Investigation into dolphin deaths in the Adelaide Dolphin Sanctuary

National Parks and Wildlife Service SOUTH AUSTRALIA

SOUTH AUSTRALIA

Government of South Australia
Department for Environment and Water

Contents

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DEW greatly appreciates the significant amount of time the experts from the investigation team have contributed to assist with this investigation over the past twelve months.

The breadth of expertise and experience across a broad range of fields has been critical in the undertaking of this investigation.

The support, discussion, research and collaboration within the team and which was provided to DEW has been greatly appreciated.

Glossary

Anthropogenic – relating to, or resulting from, human influence.

Bioaccumulation - when a toxic substance is absorbed via water and food at a greater rate than it is excreted and it accumulates in the body tissues

Contaminants of emerging concern (CEC) - CECs are any natural, manufactured or manmade chemical seldom monitored in the environment that is suspected, or known, to cause adverse ecological effects. These may include pharmaceuticals, personal care products, food additives, illicit drugs, microplastics, nanomaterials, pesticides, flame retardants, plasticisers and other industrial chemicals (Australian Water Association).

Endocrine Disrupting Chemicals (EDC's) - Endocrine-disrupting chemicals (EDCs) are substances in the environment (air, soil, or water supply), food sources, personal care products, and manufactured products that interfere with the normal function of your body's endocrine system.

Emaciation- the state of being abnormally thin.

Metagenomics - the study of genetic material recovered directly from environmental samples.

Microbiology- is the study of all living organisms that are too small to be visible with the naked eye. This includes bacteria, archaea, viruses, fungi, prions, protozoa and algae.

Necropsy - the examination of an animal to determine cause of death

Opportunistic infections- infections that occur when there is a weakened immune system

Shotgun metagenomics- shotgun sequencing is a method used for sequencing random DNA strands

Introduction

The Adelaide Dolphin Sanctuary

The Adelaide Dolphin Sanctuary (ADS) was established as a result of community concerns about the safety of dolphins living in the Port Adelaide River (Port River) and Barker Inlet and their environment. In response to widespread support for increasing their protection, the government of the day developed an Act of Parliament to provide more security for the dolphins and their environment. The *Adelaide Dolphin Sanctuary Act 2005* was proclaimed in June 2005. The objectives of the ADS Act are to protect the dolphins in the Port River and Barker Inlet area and to protect the habitat on which they rely. The protection is provided through amendments in 11 other Acts with operational and regulatory responsibilities in the area. The ADS management plan sets out how the objects and objectives of the Act are to be achieved.

The ADS is an area of 118 square kilometres, located along the eastern side of Gulf St Vincent. It includes the Port River and Barker Inlet and stretches around to North Haven Marina then north to Port Gawler and the Adelaide International Bird Sanctuary (AIBS).

The ADS is home to around 30 resident Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) with some 400 more dolphins thought to visit the area. Dolphins from within the ADS are members of a wild free ranging population and may form part of a larger dolphin population in Gulf St Vincent.

The ADS includes mangroves, seagrass, saltmarsh, tidal flats, estuarine creeks and rivers all combining to provide critical habitat for the ADS dolphins and their prey species.

The ADS is economically, socially, culturally and historically important. The area contains key infrastructure for the state including the state's busiest shipping port, electricity generation plants, and popular recreational areas. It is arguably the most intensively used marine waterway in South Australia. Despite the impact of these uses, the ADS manages to sustain an ecosystem that can support a range of marine life including bottlenose dolphins.

Managing dolphin welfare in the ADS

Day-to-day management of the ADS is undertaken through the Department for Environment and Water (DEW) by National Parks and Wildlife Service SA (NPWS) marine rangers based in Port Adelaide. ADS park management operations focus on protecting the dolphin population from harm and protecting the important marine, estuarine and coastal habitats of the area. NPWS staff conduct regular land and boat patrols of key areas, monitor the dolphin population, collaborate with the research community, engage with visitors, provide interpretive and educational resources, manage visitor interactions with dolphins, work closely with tourism operators, coordinate intervention in dolphin incidents, maintain park assets, like buoys, and maintain park presentation.

Consistent with the *National Parks and Wildlife Act 1972* and the *Animal Welfare Act 1985*, DEW's 'Marine Mammal Intervention Policy and Procedure' guides direct decisions in relation to intervention in marine mammal health and welfare issues. DEW manages the ADS dolphin population consistent with other wild animal species. Principally, intervention is not undertaken for animals experiencing a natural disease or other condition. Intervention may be considered where an animal is affected by anthropogenic factors (e.g. fishing line entanglement). This is because capture and treatment of a live, wild dolphin is complex both in terms of logistics and animal welfare considerations. Intervention, such as capture and treatment, can cause significant additional stress to a wild animal which must always be taken into account in managing the animal's welfare. Decisions regarding whether and how to intervene in a dolphin incident are unique to each situation and must consider risks and benefits including the animal's welfare, operational feasibility and human safety. Importantly, DEW relies upon veterinary advice as part of any decision to intervene.

In 2020, NPWS set up a Dolphin Veterinary Reference Group (DVRG), consisting of veterinarians and cetacean biologists, to advise on the management of health-related events affecting ADS dolphins. This group provides a forum for NPWS to seek advice on health, disease and animal welfare issues. It also provides advice to identify and prioritise emerging health issues for further research, and facilitate collaboration and coordination among dolphin health experts to improve knowledge and understanding for the management of the ADS dolphins.

Dolphin health in the ADS

In the past few years concern has been growing from the community and dolphin researchers about the health of some of the dolphins in the ADS, particularly animals residing in the inner waters of the Sanctuary. This concern is due to an increased number of recorded ADS dolphin deaths, lower than average ADS dolphin calf survival rates and the disappearance and presumed death of a number of resident ADS dolphins.

In response to this concern the previous Minister for Environment and Water launched this investigation in August 2021 into: the potential causes of the increased dolphin deaths and disappearances in the ADS; the potential causes of declining health of the dolphins and ecosystems within the Port River and Barker Inlet; and to recommend research or management responses necessary to address any issues identified.

Investigation Scope

Whilst the investigation was launched following four deaths leading up to August 2021, there have been further deaths during the course of the investigation. As a result the animals summarised in (Table 1) have been considered in this investigation.

Table 1 Summary of known dolphin deaths and disappearances of juvenile and adult dolphins around the ADS since 2021.

Name	Comment	Month/Year	Sex-Age
Doc	Missing, presumed dead	June 2021	Male 8 years
Twinkle	Missing, presumed dead	July 2021	Male 20 years
Semaphore Dolphin*	Died, body recovered	July 2021	Female adult
Tallula	Died, body recovered	August 2021	Male 12 years
Hunter	Poor condition, euthanased	October 2021	Male 6 years
Squeak	Died, body recovered	November 2021	Male 4 years
Namor	Died, body recovered	March 2022	Male 13 years

*not an ADS dolphin

The Investigation

The investigation team

To inform the investigation, a team of 32 local experts was assembled.

The investigation team included water scientists, marine mammal biologists and researchers, veterinarians, veterinary pathologists, toxicologists, zoologists, marine scientists, wildlife experts, industry and environmental managers.

- Dr Sue Gibbs, Dr Catherine Kemper and Ikuko Tomo - South Australian Museum
- Assoc. Professor Luciana Moller, Assoc. Professor Guido Parra - Flinders University
- Assoc. Professor Lucy Woolford, Dr Anne-Lise Chaber and Dr Rebecca Souter – The University of Adelaide
- Dr Mike Bossley - Whale and Dolphin Conservation
- Aaron Machado - Australian Marine Wildlife Research & Rescue Organisation (AMWRRO)
- Matthew Pellizzari and Stephanie Bolt - Flinders Ports
- Maggie Hine - Port Adelaide Enfield Council
- Dr Clive Jenkins and Matt Nelson - Environment Protection Authority (EPA)
- Skye Barrett and Annabel Jones - Primary Industries and Regions SA (PIRSA)
- Professor Simon Goldsworthy and Dr Roger Kirkwood - South Australian Research & Development Institute (SARDI)
- Tim Kildea - SA Water
- Dr Anupama Kumar - Commonwealth Scientific and Industrial Research Organisation (CSIRO)
- Dianne Hakof and Dr Ian Smith - Zoos SA
- Dr Simon Bryars, Darryl Cowan, Jon Emmett, Verity Gibbs, Jason Higham, Dr Alice Jones, Lisien Loan, Dr Chloe McSkimming and Dr Nikki Zanardo - DEW

Methodology

To help better understand the functionality and potential threats to the ADS's ecosystem, the investigation team reviewed historical data, identified trends and patterns in factors corresponding to dolphin deaths, developed a common understanding on how the ecosystems within the sanctuary work, what pressures affect them and how dolphins interact with these.

As a result of this work a conceptual diagram of the Port River and Barker Inlet ecosystem was developed and used to inform the investigation (Figure 1).

