating wounds	

	Excoriations/ Abrasions 	The lesions sometimes appear as deep penetrating, raised wounds 	
	Net marks, amputations and scars with rope/nylon filament line, propeller wound 	Anthropogenic factor involved; e.g. entanglement, rope, nylon filament line, a boat propeller.	

	Tattoo-like skin lesions 		They may be related to low salinity of the water. Status of animal's immune system may also relate to this lesion.
	Penetrating wound	Deep penetrating cut, into the blubber layer	Port River photographed 2014

	Raised vesicle (closed vesicle)	Circular, ovoid shape, single or multifocal, closed	
	Dark fringed	Circular, ovoid shape with dark border	

	Lobomycosis like skin lesion	Numerous raised, ulcerated papillary nodules, early stage 	Port Rive, Adult female 2018
	Orange- yellow discoloration	Orange-yellow discoloration cover on the skin. Aetiology has not been confirmed but diatom is suspicious	Gulf St Vincent, photographed Oct 2021 (this case was covered on to tattoo like skin lesion)