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Review of threats to dolphin health in the Adelaide Dolphin Sanctuary

Roger Kirkwood, Marty Deveney and Simon Goldsworthy

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March 2022

A Report to the Department for Environment and Water

Government of South Australia
Department of Primary Industries and Regions

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SARDI
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EXECUTIVE SUMMARY

Dolphins living in coastal areas are challenged by many anthropogenic activities, including habitat modification, boating and fishing, and introduction of toxic chemicals. This report reviews threats to Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in the Adelaide Dolphin Sanctuary (ADS), located in the Port River and Barker Inlet, South Australia, as they were understood in early 2022.

Indo-Pacific bottlenose dolphins are high trophic-level marine predators in a highly modified environment. The ADS is adjacent to the city of Adelaide, established as a British Province in 1836 and with a population of 1.4 Million in 2021. Much of the original mangrove and saltmarsh vegetation that surrounded the Port River and Barker Inlet has been removed, water courses have been altered and channels dredged, runoff from the city and discharge of chemicals and waste from numerous industries have entered or been discharged to the inlet. Attempts to mitigate potentially toxic materials entering the inlet have been ongoing for at least the last 50 years. Despite substantial improvements in water quality, anthropogenic waste and runoff continues to enter the waters, and sediments and food-chains undoubtedly continue to contain significant industrial residues.

The bottlenose dolphins that enter the Port River and Barker Inlet are a component of a broader Gulf St Vincent population. Some dolphins predominantly reside in the inlet, others come and go frequently, and some enter occasionally. Through recognition that the dolphins were important consumers in the inlet, an asset to the community of Adelaide, and threatened by on-going human activities, an Adelaide Dolphin Sanctuary was gazetted over the inlet and adjacent waters of Gulf St Vincent in 2005.

Monitoring of dolphin presence and behaviour has been conducted by interest groups and researchers since the 1980s. Many individual dolphins are recognisable. Following establishment of the sanctuary it appeared that dolphin numbers were increasing. After 2010, however, a decline became apparent. Fewer calves were being raised to weaning age and there appeared to be an increase in the mortality rates of adults. In 2021, there was four confirmed and two unconfirmed mortalities of young (<14 years old) male dolphins. Five of the six were known individuals that had appeared otherwise healthy, then suffered rapid deterioration in body condition. While the ultimate causes of the deaths could be determined for several of the dolphins (see Table 2), the reasons for initiation of the deterioration in condition were not known.

The report documents the considerable variety of factors and their sources that threaten the health of coastal bottlenose dolphins which, in turn, aims to aid investigations into a recent apparent decline of Indo-Pacific bottlenose dolphins in the ADS. The coastal environments where these dolphins reside are anthropogenically modified and contain higher than back-ground levels of toxins that could initiate poor health or accelerate deterioration following exposure to another threat. While the cause of the apparent local decline of dolphins in the ADS remains uncertain, there are several probable contributing factors. There is likely to be contributions from toxins and stress, leading to immune suppression, and factors such as pre-existing and introduced or epidemic pathogens that cause deterioration in condition and organ functions. In many instances, the ultimate cause of death may differ from the pathway(s) and factors that cause ill-health.

Keywords: *Tursiops aduncus*; Port River; Barker Inlet; legacy toxins.

1. INTRODUCTION

1.1. Background

Dolphins are small (<3 m) toothed cetaceans, which are entirely aquatic marine mammals. They feed underwater but must return to the surface to breath. Three species of dolphin are found in South Australia: the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), found in coastal waters, estuaries and embayments, the common bottlenose dolphin (*T. truncatus*), in the gulfs and along oceanic coasts, and the common dolphin (*Delphinus delphis*), in the gulfs and shelf waters (Kemper et al. 2008, Filby et al. 2010, Parra et al. 2021). Offshore, common dolphins are more abundant than bottlenose dolphins. All three species are globally abundant but have genetically distinct regional populations.

Globally, the International Union for Conservation of Nature (IUCN) class Indo-Pacific bottlenose dolphins as 'Near Threatened', and both the common bottlenose dolphin and common dolphin as 'Least Concern' (IUCN 2021). The IUCN conducts regional assessments of conservation status in addition to species-level assessments, in recognition of the possibility and significance of extinction of distinct locally adapted populations (Currey et al. 2009a). There are, however, insufficient data on many populations of Indo-Pacific bottlenose dolphins to assess their risk of extinction (Braulik et al. 2019).