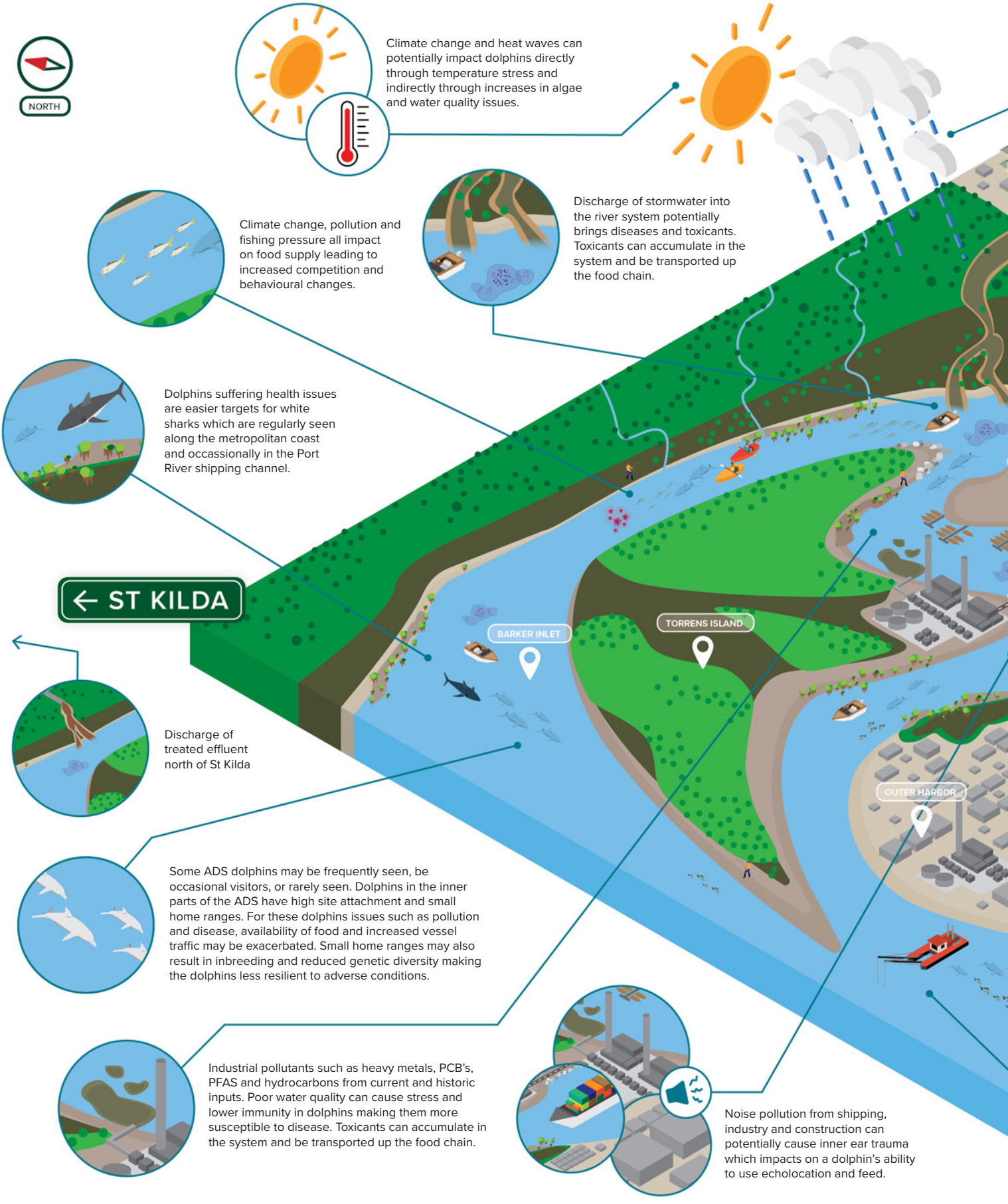
The conceptual diagram was used to inform a literature review *Threats to dolphin health in the Adelaide Dolphin Sanctuary* (Appendix 1) undertaken by the South Australian Research and Development Institute (SARDI). The review examined dolphin health literature from around the world along with data and reports about the local ADS dolphin population, providing a robust review of potential threats to ADS and coastal dolphins to inform the investigation. The report also highlighted similar dolphin mortality events around the world where the difficulties in finding a definitive cause of these events was identified.

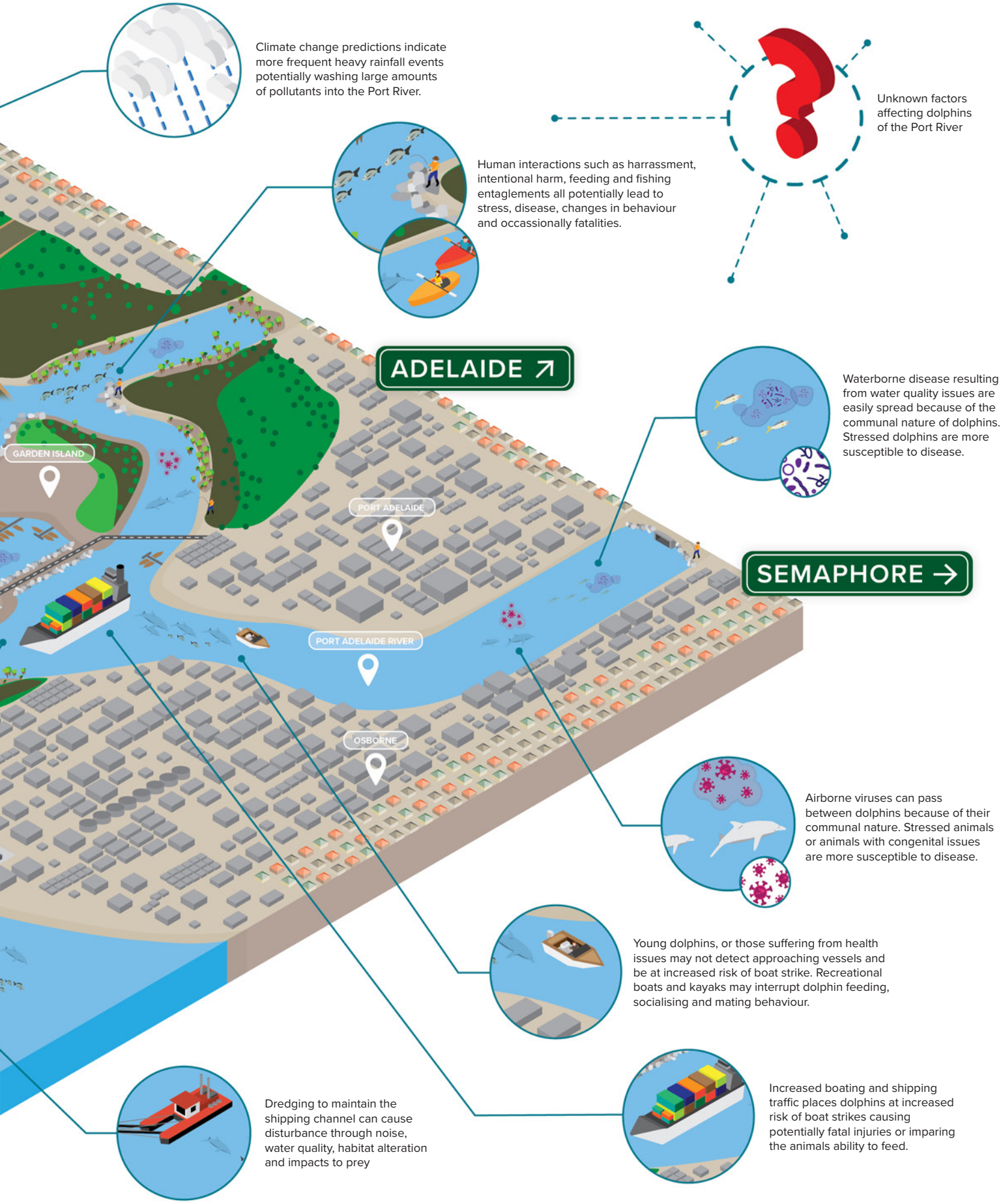
Using the conceptual model (Figure 1) and SARDI review (Appendix 1), the investigation team identified the following key lines of enquiry to pursue in the investigation:

- nutrients and toxicology
- disease
- harmful algae
- trends in ADS mortalities
- ADS population and structure

Each line of enquiry aimed to assess what potential impact these factors could be having on dolphin health in the ADS. Members of the investigation team then carried out studies and research in each of the respective lines of inquiry dependent upon their relevant expertise.

Figure 1 Conceptual diagram pressures and threats to ADS dolphin




Climate change predictions indicate more frequent heavy rainfall events potentially washing large amounts of pollutants into the Port River.
Unknown factors affecting dolphins of the Port River
Human interactions such as harrassment, intentional harm, feeding and fishing entaglements all potentially lead to stress, disease, changes in behaviour and occassionally fatalities.
ADELAIDE
Waterborne disease resulting from water quality issues are easily spread because of the communal nature of dolphins. Stressed dolphins are more susceptible to disease.
GARDEN ISLAND
PORT ADELAIDE
SEMAPHORE
PORT ADELAIDE RIVER
OSBORNE
Airborne viruses can pass between dolphins because of their communal nature. Stressed animals or animals with congenital issues are more susceptible to disease.
Young dolphins, or those suffering from health issues may not detect approaching vessels and be at increased risk of boat strike. Recreational boats and kayaks may interrupt dolphin feeding, socialising and mating behaviour.
Dredging to maintain the shipping channel can cause disturbance through noise, water quality, habitat alteration and impacts to prey
Increased boating and shipping traffic places dolphins at increased risk of boat strikes causing potentially fatal injuries or imparing the animals ability to feed.

Lines of enquiry

Nutrients and Toxicology

Nutrients (such as nitrogen and phosphorus) are essential to a healthy marine ecosystem but excessive nutrient loads can be detrimental (as "stressors", but not as toxicants). Also, numerous anthropogenic chemicals and enhanced concentrations of certain elements, such as metals and metalloids, can be toxic to marine life and effect the health and productivity of an entire ecosystem.

Key sources of nutrient pollution can be 'point source' wastewater treatment plants, various industries, many 'non-point sources' in urban catchments and the sediments within the Port River itself.

Over the last 20 years, nutrient input from industrial sources into the ADS have been reduced, for example, through the closure of the Port Adelaide Wastewater Treatment Plant (2004), closure of the Penrice Holdings soda-ash plant at Osborne (2013) and SA Government-funded improvement of processes at the Bolivar Wastewater Treatment Plant. Those nutrient load reductions can be attributed in part to the Port Waterways Water Quality Improvement Plan (EPA 2008). The nutrient contribution from stormwater inputs to the Port River is also likely to have been reduced by the construction of several urban wetlands (during the period 1996-2005) which treat stormwater flows before they enter the Port Waterways.

Sources of potentially toxic pollutants also include industrial activities and diffuse stormwater sources. That said, the variety and complexity of potentially toxic pollutants is very difficult to quantify.

Industrial activities that use or produce nutrients or potentially toxic chemicals are closely regulated by the EPA to ensure their pollution control systems are well designed and operating effectively. It is important to note here that an unauthorised discharge of industrial wastewater to the environment is an offence and can attract significant enforcement provisions under the *Environment Protection Act 1993*.

The sediments within the Port River have been monitored and reported previously by the EPA (1997) for organochlorine pesticides (chlordane, DDT, dieldrin, heptachlor, lindane, aldrin), polychlorinated biphenyls (PCBs), herbicides (atrazine), metals (lead, zinc, copper, mercury, cadmium and arsenic) and organotins. The sediments in the Port River are an important part of the ecosystem, with many biota using it has habitat and food source, so it is therefore logical to consider this in the context of dolphin dietary uptake within the conceptual model.

Most metal pollutants and 'persistent organic pollutants' or POPs are at extremely low concentrations in stormwater and marine waters and detailed scientific knowledge relating to their biological effects is incomplete. However, Dolphins are at the top of the food chain, and are therefore susceptible to potentially toxic pollutants such that may biomagnify through the food chain. The bioaccumulation of pollutants in Port River dolphins and fish has been reported previously by the EPA (in 2000 and 2005). These reports confirmed the presence of key pollutants in dolphins although the levels were approximately the same as had been measured in dolphins found in other urbanised coastal areas around the world. While bioaccumulation of pollutants in dolphins is clearly an effect that should be minimised, the EPA has not been able to quantify the actual risk of harm to Port River dolphins because there hasn't been a sufficiently clear basis linking pollutant body burden (i.e. the amount of pollutants that have bioaccumulated) to dolphin health status. Moreover, there is the added uncertainty in harm assessment due to bioaccumulation of pollutant combinations.

An EPA assessment of persistent organic pollutants in marine fish was also conducted in 2012 and presented at an international symposium. That study collected several fish species from various South Australian estuarine and coastal regions, including the Port River. Pollutant analyses included tetra- through to octa-chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and "dioxin-like" PCBs.

That purpose of that assessment was to inform the risk of people consuming whole fish caught by recreational fishers. The results from the 2012 fish survey indicated that the contamination of dioxins, furans and dioxin-like PCBs of estuarine and coastal fish in South Australia were not high enough to warrant any food advisories with regard to recreational fishing.

The EPA also conducted a bioaccumulation study of trace elements and metals in mussels (2009-2010) and published a paper in 2011. The measurement of metal accumulation in mussels has been a routine and highly regarded method for the assessment of metals in marine environments around the world. That South Australian study included several South Australian marine environments, including the Port River.

Marine mussels are not a food source for dolphins, but the measurement of pollutant bioaccumulation in mussels does confirm pollutant bioavailability, with some advantages over fish monitoring: mussels are sentinel and they can accumulate pollutants to relatively high levels.

While these studies were being conducted, there were no indications that Port River dolphins were subject to increasing rates of disease or otherwise suffering unusually. Rather, the indications were that the Port River dolphin population was increasing during the period 2000-2018. That said, it may be feasible to use all these historical data as a baseline for future monitoring of pollutant bioaccumulation in the Port River, leading to an assessment of changes in pollutant levels that dolphins may be exposed to as a result of their dietary uptake.

Stormwater is definitely the largest volumetric input the Port River; there are numerous drains that input directly and many others flow into West Lakes which subsequently flows in the Port River at Bower Road. That's not to say that stormwater inputs are definitely the main source of pollutants (with the exception of sediments) although from the outset of the investigation, the influence of stormwater and its potential to be a source of many pollutants, pathogens and disease which could impact upon the health of the ADS dolphins was recognised.

Over time, there has been significant changes in land use in the surrounding catchments feeding into the ADS. There has been an increase in impermeable surfaces across the broader Adelaide metropolitan area leading to an increase of approximately 20% in stormwater runoff over the past ten years (pers comm. EPA).

Pollutants in stormwater have the potential to cause significant ecological changes to urban creeks and coastal waters, including:

- Increase in algal blooms from increased nutrient levels
- More frequent algal blooms reducing oxygen availability for aquatic organisms
- Introduction of pollutants such as metals, hydrocarbons and numerous others
- Increased sediment can smother habitats
- Increased turbidity (caused by fine suspended sediments) can reduce sunlight penetration and photosynthesis of seagrass.
- The investigation team recommended testing fish and other dietary items as well as dolphins' tissues and organs which can accumulate pollutants to understand:
- If temporal changes in potentially toxic pollutants in dolphin diet and in dolphins themselves, and if the extent of bioaccumulation is changing over time?
- Comparative assessments of potentially toxic pollutant bioaccumulation with other equivalent assessments around the world to understand if the Port River unusual in terms of pollutant bioaccumulation?

Disease

There are infectious diseases known to commonly affect cetaceans, including morbillivirus infection (a measles like disease that can cause widespread mortalities) and emerging diseases of concern including Toxoplasmosis and Brucellosis. Whilst these latter two are more commonly associated with terrestrial species they are becoming detected more frequently in marine species. The single-celled parasite *Toxoplasma gondii* that causes toxoplasmosis has become widespread among marine mammals, largely because of the number of cats living within catchments.

Cetacean morbilliviruses, Brucella spp. and Toxoplasma gondii are all thought to interfere with population abundance by inducing high mortalities, lowering reproductive success or by synergistically increasing the virulence of other diseases (Van Bressem et al. 2009).

Screening for known and emerging parasites to identify if there was a single disease responsible for, or at least present in, all the dolphin deaths was identified by the investigation team as a critical component of the investigation.

Biotoxins from Harmful Algae

Harmful algal blooms occur naturally but can also be caused or enhanced by human activities. Products from harmful algae can be toxic to shellfish, fish and cetaceans (Wang et al 2015, Brown et al. 2018, Brown et al. 2021), and accumulate through the food chain. An increase in nutrients or changes in water temperatures can cause the number of algae to increase, leading to harmful algal blooms and the production of high concentrations of these biotoxins that can reduce dolphin health.

The investigation team recommended to test the water for harmful algae and tissue samples from collected dead dolphins for biotoxins from harmful algae, as a potential cause of dolphin morbidity or mortality.

Mortality trends

Understanding the context of dolphin deaths since June 2021 and comparing this to historical data and observations since 1987 to determine if they were within normal ranges, was another important line of enquiry identified by the investigation team. The investigation team also sought to understand if emaciation seen in dolphins was a new symptom in observed deaths or had commonly been recorded over time.

ADS Population

Understanding home ranges of dolphins is important to determine if deaths are concentrated among those dolphins using particular areas of the ADS, such as the inner waters. Also of importance is to determining the longer-term viability of dolphins in the ADS is understanding the overall structure of the ADS dolphin population in Gulf St Vincent.

Given the large size of Gulf St Vincent, monitoring the dolphin population and understanding how dolphins use its' varying habitats is challenging. Dolphin researcher Dr Mike Bossley has been undertaking dolphin surveys of the inner waters of the ADS since the 1980's. In addition, NPWS marine rangers have undertaken population monitoring surveys in both inner and outer waters of the ADS since 2015.

Investigation Results

Toxicology

In 2005, the EPA released the report *Heavy metal status of South Australian dolphins* (Butterfield and Gaylard, 2005), which found the majority of dolphins tested had metal concentrations comparable to dolphins from other parts of the world. Of 83 dolphin livers and 62 dolphin bones analysed, there were 30 high concentrations, which could have been due to natural variation within the population, natural sources of metals in the marine environment or anthropogenic pollution. There were significant differences between metal concentrations in dolphins from different regions, which suggested local sources of metals from geological and anthropogenic activities were being accumulated in dolphin tissues.

Currently, potential anthropogenic sources of metals within the ADS are from Bolivar wastewater treatment plant, the Torrens Island and Pelican Point power stations, urban stormwater discharge, and accumulations in sediments from past industries. High levels of cadmium, lead and copper have been recorded in several fish species from Barker Inlet (Edwards et al. 2001). These fish represent a prey source for dolphins, and metals in fish could bio-accumulate in the dolphins.

The testing of metals in dolphins since the 1990s (included in the *Heavy metal status of South Australian dolphins* study) allows levels of contaminants to be tracked over time in the ADS dolphin population.

As part of this investigation, extensive toxicology testing was recommended and undertaken on Hunter, Tallula and the Semaphore dolphin (not a resident ADS dolphin). The testing of chemical residues in the liver was analysed by the National Measurement Institute and the Department of Industry, Science, Energy and Resources.

As part of this investigation, the South Australia Museum (SAM) collated all historical toxicant testing that had occurred on bottlenose dolphins in the ADS. This enabled the investigation team to compare the toxicant testing that was undertaken on Hunter, Tallula and the Semaphore dolphin.

Below is a list of the range of liver toxicology tests undertaken on Hunter, Tallula and Semaphore dolphin by the National Measurement Institute (NMI), Sydney.

- Metals – Arsenic, mercury, copper, lead, zinc, cadmium, chromium, nickel, platinum, molybdenum, antimony, aluminium, iron, cobalt, lithium, manganese, selenium, strontium, tin, vanadium. – (some detected)
- PFAS - (some detected)
- PCB congeners – (non-significant levels detected)
- Organochlorine (OC) Pesticides - (non-significant elevations)
- Herbicides – (below levels of detection)
- Polycyclic aromatic hydrocarbons - (below levels of detection)
- Carbamates – (below levels of detection)
- Organophosphate (OP) Pesticides – (below levels of detection)
- Synthetic Pyrethroids – (below levels of detection)
- Fungicides – (below levels of detection)
- Phenols – (below levels of detection)
- Phthalates – (below levels of detection)
- Chlorinated Hydrocarbons – (below levels of detection)
- Ethers – (below levels of detection)
- Amines, Nitroaromatics & Nitrosamines – (below levels of detection)
- Dioxins- (some detected)b

Toxicology Test results

The extensive testing revealed bioaccumulation of metals, persistent organic pollutants and selected Contaminants of Emerging Concern (CEC) in the dolphins.

Trace metals and some persistent organic contaminants were at low concentrations (Table 2). The levels detected were similar or lower than levels reported by other monitoring studies in Gulf St Vincent bottlenose dolphins. Table 3, reproducing data from the 2005 report, shows the classifications for metals in dolphins in South Australia.

The results from the testing concluded that CECs such as phthalates, short-chain paraffin, and pesticides in the liver samples of the two ADS dolphins were below detection limits.

PFAS

Per- and poly-fluoroalkyl substances (PFAS) are a group of human-made chemicals that have been used since the 1950s in non-stick cookware, waterproof clothing and fabric stain protection. However, the most problematic use of PFAS has been in aqueous film-forming foam (AFFF), which is used to fight liquid fuel fires.