Human activities can threaten the survival of coastal dolphins. Impacts on water and sediment quality, and fishing activity, can threaten the food chain, while boating and fishing activity can result in noise-induced exclusion, collisions and entanglements (Reeves et al. 2003, Braulik et al. 2019). Coastal dolphins are exposed to marine pollution in food in a similar manner to humans who frequently consume seafood, but also live in and drink waters that receive pollution. Indications of disease in dolphins, therefore, has implications for humans who eat regularly from, or live in, the same areas (Durie & Jones 2006, Mancia et al. 2015). Declines in coastal dolphin populations may be evident but difficult to attribute to a cause. For example, in New Zealand, a 7.5% decline per year over 26 years of a population in the Bay of Islands was attributed to 'change in habitat, mortality and possibly low recruitment' (Tezanos-Pinto et al. 2013).

The Adelaide Dolphin Sanctuary was gazetted in 2005 to aid protection of Indo-Pacific bottlenose dolphins in the Port River and Barker Inlet, South Australia (Adamczak et al. 2018). Decreasing trends in populations of coastal bottlenose dolphins have been recorded in Shark Bay, Australia (Bejder et al. 2006b), Sado Estuary, Portugal (Augusto et al. 2012), Bay of Islands, New Zealand

(Tezanos-Pinto et al. 2013) and Guayaquil, Ecuador (Félix et al. 2017). They have been attributed to a combination of impacts, such as bycatch, vessel activity, pollution, and habitat degradation. Few studies assess the potential stressors individually and collectively to a depth that could inform a comprehensive management response to mitigate individual and cumulative impacts (Félix et al. 2017).

A key impact on coastal dolphins that is difficult to attribute to a single cause is immune-suppression (Desforges et al. 2016). Marine mammal exposure to several environmental pollutants is associated with changes to innate and adaptive immunity, which include cellular and humoral (body fluid) immunity (Desforges et al. 2016). Immunocompromise can be caused by multiple sources including disease (viral – most common, bacterial and parasitic); pollutants (e.g., PCBs, hydrocarbons, heavy metals and toxic algal blooms); or other stressors, such as food shortage or altered water quality. Due to their ecology and life-history, marine mammals accumulate some of the highest levels of environmental contaminants of all wildlife (Desforges et al. 2016).

1.2. Objectives

This report derives from an initiative to review the global literature on threats to coastal dolphins to aid investigations into the recent decline of Indo-Pacific bottlenose dolphins in the Port River and Barker Inlet.

The review aims to summarise the broad scale challenges and potential impacts on coastal dolphins from anthropogenic activities, with a focus on the Indo-Pacific bottlenose dolphins residing in the Port River and Barker Inlet, South Australia. To direct the review, the South Australian Department for Environment and Water (DEW) in consultation with many experts, developed a conceptual model of the potential impacts on bottlenose dolphins in the Port River and Barker Inlet (Appendix 10.1, Figure 10).

2. PORT RIVER AND BARKER INLET

The Barker Inlet of the Port Adelaide River estuary is characterised by mudflats, adjoining seagrass meadows, intertidal saltmarsh, grey mangrove forests, and supratidal samphire wetlands (Figure 1) (Thomas et al. 2001).

Prior to European settlement, sand plains ran between the coast of Gulf St Vincent and the Port River, with an extensive area of supratidal samphire saltmarsh occurring inland from the sand bar (Belperio & Rice 1989). Most of the low-lying coastal region was covered by saltmarsh. Adjacent to the Port River and the network of tidal creeks comprising the Barker Inlet, was an intertidal mangrove woodland. The surrounding natural catchments provided freshwater runoff into the area, creating a biologically diverse habitat (Edyvane 1999, Harty 2004).

In 1895, a coastal embankment (bund wall) was constructed around Barker Inlet, on the landward side of a mangrove woodland (Burton 1984). The aim of the embankment was to drain and claim this low-lying coast for industrial purposes. Aided by breaches and subsidence around the embankment, mangrove woodlands were able to expand inland into former saltmarsh areas. In 1935, at a distance inland from the colonising mangroves, levee banks were constructed to create evaporation basins for salt harvesting. The Dry Creek Salt fields stretch for 35 km along the coast in a narrow strip (Coleman 2013, in Thomas et al. 2001). Further aided by the altered tidal regimes of the salt fields and further subsidence due to ground water extraction, mangrove woodlands continued to expand inland over former saltmarsh habit to the levee banks surrounding the salt evaporation ponds (Burton 1984, Belperio 1993).