From the 1970s, AFFF was used by the defence forces for firefighting activities and firefighting training. As a consequence, increased PFAS levels have been detected at defence bases. Other affected sites include airports and locations where firefighting training takes place (Australia State of the Environment Report, 2021).

Increased levels of PFAS are known to be toxic to a range of animals; however, the impact on human health is unknown. The Australian National University was commissioned by the Australian Government to undertake a PFAS Health Study to examine possible links between PFAS exposure and health in communities in Williamtown, Oakey and Katherine; (i.e., associated with use at defence bases). Initial findings linked high cholesterol levels and high levels of PFAS, and possible links with reduced kidney function, testicular cancer, and the immune response to diphtheria and rubella vaccines (Kirk et al. 2018, Banwell et al. 2019).

In 2017, the EPA released the report *Per and polyfluorinated alkyl substances (PFAS) in the marine environment*. The liver samples from 44 dolphins (including nine dolphins from the ADS) were tested for PFAS levels, including Perfluorooctane sulfonic acid (PFOS), which constitute greater than 90% of total PFASs (Table 4).

Table 2 Summary of total recoverable trace elements in liver in ADS Dolphins by Inductively Coupled Plasma (ICP), Source: Dr L. Woolford. Low, medium and high classification scheme for metals in South Australian dolphins from Butterfield and Gaylard (2005)

Dolphin		Arsenic (mg/kg)	Cadmium (mg/kg)*	Copper (mg/kg)	Lead (mg/kg)*	Mercury (mg/kg)*	Zinc (mg/kg)*
Hunter		0.34	0.04	5.9	0.13	10	44
Tallula		0.24	0.05	8.7	0.24	28	38
Semaphore		0.35	0.81	32	0.05	300	290
Oriana (2018)		0.29	0.14	18	0.08	68	94
Classification scheme	Low		<60		<4	<400	<400
	Medium		60-150		4-10	400-1600	400-700
	High		>150		>10	>1600	>700

*dry weight

Table 4 Published PFOS concentrations (ng/g) in dolphin livers. Source:EPA

Species		n	PFOS (ng/g)	Range	Source
Dolphin[11]	Adeldie metropolitan, SA	5	436	290-690	This survey
	Port River, SA	9	1,986	510-5,000	This survey
	West Coast, SA	6	7.250	<5-13	This survey
	Bunbury, WA	8	36.92	<5-97	This survey
	Mandurah, WA	2	227	34-420	This survey
	Swan River, WA	4	6,975	2,800-14,000	This survey
Dolphin[12]	Offshore NSW	7	705.1	58-1,800	This survey
	Tasmania	3	46	11-71	This survey
Dolphin[13]	NW Atlantic	20	489	48-1,520	Kannan *et al* 2001

Table 4 PFAS levels recorded in the investigation

	PFOS mg/kg	PFOS ng/g
Hunter	0.43	430
Tallula	0.3	300
Semaphore dolphin	0.041	41

The study found that dolphins from the Port River had significantly higher PFOS levels than animals from the Adelaide metropolitan coast, and both groups had significantly higher levels than dolphins from the west coast of SA. This likely reflects the proximity to PFAS sources, including the Inner Port fuel berths and stormwater wetlands in the ADS, as well as the lower levels of flushing through the Port River and Barker Inlet system compared to the Adelaide metropolitan coast and west coast of SA.

The 2017 EPA study reported PFAS levels in livers of nine dolphins from the Port River ranged between 510–5,000 ng/kg and an average of 1986 µg/kg (Table 4). In the present investigation, PFOS levels in the livers from of the dolphins Hunter and Tallula varied between 300- 430 ng/kg. The lower levels in the dolphins from 2021 may indicate PFOS levels are declining in ADS dolphins, which is consistent with PFOS being phased out of industrial and fire-fighting applications. Although the most recent data is based on only two samples, and bioaccumulation of PFAS can differ throughout age and gender of dolphins.

It is unclear if the levels of the contaminants of concern measured in dolphins that died in 2021 were adversely affecting the dolphins. To understand the implication of the detection of multiple contaminants in dolphin livers, further monitoring to assess the levels of these contaminants in the food sources of the dolphins including marine invertebrates and fish at different trophic levels is needed.

The toxicological testing undertaken in this investigation was wide reaching but was not exhaustive. Further tests can be undertaken, particularly in the areas of CECs. The toxicology testing undertaken has required methodologies based on sampling water and sediments be adapted to detect CECs on marine mammals. They are not yet validated. The investigation team worked with the NATA accredited national laboratory to develop reliable methods for measuring these CECs in biota.

It is unknown whether or not contaminants are interacting and further investigation to determine the potential effects in combination will be performed in collaboration with CSIRO.

Disease

The major pathological findings in each of the dolphin bodies studied by the South Australian Museum and University of Adelaide are summarised in Table 6. Although the body of Doc was not recovered this dolphin is included in this table because samples were collected by a wildlife vet when it was captured to remove a fishing line entanglement two days prior to its disappearance.

Table 6 Major pathological findings and presumed cause of death of five ADS dolphins sampled between June 2021 and March 2022

Dolphin	Major Pathological findings, Circumstance of Death (CoD)
Hunter	This six-year old dolphin was a known resident of the ADS and had been observed looking very thin, with a lesion on its flank, raising concerns about the animal's health. It had been reported as appearing lethargic, emaciated, and spending more time than usual on the surface. Hunter had a congenital jaw deformity which may have reduced the efficiency of foraging. Following consultation with an expert wildlife veterinarian, the Department for Environment and Water decided to euthanise this dolphin to prevent further suffering. The post mortem found the animal was in very poor health and was unlikely to have lived much longer. Several small prawns found in the dolphin's stomach were identified as two local endemic species (*Metapenaeus bennettae* and *Alpheus novaezelandiae*). Research based on stomach contents analysis of ADS dolphins during past necropsies has found that prawn is not considered part of an ADS dolphin's typical diet. Foraging may have been atypical, with it being difficult for the animal to catch fish originally (due to jaw deformity) then enhanced while it was unwell, which may explain why prawns and little else were found in its stomach. The necropsy report, prepared by the University of Adelaide in conjunction with the SA Museum, showed the dolphin had multiple infections (inner ear, skin, gastrointestinal tract) which were adversely impacting it, and were likely connected to its significant weight loss. For further information see microbiology section. • CoD: euthanasia • Blood results: mild stress leukogram, dehydration • Skull: brachygnathia superior (congenital snout deformity); Left eye: chronic focal corneal scarring • Opportunistic bacterial infections – Skin: multifocal cutaneous ulcerations with subcutaneous abscesses - Vibrio harveyi and invasive protozoa – Left ear: bacterial otitis media and interna –*Edwardsiella tarda* – Pyloric stomach: haemorrhagic gastritis with mineralisation and intralesional– *Clostridum perfingens* type A – Intestinal tract: largely empty, mild to moderate diffuse enteritis – Liver: chronic-active periportal hepatitis and mild periportal fibrosis
Doc (caught and blood sample taken Jun 2021, body not seen)	Due to apparent poor condition, this dolphin was captured on xx June and a blood sample was obtained. The dolphin was considered to be in sufficient condition to survive so was released. Blood results showed: stress leukogram, dehydration. • CoD: not investigated – Doc went missing (no body was recovered)

Dolphin	Major Pathological findings, Circumstance of Death (CoD)
Squeak (body recovered Nov 2021)	A post-mortem examination was conducted by the SA Museum and the University of Adelaide. Initial findings from the necropsy report showed that this dolphin's body was very emaciated. There was severe deep blunt trauma observed on the right side of the head and neck area, but no obvious external injuries, therefore the cause of this trauma is unknown. Bacteria were identified from ear, lung and skin. Some bacteria were pathogenic (disease/ infection), some were likely to be secondary and not related to the cause of the death. A full necropsy report will be available following the analysis of test results (for diseases, histology and bacteria) and more detailed analyses of organs. Despite this dolphin's emaciated condition, its stomach appeared to contain solid food material, and faeces were found in the intestines. The spleen was atrophied and adrenals were enlarged. A chronic condition may have been evident, but there were no signs of significant infection. The stomach contents will be analysed by specialist researchers. **Other necropsy notes:** CoD: not determined (moderate decomposition). Skin ulcers and subcutaneous abscesses.
Tallula (body recovered Aug. 2021)	The post-mortem examination conducted on 25 August found that the body of this dolphin was very emaciated. One large fishing hook was discovered within the stomach (no signs of obstruction or infection were present). A chronic (long term) condition for this animal was possible because adrenal enlargement was observed. Testing for a range of diseases known to be present in marine mammals was undertaken. While these tests indicated that this dolphin was negative for cetacean morbillivirus (a measles like virus affecting cetaceans), the results indicated the presence of a disease called toxoplasmosis (a disease caused by *Toxoplasma gondii,* a protozoan parasite derived from cat faeces). However levels of antibodies detected were not high enough to indicate an active toxoplasmosis at the time of death. **Other necropsy notes:** • CoD: not determined: Fishing hook within stomach • Liver: mild to moderate periportal to rarely bridging fibrosis; hepatocellular atrophy; • Oral mucosa and blow hole: hyperplastic mixed ulcerative and hyperkeratotic dermatitis
Namor (body recovered Mar. 2022)	The post-mortem examination conducted on 18 March showed bruising on this dolphin's lower jaw, which could indicate trauma from an unknown cause. However, this was not considered significant enough to have caused death and definitive diagnosis is pending further examination and infectious disease screening test results CeMV testing was negative. A pure growth of E. coli was obtained from the abscess in the left lung, confirming bacterial aetiology. **Other necropsy notes:** • CoD: not determined. Advanced post mortem decomposition (brain was not examined). • Focal bacterial subpleural abscess and localised pneumonia may have contributed to morbidity and/ or mortality, however the lesion was localised and may be an indicator of poor health rather than cause of death in this individual. Mandibular oedema and haemorrhage suggesting recent trauma to this location, however cause not evident. No internal traumatic injuries were found. Namor had a lump on his flank caused an imbedded ray barb. The barb had been present for two years and was encapsulated. It was not considered a contributing factor to the cause of death.

Outstanding Disease investigations

There has been routine examination of the brains in these dolphins, however more detailed studies of neuropathological changes in these dolphins are presently underway in conjunction with specialist veterinary neuropathologists at the University of Adelaide and SA Pathology.

Inner ear examinations are also pending for some dolphins, which are an important focus for investigating for evidence of acoustic trauma. This will be performed in collaboration with Dr Maria Morell, PhD, Research Associate at the Institute for Terrestrial and Aquatic Wildlife Research (ITAW), University of Veterinary Medicine Hannover. Dr Morell and her laboratory are world renowned for their work in the detection of acoustic injury and degenerative disease in cetaceans.

Detailed comparative morphometric analysis of endocrine, reproductive and lymphoid organs of ADS dolphins and non-ADS dolphins is also ongoing, and will be interpreted in conjunction with blood and blubber hormone analysis.

Microbiology (bacteriology, virology and metagenomics)

Dolphins are often considered a sentinel species for the health of coastal environments with those living near major cities particularly vulnerable to environmental stressors. In recent decades there has been an increase in the incidence and spread of infectious diseases in these animals around the world (Moller 2022, Appendix 2).

In 2013, cetaceans South Australia experienced an Unusual Mortality Event (UME), the first recognised outbreak of cetacean morbillivirus, a measles like disease. The disease resulted in the death of up to 50 dolphins across the state, 41 being mostly young Indo-Pacific bottlenose dolphins including eight from within the ADS. The UME lasted approximately seven months though was concentrated in two periods (Kemper, et al. 2016).

There are a number of infectious diseases that can affect cetaceans (whales and dolphins) including Toxoplasmosis, Chlamydia, herpesvirus, and Brucella.

To understand if disease was a cause of death in dolphins during the present investigation, tests (Table 7) were performed on samples from Doc, Hunter, Tallula, Squeak, Namor and a dolphin collected just outside the ADS boundary at Semaphore (Semaphore Dolphin).

Cetacean Morbillivirus

Cetacean morbillivirus can affect the lungs and brain of an infected dolphin. The dolphin may present with poor body condition, skin lesions, breathing difficulties and susceptible to other illnesses due to the virus weakening their immune system.

Testing was undertaken on lung (indicates acute infection) and brain (indicates chronic infection). Test results for Hunter, Tallula, Squeak, Namor and the Semaphore dolphin all returned negative results.

Toxoplasma Serology

Toxoplasmosis is a disease that results from infection with the *Toxoplasma gondii parasitea* parasite. Symptoms in dolphins may include stranding, emaciation, altered mental state and impaired navigation (Bowater et al. 2003; Donahoe et al. 2014; Cooper et al. 2016).

Test results for toxoplasma serology revealed Hunter was negative, Tallula returned a marginal positive (marginal at 10x dilution) and the Semaphore dolphin returned a strong positive. In the dolphins that returned a strong positive, it does not mean that this was the cause of their death, but rather that they had been exposed to toxoplasma. No lesions consistent with an active or fulminant toxoplasmosis were seen in these dolphins. Therefore it is less likely that toxoplasmosis contributed to death in these animals.

Brucella Serology

Blood samples from Doc and Hunter were tested for the presence of *Brucella* spp by Rose Bengal serological test. Hunter tested negative and Doc tested positive – the first detection of *Brucella* exposure in a SA dolphin.

Brucellosis is a bacterial infection caused by *Brucella* bacteria. It is a zoonotic bacterium that in domestic livestock can cause abortions and infertility, so is a notifiable disease (Wildlife Health Australia). In dolphins, brucellosis has been linked with abortions, male infertility, bone and skin lesions, and death (C.Guzmán-Verri et al, 2012).

A blood sample was also taken from Hunter during euthanasia and this returned a negative result.

Chlamydia abortus

Infection with *Chlamydia* abortus has been reported as a cause of disseminated infection and death in stranded dolphins in the Northern hemisphere (Santoro, 2019).

Metagenomics testing undertaken by Flinders University revealed sequences consistent with *Chlamydia* abortus in the liver and other tissues of Hunter. *Chlamydia* abortus is a nationally notifiable disease.

However targeted PCR testing for Chlamydia spp. in Hunter performed by the national veterinary reference laboratory for Chlamydial testing in Australia returned a negative result (these tests were being repeated given the detection in the metagenomics tests).

Herpesvirus

Herpesvirues (including chickenpox, shingles, cold sores) have commonly been detected in cetaceans and can cause skin lesions. In dolphins with strong immune systems, these viruses likely do not present a significant risk factor, however, the viruses may pose an increased risk in young or individuals with poor immune responses.

Samples from the spleen of Hunter, Tallula and Squeak were analysed for the presence of herpesvirus/s using broad spectrum PCR by collaborators at the University of Melbourne (Prof. J Devlin) and all returned a negative result for herpesviruses.

Table 7 Summary of disease testing

Dolphin	Morbillivirus (Lung)	Morbillivirus (Brain)	Influenza A	Brucella (PCR	Brucella (Serology)	Toxoplasma	Coxiella
Hunter	negative	negative	negative	negative	negative	negative	negative
Squeak	pending	negative	-	-		negative	negative
Tallula	negative	negative	-	-	-	positive	negative
Namor	negative	negative	-	-	-	pending	negative
Doc	n/a	n/a	n/a		positive	negative	negative

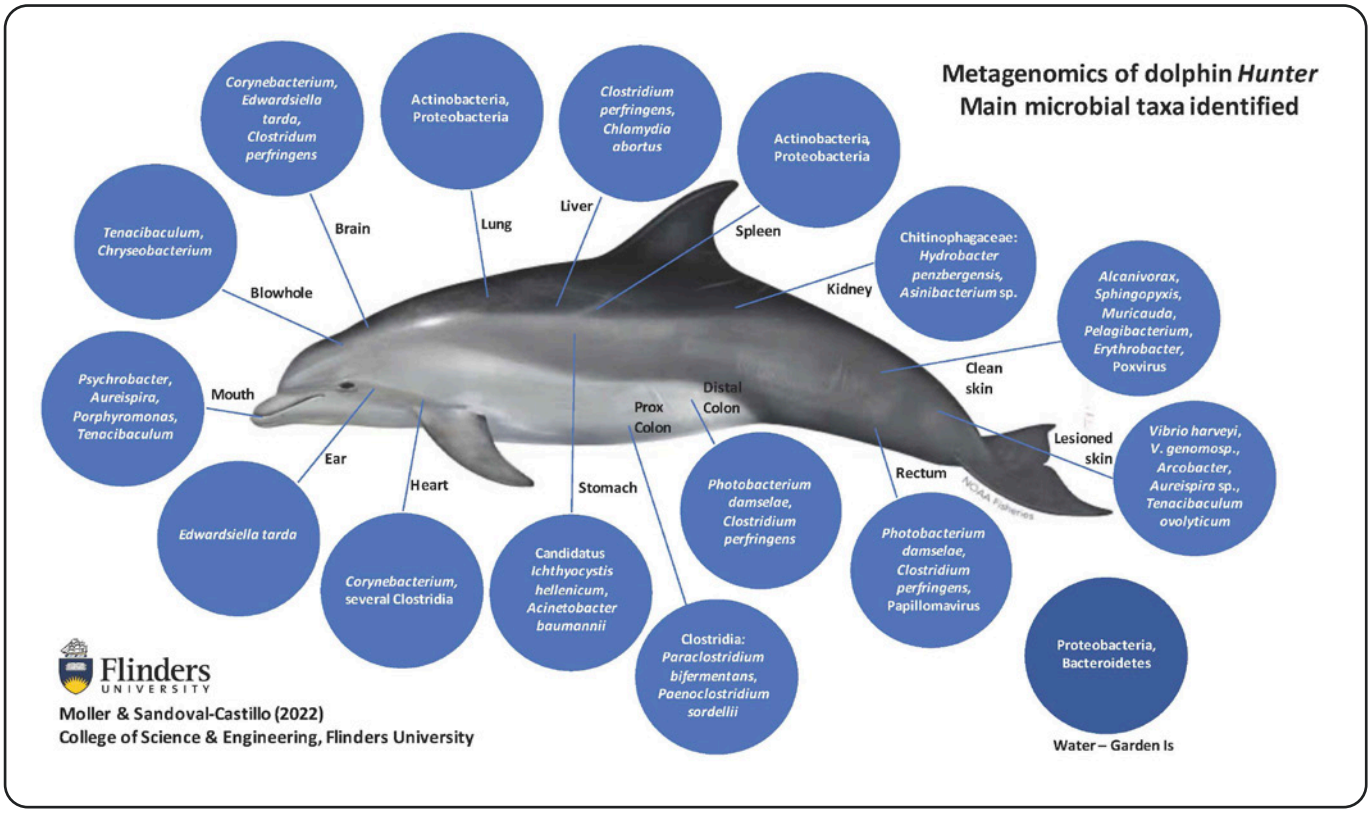


Figure 3 A summary of the main microbial taxa found in tissue samples from the ADS dolphin Hunter (Moller et al., 2022).

Metagenomics

Metagenomics can be used to study the genomic composition of an entire organism, including the community of microorganisms that exist within it.

As part of this investigation Flinders University undertook metagenomics testing of the dolphin Hunter from the ADS (Appendix 2). Swab and tissue samples were collected from Hunter and water samples of the local environment were analysed. This was undertaken to assess microbial diversity and abundance in different dolphin body regions (integumentary, respiratory, digestive, sensory, lymphatic, reproductive, urinary, central nervous and circulatory systems), and in the seawater.

Bacteria were the most abundant taxa overall, followed by small representations of viruses, fungi and *archaea*. Proteobacteria were extremely abundant in the ear sample, and abundant in the rectum, mouth, non-lesioned and lesioned skin, and the seawater. Bacteroidetes were very abundant in the kidney, and abundant in the blowhole and one skin lesion. Firmicutes were dominant in the colon samples and also abundant in the rectum, while Actinobacteria had high representation in the brain and heart.