Up to the 1950s, the area of mangrove woodland in the Port River and Barker Inlet had likely increased from pre-European extents (Cann et al. 2009), although there was also some clearing of mangrove for access and industry. In contrast, there was considerable reduction in the salt-tolerant samphire vegetation. By 1984, there had been an estimated 80% reduction in the area of samphire from pre-European times (Fotheringham 1994). Since the 1950s, the extent of the mangrove vegetation in the estuary has also declined, however, largely due to pollution. Outflow from the Bolivar waste treatment plant, high pH and hypersaline discharge from the salt evaporation basins have been implicated in die-offs of mangrove vegetation (Burton 1984, Overton 1993, Thomas et al. 2001).

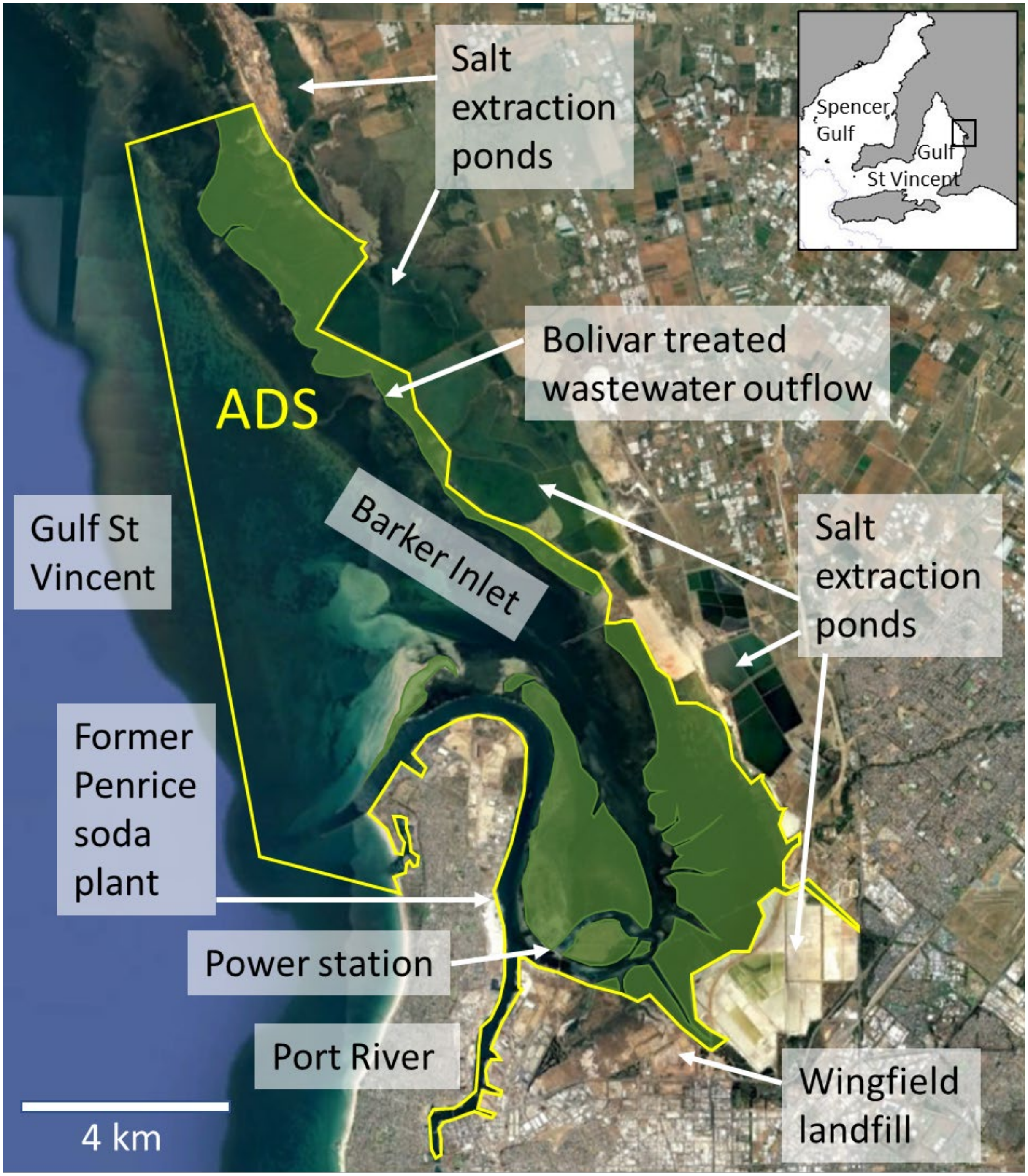

Figure 1. The Adelaide Dolphin Sanctuary (ADS, indicated by yellow outline), Adelaide, South Australia. Light-green areas indicate islands and intertidal mangrove. In the water, dark areas indicate seagrass or deeper water and lighter areas are sand bottom.

An outcome of construction of the embankments and levee banks around Barker Inlet was the exposure and drying of acid sulfate soils in the drained areas, exposing pyrite in the organic rich mangrove and samphire soils (Harbison 1986). Pyrite oxidation leads to the production of sulfuric acid, causing acidification of soils and interstitial waters, leading to degradation of the immediate and receiving environments (Harbison 1986, Thomas et al. 2001). Groundwater in the area has regularly reached pH of <3.5 (Thomas et al. 2001). Thomas et al. (2003) developed a conceptual model illustrating chemical and physical changes that occur when tidal influences are excluded from sulfidic materials in mangrove sediments (Figure 2).

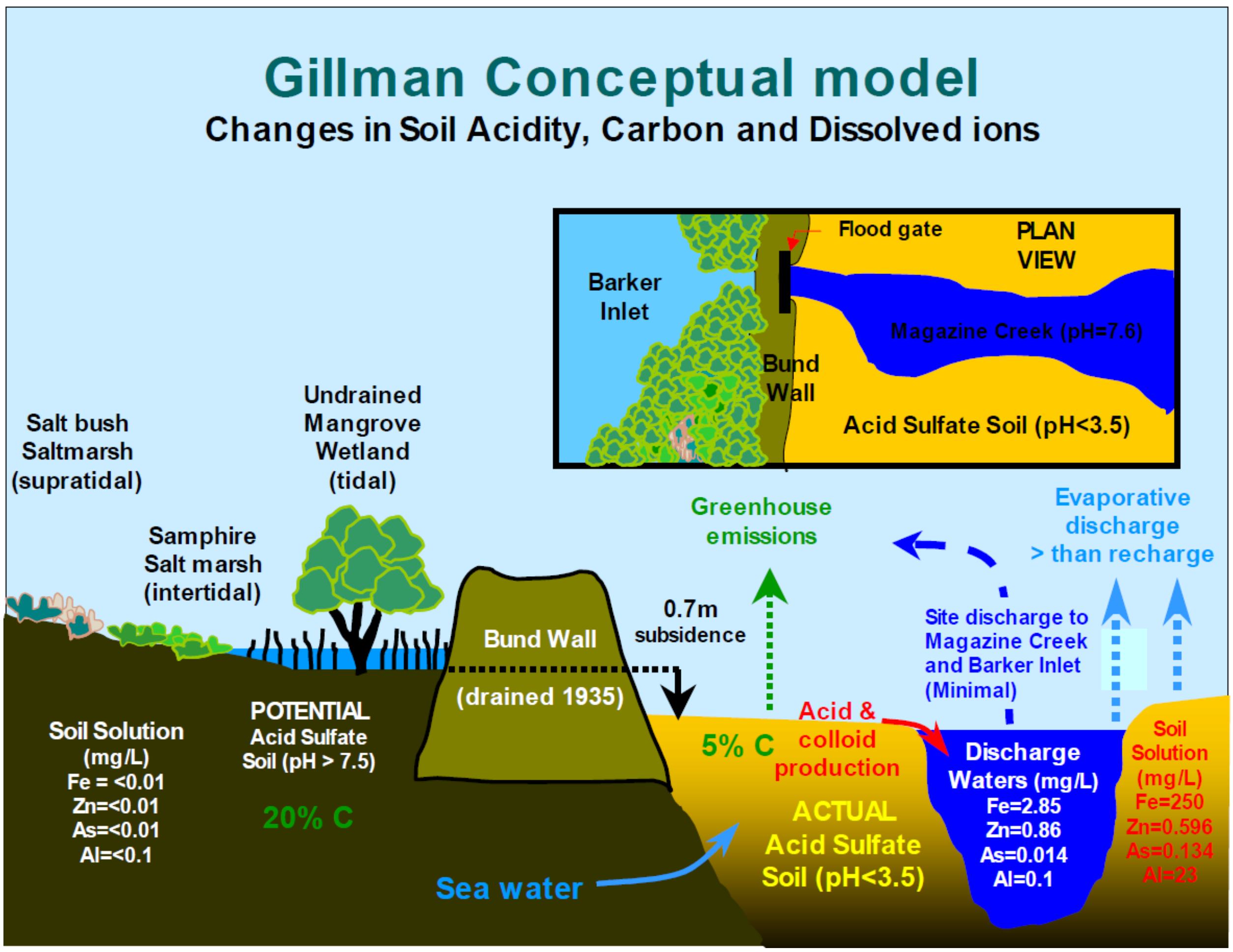

Figure 2. Conceptual model illustrating chemical and physical changes that occur when tidal influences are excluded from sulfidic materials in mangrove sediments (Thomas et al. 2003).

Examples of metal pollutants recorded in the Port River and Barker Inlet include high concentrations of aluminum, arsenic, iron, zinc, lead and manganese in sediments or adjacent ground water (Harbison 1986, Thomas et al. 2001). Anomalous levels of arsenic in the water column at several sites along the Port River estuary were linked to an old Acid Plant at Snowden's Beach (referenced Kinhill Delfin Joint Venture 1990, in Thomas et al. 2001). Increased nutrients from sewage treatment works, industrial discharge and stormwater runoff support occasional toxic and non-toxic algal blooms, which may have exacerbated low oxygen levels in the water column, leading to increased release of metals and further nutrients from the sediments.

Because the waterways do not have significant riverine inputs, the Port waterways (including the Barker Inlet) are best described as an embayment rather than an estuary (Pfennig 2008). The absence of freshwater environmental flows is not as important in this system as it might be in other river catchments and estuaries. A saltwater flow-through system was constructed in the 1970s that draws 500 million litres of seawater per day into West Lakes and discharges it through the Port River (EPA 2003). Salinity in the inlet varies seasonally between 35 and 41 ppm with no significant geographic variation during winter months and with only slightly higher salinities in the inner and mid parts of the estuary during warmer months (Jones et al. 1996).