Chlamydiae also had a moderate contribution to the micro-community in the liver. Several microorganisms of pathogenic potential, especially associated with gastro-intestinal diseases and including zoonotic agents, were identified. Bacteria associated with polluted environments were also observed in the dolphin skin.

A summary of the main microbial taxa found are illustrated in Figure 3

The study demonstrates that metagenomics is a valuable tool for microbial and pathogen surveillance in marine mammals and estuarine environments. Information from the study is important for consideration in the management of illness in ADS dolphins, in possible intervention and in mitigating risks to wildlife.

Biotoxins from Harmful Algae

Liver samples from Hunter, Tallula, and the Semaphore dolphin were conducted through the South Australian Shellfish Quality Assurance Program (SASQAP) for the presence of biotoxins that can be concentrated in seawater by algal blooms, including Paralytic shellfish poisons (saxitoxin equivalent), Neurotoxic shellfish poisons, Amnesic shellfish poisons (domoic acid equivalent), and Diarrhetic shellfish poisons. All tests were negative.

Water samples collected by Flinders University at Garden Island and Snowden Beach (in the ADS) were analysed for phytoplankton species through the NATA-accredited Microalgal services laboratory in Melbourne. The Garden Island sample (but not the Snowden Beach sample) revealed the presence of two potentially harmful species of phytoplankton (a dinoflagellate *Dinophysis acuminata* and a diatom *Pseudo-nitzschia pungen*/multiseries). While the phytoplankton were found within the water sample, their biotoxins were not detected within liver samples from Hunter, Tallula or the Semaphore dolphin as examined by PIRSA and Analytical Services Tasmania (analysed in duplicate at SASQAP, Port Lincoln and by alternative methodology at Analytical Services Tasmania).

Mortality trends

Understanding trends in dolphin mortalities over time in the ADS was important for this investigation to confirm whether the mortalities since June 2021 were atypical. The SA Museum has had a long interest in studying the dolphins of the Port River and Barker Inlet, long before it was declared a Sanctuary. They have built an extensive database of tissue samples and historic records of investigations and reports into dolphin mortalities covering a period of more than 30 years.

To determine the mortality trends, DEW commissioned the SA Museum to analyse their database. The resulting report *Dolphin mortalities and pathology in Adelaide Dolphin Sanctuary 1987 - 2020* prepared by I. Tomo, and C. Kemper of the South Australian Museum (Appendix 3) describes necropsy and life history data for dolphins stranded or dead and floating in the ADS between June 1987 and December 2020. This includes a total of 93 records comprising two cetacean species. 74 of these (66 Indo Pacific bottlenose dolphin *(Tursiops aduncus*) and 8 common dolphin (*Delphinus delphis*) were examined post-mortem at either the SA Museum, the University of Adelaide or the Australian Marine Wildlife Research and Rescue Organisation.

Objectives of the analysis 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). A database framework has been established by the SA Museum, containing information on anthropogenic events, life history data and results of pathology investigations, and a system of categorising 'circumstance of death'.

The circumstance of death for *Tursiops aduncus* was non-anthropogenic in 53.5% (n=35) of cases anthropogenic (e.g. shooting, stabbing, and fishing gear entanglements) in 17.5% (n=11), and 'unknown circumstance' in the remaining cases (30%). Non-anthropogenic causes included disease which was 40% (n= 26).

Researchers from the University of Adelaide and the SA Museum have made the observation during post mortem examinations that several male ADS dolphins had not reached the expected sexual maturity for their age (despite their known age) when compared to other wild South Australian populations. During the post mortem examinations of Hunter, Squeak and Namor it was noticed that the size of their spleen was also smaller in comparison to dolphins of similar size/age class from other areas of South Australia. The cause of this is presently unknown. The sample size is too small to draw any conclusions. Further analyses to understand what may be causing this is required. Research considerations will include genetics along with the effects of endocrine disrupting chemicals potentially leading to a range of long term adverse health outcomes.

Circumstance of death

The circumstances of death of 74 dolphins recovered from the ADS area between 1987 and 2020 are summarised in Table 7.

Table 7 Circumstance of death in ADS dolphins

Type of death	*Tursiops aduncus*	*Delphinus delphis*
Disease	26	4
Intentional killing	3	
Known entanglement	2	
Live stranded	3	
Other natural	6	
Other intentional	6	1
Unknown	20	3
Total	66	8

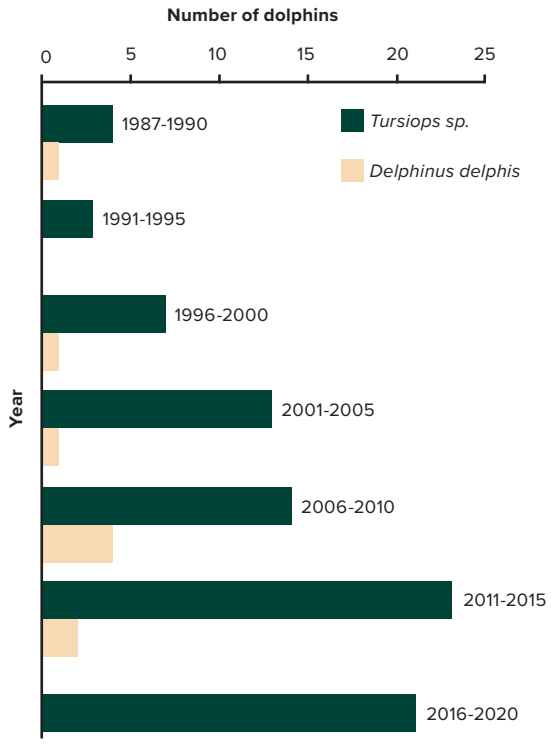


Figure 4. Frequency of dolphin mortalities in the ADS area recorded by the SA Museum between 1987 and 2020. Tursiops sp. likely were mostly T. aduncus but could include some T. truncatus.

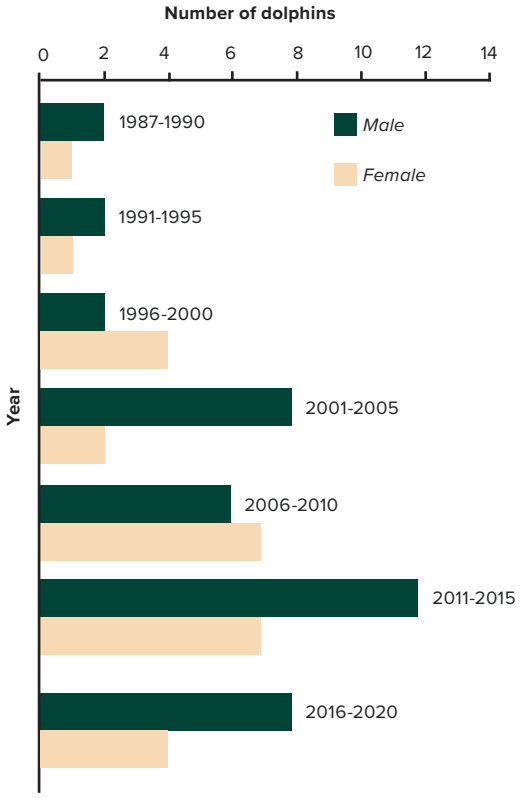


Figure 5 Sex of Tursiops sp. recovered from the ADS between 1987 and 2020.

A common observation in the recent dolphin deaths was emaciation (poor body condition). To determine whether the number of emaciated dolphins in the ADS seen recently was unusual, the SA Museum reviewed historical data and the body condition score of all examined dolphins from the 1980's and prepared a Report (Appendix 4).

Photos and notes on the external appearance of the body were made at the post mortem examination of 52 dolphins collected from 1987-2021. In some cases, additional information was sourced from photos of animals before they were made available to the Museum. Observations made at the time of necropsy were verified using the photographs.

The report found that moderately/very emaciated dolphins were found in all time periods since 1987 but the proportion appears to have been greater since 2005. The assumption is that the data after 2005 are the most meaningful because observer effort is likely to have been consistent after the creation of the ADS in 2005. 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.

ADS body condition summary (SAM Specimens, non-neonate)

Figure 6 ADS body condition summary

ADS dolphin deaths (June 2021-March 2022)

A total of six male dolphins (including one juvenile, four sub adult and one young adult dolphin) died or went missing during the period of the investigation (Table 8). Four of the six bodies were able to be recovered and post mortem examinations conducted. This included gross post mortem examination findings, infectious disease testing, toxicology testing and harmful algal bloom toxin testing. A common observation in five out of the six dolphins was poor body condition of the animals, with three rated as emaciated and two as thin. A common presentation in these dolphins was an observed weight loss/wasting 2-4 weeks prior to death.

Potential causes of emaciation can include:

- decreased food availability
- habitat degradation
- fish kills and declines in food availability
- reduced feed intake due to gastrointestinal, renal, respiratory, musculo-skeletal, and neurological disease which can interfere with feeding behaviour
- chronic disease contributing altered metabolism, or neoplasia contributing to cachexia
- toxicity
- chronic stress e.g. anthropogenic-noise, harassment from human approaches, inter species interactions.

Table 8 Summary of deaths and disappearances since 2021

Dolphin Name	Circumstance of death	Month/Year	Sex/Age class	Body Condition Score (/4)
Doc	Missing, presumed dead (health testing undertaken during a disentanglement)	June 2021	Male subadult (8 years)	Thin (2)
Twinkle	Missing presumed dead (last seen in very unwell state)	July 2021	Male adult (20 years)	*thin
Tallula	Died	August 2021	Male subadult (12 years)	Emaciated (1)
Hunter	Euthanased	October 2022	Male subadult (6 years)	Emaciated (1)
Squeak	Died	November 2021	Male juvenile (4 years)	Emaciated (1)
Namor	Died	March 2022	Male subadult (13 years)	Normal/robust (3)

*body condition score could not be assigned as body was not retrieved but photos show apparent loss in body condition

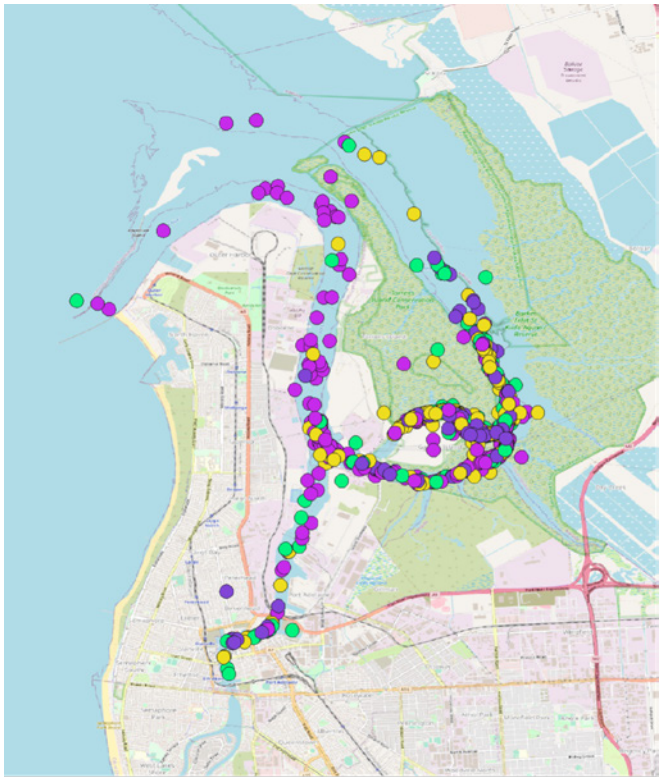


Figure 6 Core home ranges of dead male dolphins until Dec 2020 (Hope, Tallula, Twinkle, Squeak and Doc). Source: Dr Bossley

Home Ranges

Dr Mike Bossley's long term research has revealed that the five dolphins, Doc (last observed 27 June 2021), Twinkle (last seen 7 July 2021) and Tallula, Hunter and Squeak all had a core home range of the inner part of the Barker Inlet estuary. Hunter and Squeak had a core home range including North Arm, Angas Inlet and Inner Barker Inlet. This was of particular interest to note and will inform future research into the area including toxicological studies of prey species in this area.

In addition to the observations of the former home ranges of the dead dolphins, the number of weaned calves surviving in the ADS inner waters has declined since 2017. Dr Mike Bossley has been observing the dolphins and their calves, and has recorded that no calves from inner and outer ADS waters have survived to weaning since 2017. The northern waters of the ADS are harder to monitor as frequently as the inner and outer waters of the ADS and knowledge of births and calf survival in these outer waters is not well known. Calves have been observed in outer ADS waters since 2017 however they are not sighted as frequently on surveys to monitor their movements.

Seasonality

The wasting and mortalities seen in ADS dolphins in 2021 all occurred during the winter-spring period, with mortalities reported in June (1), July (1), August (1) and November (1). In 2022, a single case of wasting and death occurred in August (data not included in this report). Further investigations are underway to determine significance in seasonality of deaths and the importance of factors such as rainfall, stormwater volume, water temperatures, and seasonal activities within the Port River area.

ADS population

The 2008 ADS management plan promotes non-invasive research methodologies and as such most of what we know about the health of the dolphins which inhabit the ADS has been gained through the collection and studies on dead animals. The SA Museum has been undertaking post mortems (necropsy) on the ADS dolphins since the 1980's. Post mortem studies are critical to understanding a range of factors that may have contributed to the cause of death of a dolphin. More recently, the University of Adelaide's Veterinary Health Centre at Roseworthy have also been undertaking the examination and study of dolphin health in partnership with researchers from the SAM and Flinders University.

Throughout the investigation, all retrieved dolphin bodies have been studied collectively by scientists and Veterinary Pathologists at the SA Museum, Adelaide and Flinders' Universities. All post mortem examination reports have been made available on the Department for Environment and Water's website (Appendices 5,6,7,8,9).

Researchers are in a fortunate position to be able to combine post mortem findings with life history data of ADS dolphins. Dr Mike Bossley has been studying the Port River Dolphins for 34 years. The non-invasive field research that Mike has conducted over the years has developed an important baseline of knowledge about the way dolphins use their habitat, their social structures and the threats they face. These boat based surveys have focused on the Port River, Barker Inlet and Outer Harbor waters.

In addition to Dr Bossley's research since 2015, the NPWS rangers have been conducting monthly boat based surveys of the ADS, which takes in the full range of the Sanctuary including Port River, Barker Inlet, and Outer Harbor to the waters near Port Gawler.

Volunteers from Whale and Dolphin Conservation (WDC) and the ADS Action Group also regularly monitor the dolphins from land based locations in the inner water of the Port River, Garden Island and Outer Harbor. This monitoring will provide important information about the movement patterns of individual dolphins occupying these waters.

It is not yet known what proportion of the total dolphin population the deaths recorded in this investigation make up.

Researchers have commenced analyses of the collected dolphin population data to estimate the total abundance of dolphins within the ADS over that time period. Further studies by Flinders University are also underway to determine population ecology and viability, ranging patterns and population structure, genetic diversity and inbreeding levels of bottlenose dolphins from the Port River system.

Conclusion

1. The research so far has not found evidence of a single cause responsible for the recent deaths.

Extensive testing has not been able to determine a single common link between dolphin deaths however there are some commonalities. All deaths (from June 2021-March 2022) were young males with a similar home range in the inner waters of the ADS. There was noticeable wasting of body condition observed over a short period of time, and there were opportunistic infections. With the exact cause of death being inconclusive it is likely multiple factors in combination led to the death of each individual.

Despite extensive testing as conducted through this investigation it is not unusual to not be able to find identify a cause of death in cetaceans. Unusual Mortality Events (UME) have occurred in dolphin populations elsewhere in the world, particularly over the last two decades and often without a cause being identified.

2. The ADS Dolphins are exposed to a greater range of anthropogenic stressors than offshore populations in South Australia. There are likely to be multiple contributors to dolphin morbidity and mortality not just a single source.

Wild marine mammals are exposed to multiple natural and anthropogenic environmental stressors. Concerns about anthropogenic stressors faced by wild marine mammals include increased environmental exposures to pathogens, pollution, and noise (Fair and Becker, 2000). There remains a large gap in knowledge about the effects of both acute and chronic stress in marine mammals.

The ADS dolphins live within a heavily industrialised environment with legacy pollutants at the end of one of SA's most urbanised catchments and busiest shipping ports. As a result the dolphins are exposed to waterfront industry, noise pollution, and high recreational water uses such as boating, fishing and kayaking. Although some of these threats may also affect other dolphin populations, they are likely not to the same extent of exposure that ADS dolphins experience in comparison to other wild dolphin populations in SA.

3. It is hypothesised that the ADS dolphins with a home range focused on the inner part of the Port River and Barker Inlet may have a compromised immune response.

Toxicological investigations, whilst extensive, have been non-exhaustive and further work is ongoing (e.g. using non-targeted assays, development of a Cumulative Effects Model to examine the effects of compounding stressors and toxicant exposure). Further research into endocrine disrupting chemicals (EDC's) in ADS sediments and water and their potential effects on ADS dolphin health is required. Understanding the pathways of bioaccumulation and biomagnification of contaminants though the food chain for dolphins as top order predators is recommended. If there is low genetic diversity and a degree of inbreeding in the ADS dolphins, they may exhibit low genetic variation at immuno-related genes, which could lead to greater susceptibility to disease. Further genetic research of ADS dolphins is required to clarify this.

4. Intentional harm is not the cause of dolphin deaths

In all of the post mortem examinations of dolphin deaths conducted from June 2021-March 2022 by veterinary pathologists there was no indication of intentional harm in any of the dolphins. In the circumstance of death data recorded by the SAM it describes a decline in intentional killing after the ADS was established in 2005.

Number of indo-Pacific bottlenose dolphons related to circumstance of death in the Adelaide Dolphin Santuary South Australia before (n = 20) and after (n = 27) gazetting.

Circumstance of death	1987-2004	2005-2013
Unknown	5	5
Anthropogenic		
Intentional Killing	2	0
Entanglement	1	0
Other unintentional	3	2
Non-anthropogenic		
Disease	4	17
Other natural	4	2
Live stranding	1	1

Figure 7 Circumstance of death of ADS dolphins. Source: S.K. Adamczak et al 2018

There have been no known intentional killings of dolphins in the ADS since they were reported in 1998. Strict enforcement of the law is the most likely reason for the decrease in anthropogenic related deaths in the sanctuary (Adamczak et al, 2018).

Recommendations

A number of studies have been initiated to examine potential causes of dolphin deaths.

Toxicological studies and the necropsies of the recent mortalities have not identified a single common cause of death for all animals. Current expert advice suggests immunosuppression as a key factor and investigations into possible causes, such as long lived chemicals and pollutants present in the ADS environment are being followed up. The studies listed below are now underway and seek to provide greater insight into the presence of and possible pathways of a variety of contaminants potentially affecting the dolphins, through both toxicology studies of dolphin samples, novel genomic studies, monitoring of dolphin skin lesions and body condition, sampling of fish (food pathways) and reviewing past studies of dolphins, sediments and water.

The pollution and other studies are complemented by a series of longer term examinations of the Port River dolphin population and their associated environment in collaboration with Adelaide and Flinders Universities and Dr Bossley. These studies will continue for the next three years.

Research and monitoring program recommendations

- Continue to monitor the ADS dolphin population, including early signs of ill health in individuals such as through the presence of skin lesions and assessment of body condition.
- Until a known cause for the current trends of dolphin deaths is identified efforts should continue to conduct post mortems on all dolphin deaths in the ADS, including microbiology studies.
- Undertake research on potential factors that may be contributing to immune suppression and delayed sexual maturity and spleen size in some ADS dolphins, which may include genetics, endocrine disrupting chemicals and emerging contaminants of concern.
- Further research should be initiated to compare dolphin immune response in young adults and calves to better understand why so few calves are surviving to weaning.
- Targeted and long-term monitoring of stormwater and microbiology studies should be undertaken by researchers to understand sources of disease and pathogens entering the Port River and Barker Inlet (given their potential to cause secondary infections in ADS dolphins).
- Targeted monitoring of dolphins' dietary sources for a range of pollutants that have potential for bioaccumulation through the food chain. To build on previous monitoring programs and inform the assessment of pollutant availability to dolphins via dietary uptake.
- Targeted monitoring of translocated mussels for a range of pollutants that have potential for bioaccumulation through the food chain. To build on previous monitoring programs and inform the assessment of pollutant bioaccumulation.