Within the sheltered shallow creeks of the Port River and Barker Inlet, mangroves provide important nursery and feeding areas for several economically important species such as King George whiting (*Sillaginodes punctatus*), western king prawn (*Penaeus latisulcatus*), yellowfin whiting (*Sillago schomburgkii*) and southern sea garfish (*Hyporhampus melanochir*) (Jones 1984, Jones et al. 1996). The adjoining Gulf St. Vincent also contains some of the largest and most diverse areas of temperate saltmarshes in Australia (Edyvane 1999).

Wastewater and stormwater inputs off the Adelaide metropolitan coast have led to periodically degraded water quality, and contributed to the loss of over 5,200 ha of seagrass and significant macroalgal reef degradation along the metropolitan coast (Gaylard 2009). After recognition and amelioration of anthropogenic pollutants commenced in the late 1900s, sources of pollution into the Port River and Barker Inlet have undoubtedly reduced (for example, through stricter stormwater runoff controls, removal through dredging of sediments that could contain legacy toxins, and improved effluent treatment processes). Some pollutants, however, will continue to find their way into the inlet, while those that have accumulated in sediments and food-chains may continue to impact marine and inter-tidal flora and fauna, including dolphins. In addition, new types of pollutants and their sources are likely to be identified.

3. ECOLOGY OF COASTAL BOTTLENOSE DOLPHINS

Indo-Pacific bottlenose dolphins are morphologically distinguishable from common bottlenose dolphins. They are slenderer, their beak is longer, their teeth are finer and adults have black spots or flecks on their bellies (Wang & Yang 2009). Indo-Pacific bottlenose dolphins are also lighter blue in colour, have a distinct cape and a light spinal blaze extending from the head to below the dorsal fin (Wang & Yang 2009). They likely became genetically distinct from common bottlenose dolphins around 1 million years ago (Moura et al. 2020), although the species' contemporary ranges overlap.