- A risk assessment of the potential leachate seeping from landfill sites (including the closed Garden Island and Wingfield landfills) into adjacent marine waters should be considered. The flux rate of pollutants from landfills into nearby marine waters is not anticipated to be significant, but given the context there is justification to set up an initial desktop assessment (using currently available data) of potential for pollutant source, pathway and receptor (ADS dolphins and their prey) connectivity. That assessment could then provide decision support for further work if warranted.
- The use of new technologies should be encouraged, e.g. drones to understand dolphin behaviour and assess body condition, assess water quality, harmful algal blooms, biotoxins and climate change impacts on the ADS environment.

Management recommendations

- Polluted stormwater can cause significant ecological changes to urban streams and coastal waters. In South Australia, stormwater management is a responsibility that is shared between the state government, local government and private landowners. The management of stormwater is therefore complex, involving multiple jurisdictions and council areas. It is recommended that DEW, EPA and relevant local governments work collaboratively on improving local stormwater quality and management. A targeted approach to the monitoring of stormwater may be of benefit to potentially target and reduce secondary infections from disease pathogens entering the system through stormwater.

  There are many challenges in how to deal with long-term environmental and health impacts of legacy pollutants, particularly within a marine environment. Remediation measures such as removal of sediments can exacerbate their effect, through the resuspension of contaminated sediments in the water column allowing for them to again become bioavailable to a range of species. The EPA requires all licensed dredge operators to develop a Dredge Management Plan. These plans take into consideration studies of benthic habitat and assessments of the sediments and any contaminants present prior to dredging to inform methods of removal which reduce resuspension and disposal options. Based on that information the impact to marine environments including the Port River can be minimised or prevented.

- The investigation team noted that because the deaths by disease cannot currently be attributed to a specific cause, and that immune system deficiency may be an underlying issue for the ADS population, undertaking operations which cause additional stress through capturing animals for testing was not recommended at this point in time. The team noted that there could still be benefits to undertaking this testing in the future in order to understand contributing health factors, particularly if the deaths or trends in unexplained poor body condition continued. Consideration should be given to undertaking tests for sick dolphins and the critical health related information they could provide including a base level of information in the case that the dolphin died and the body could not be recovered. Consideration should also be given to the taking of samples from healthy dolphins within the Sanctuary as part of a dedicated dolphin health project which could improve knowledge of genetic diversity and health parameters within the Sanctuary's dolphins.

Research in the ADS

The following future research and monitoring projects are committed to and will occur within the ADS to address gaps in knowledge which could lead to important management outcomes for the Sanctuary.

Flinders University

- Fishes as indicators of dolphin health within the Adelaide Dolphin Sanctuary, South Australia
- ADS Dolphin Health and Port River Study
- (PhD Kennadie Haigh) Population ecology and viability of Indo-Pacific Bottlenose Dolphins (*Tursiops aduncus*) in the Adelaide Dolphin Sanctuary.
- (Honours) Skin lesions as an indicator of health in Indo-Pacific Bottlenose dolphins (*Tursiops aduncus*) of the Adelaide Dolphin Sanctuary.
- (Honours) What is the state of harmful algal blooms (HABs) in the ADS and Port River system?
- (Honours) Ranging patterns of Indo-Pacific Bottlenose Dolphins (*Tursiops aduncus*) in the Adelaide Dolphin Sanctuary
- (Honours) Population structure, genetic diversity and inbreeding levels of bottlenose dolphins from the Port River system.

Adelaide University

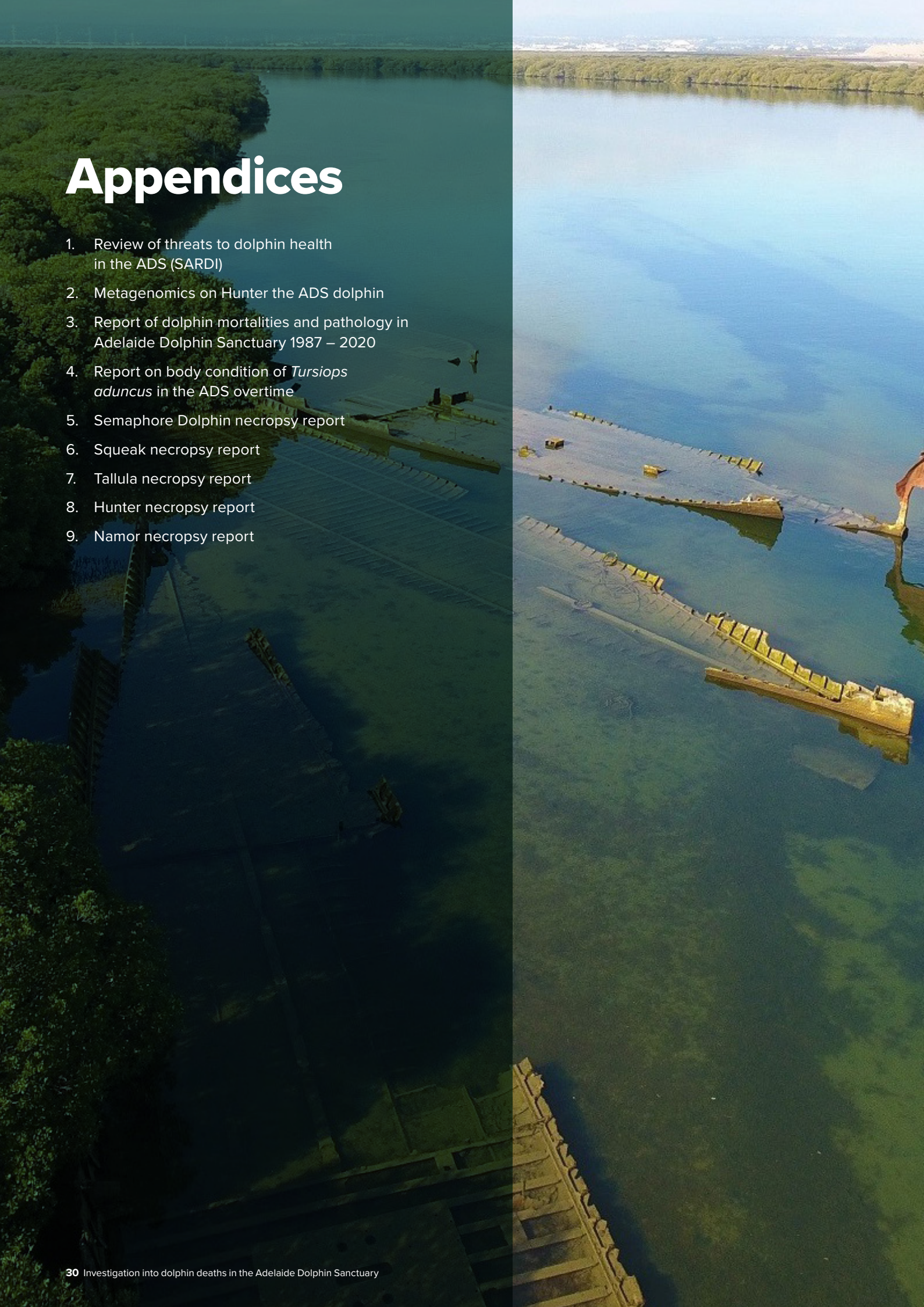
- PhD (Rebecca Souter). This project will work to elucidate the causes of morbidity and mortality in the ADS dolphins, as well as examine the health and threats to health and welfare of dolphin species across South Australia more broadly. This work will complement the PhD project examining environmental health in the ADS led by Flinders University. Including:
  - Investigation of effects of weather events on dolphin mortality events
  - Identification of toxicants of significance and their effect on disease expression and the endocrine and immune systems of dolphins
  - Investigation of the effect of Endocrine disrupting chemicals with Dr Anu Kumar (CSIRO) and Griffith University
  - Ongoing pathological surveillance of *Tursiops* and *Delphinus* species mortality events in South Australia
- PhD (Sophie Dolling). This project is assessing whether microplastics can act as a vector for the transportation of PFAS pollution through the Port River and Barker Inlet systems. The project aims to:
  - Identify whether PFAS is accumulating on microplastic pollutants at a higher concentration than surrounding waters in the Port River and Barker Inlet systems.
  - Analyse microplastic and PFAS pollutant load in common prey items of the bottlenose dolphin in the Port River and Barker Inlet systems.
  - Evaluate whether plastic pollution may be contributing to bioaccumulation and biomagnification of PFAS in the bottlenose dolphin population in these systems.

Government programs

- Metals in mussels (EPA). The EPA has assessed metal levels in translocated blue mussels in estuarine and coastal environments across South Australia. Mussels are recovered after a period of time and analysed for total metal load in the flesh of the mussel. In the past results have showed vast differences in metal concentrations between different regions and also compared to the baseline metal concentration. Many of these spatial differences can be attributed to the level of industrial activity in the different regions. Sampling for this program was last performed in 2009 and the EPA is considering a repeat of this work and possibly expand it to include persistent organic pollutants as well.
- Metals and persistent organic pollutants in fish (EPA). This is a program that the EPA has previously coordinated. The EPA have engaged skilled PIRSA expertise to collect fish from various estuarine and coastal environments (similar to the mussel monitoring program). Fish monitoring for pollutants is more complex that mussels because fish are mobile and their sampling cannot guarantee uniform fish age. So data interpretation needs to be nuanced, although it is useful for recommendations with respect to food advisories and to inform dietary uptake by dolphins. Sampling for pollutants in fish was last performed in 2012 and the EPA is considering a repeat of this work.
- National Parks and Wildlife on-going monthly dolphin population surveys.

Appendices

1. Review of threats to dolphin health in the ADS (SARDI)
2. Metagenomics on Hunter the ADS dolphin
3. Report of dolphin mortalities and pathology in Adelaide Dolphin Sanctuary 1987 – 2020
4. Report on body condition of *Tursiops aduncus* in the ADS overtime
5. Semaphore Dolphin necropsy report
6. Squeak necropsy report
7. Tallula necropsy report
8. Hunter necropsy report
9. Namor necropsy report



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