Populations of Indo-Pacific bottlenose dolphins inhabit many bays and estuaries around the Indian and eastern Pacific Ocean: including the west African coast (Christiansen et al. 2010), India and south-east Asia, China, Japan and Australia (Culik 2004, Wang & Yang 2009). Coastal populations in Australia are found in all states and have been specifically studied in Queensland (Chilvers et al. 2005, Ansmann et al. 2013), New South Wales (Fury & Harrison 2008, Wiszniewski et al. 2009, Fury & Harrison 2011b), Victoria (Charlton et al. 2007, Charlton-Robb et al. 2011), Western Australia (Chabanne et al. 2012, Chabanne et al. 2017, Haughey et al. 2021) and South Australia (Möller et al. 2006, Kemper et al. 2008, Bossley et al. 2017, Bilgmann et al. 2019). A hierarchical metapopulation structure was revealed along southern Australia, with at least six genetically distinct populations, inferred from mitochondrial DNA control region sequences and microsatellite loci, occurring between Esperance (WA) and southern Tasmania (Bilgmann et al. 2007, Pratt et al. 2018). A population phylogenetic study showed that dolphins in the Port River and Barker Inlet clustered with other Gulf St Vincent groups (Port Wakefield, Stansbury and Cape Jervis) and were distinct from populations in adjacent Spencer Gulf (Pratt et al. 2018).

Maturity in Indo-Pacific bottlenose dolphins occurs at 5-12 years for females (Kemper et al. 2019) and 10-12 years for males (Connor et al. 2019). Females are pregnant for 12 months, usually give birth in late summer (December to April), and suckle calves for up to 18 months. First-year calf mortality (30%) and pre-weaning mortality (46%) were higher in the Port River than have been described for other locations (Steiner & Bossley 2008). Calf mortality is particularly high for primiparous (80%) and old multiparous females (73%) (Crook 2020). The inter-birth period for Indo-Pacific bottlenose dolphins in South Australia is estimated to be 3.8 to 4 years (Steiner & Bossley 2008, Kemper et al. 2019). Indo-Pacific bottlenose dolphins grow to around 2.7 m long and 200 kg in weight and have a lifespan of 30-40 years (Wang & Yang 2009, Kemper et al. 2019).

Coastal bottlenose dolphins appear to form long-term social groups based on sex and age (Shane et al. 1986). Groups are mostly of mixed sex or of females and calves (Fury et al. 2013). Habitat use by female dolphin groups suggests that shallow tributaries may provide a sanctuary from aggressive males (Fury et al. 2013). Movement patterns of individuals are extremely variable from location to location but are relatively predictable at any given site. Food resources are one of the most important factors affecting movements. Bottlenose dolphins are generally active during the day and night. Interactions with humans are frequent, with human activities being potentially helpful, harmful or neutral to the dolphins (Fury et al. 2013). One study has found that bottlenose dolphins occurring in environments with less anthropogenic pressure exhibit a higher behavioural complexity so are considered to be less disturbed (Cribb & Seuront 2016).

Along the Adelaide metropolitan coast (not including the Port River and Barker Inlet), bottlenose dolphins favour shallow nearshore areas and reefs in summer, and deeper waters in winter (Zanardo et al. 2017). Abundance estimates based on capture-recapture models ranged from 95 individuals in winter to 239 in summer, comprising year-round residence, seasonal visitors and occasional visitors (Zanardo et al. 2016). The seasonal change in distribution appears to be driven by prey availability. Within the 30 km coast, two distinct socially cohesive communities have been identified, a northern and a southern-offshore group (Zanardo et al. 2018). The relationship between these dolphins along the metropolitan coast and dolphins that enter the Port River and Barker Inlet has not been investigated. The fine scale variations in distribution and social network highlight the importance of identifying the relevant local population when investigating impacts on a population (Chabanne et al. 2017). The individual and population level consequences of behavioural changes in response to human activity are difficult to quantify.

4. COASTAL DOLPHINS IN THE ADELAIDE DOLPHIN SANCTUARY

It is likely that Indo-Pacific bottlenose dolphins frequented the Port River and Barker Inlet for thousands of years prior to European colonisation. Subsequent fishing and removal of fish habitat (seagrass and mangroves) in the Inlet would have reduced its dolphin carrying capacity, and effluent from industry and suburban runoff could further have reduced dolphin numbers before any monitoring of their residence. During the 1950s, for example, dolphins were rarely sighted in the Port River and Barker Inlet (Peter Shaughnessy, pers. comm.). Since the 1950s, efforts have been made to reduce the input of waste and toxins into Inlet waters. The gradual clean-up of water quality may have assisted the return of the dolphins. Current occupation by dolphins, which are sentinels for a healthy marine ecosystem (Wells et al. 2004), attests to a level of quality of the habitat.

Dolphins in the ADS have been monitored through near-monthly surveys conducted since 1990, each survey lasting 3-4 hours, along the same 40 km route, with Beaufort sea-state <3 (Bossley et al. 2017). On average, five groups of Indo-Pacific bottlenose dolphins were encountered per survey, with an average group size of 3.7 dolphins. The dolphins exhibit a mosaic of home ranges (Bossley & Rankin 2015). Some predominantly reside in the estuary, others come and go frequently, and some enter occasionally. The age structure of dolphins in the survey area in 2015 was: 42% adult (>10 years old), 26% sub-adult (4-10 years), 20% juvenile (0.5-3 years), and 4% calves (Bossley et al. 2017).

The Adelaide Dolphin Sanctuary (ADS) was declared through a State Government Act[1] in 2005 '*to protect the dolphin population of the Port Adelaide River estuary and Barker Inlet and its natural habitat, to provide for the protection and enhancement of the Port Adelaide River estuary and Barker Inlet; to amend previous acts*'. The Act established an Advisory Board to provide guidance to the Minister for Environment and Conservation on matters relating to the Sanctuary. It also outlined a General Duty of Care (Part5) obliging persons '*to take all reasonable measures to prevent or minimise any harm to the Sanctuary*' (Appendix 10.2). Limiting the reach of the Act is that harm must be '*more than transient or tenuous in nature*', and what may or may not be transient and tenuous in nature can be argued. Following legislation, an ADS Management Plan was released in 2008 (Anonymous 2008). This outlined six objectives and 21 issues related to the residence and proliferation of dolphins in the Sanctuary (Appendix 10.3).

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1 https://www.legislation.sa.gov.au/lz?path=%2FC%2FA%2FADELAIDE%20DOLPHIN%20SANCTUARY%20ACT%202005

Up to 2010, an increase was apparent in dolphin numbers seen in the Sanctuary, from 4-15 dolphins per survey in the early 1990s up to 20-30 dolphins per survey in the early 2000s (Bossley et al. 2017). The increase was attributed to both establishment of the Sanctuary, limits to human activities associated with this, and improved water quality.

An assessment of dolphin deaths in the ADS before (1987-2004) and after (2005-2013) its establishment, however, showed an increase in mortality, along with an increase in disease (pneumonia or infection) as a cause of mortality, post ADS establishment (Figure 3, Figure 4, Table 1) (Adamczak et al. 2018). In part, this increase possibly related to the increased usage of the ADS by dolphins and an increased scrutiny of carcasses.

After 2010, the numbers of dolphins seen on surveys began to decline (Mike Bossley, pers. comm.). There was also an apparent increase in mortality rates, although this could not be confirmed as dolphins no longer seen during surveys might have died or might have moved away. A decline also became evident in the numbers of females with calves and in calf survival (Mike Bossley, unpublished data) (Figure 5), further suggesting there were issues with the dolphin population. For example, only two (13%) of 15 calves produced from 2016 to 2020 survived to weaning. In the previous five years, 2011 to 2015, 11 (48%) of 23 calves survived to weaning, and in the five years before that, 2006 to 2010, 16 (62%) of 26 calves born survived to weaning.

In 2021, an estimated 10-20 dolphins are largely resident in the ADS (Mike Bossley, pers. comm.). The majority are individually recognisable and are regularly re-sighted during surveys. In 2021, there were six recorded mortalities (including two assumed mortalities) of Indo-Pacific bottlenose dolphins in and around the ADS (Table 2).

Within the ADS, several benthic habitat types are available for the dolphins. A higher dolphin frequency was observed over bare sand habitat than seagrass (Cribb et al. 2013). Potentially, bare sand provide a less complex habitat than seagrass in which to feed (Cribb et al. 2013), particularly as seagrass beds may impair their ability to echolocate to find prey (Nowacek 2005).

The diet of Indo-Pacific bottlenose dolphins in Gulf St Vincent has been investigated through the stomach contents of mortalities (stranded individuals and some fisheries bycatch) taken to the South Australian Museum (Gibbs & Kemper 2018). This gives an indication of prey of the dolphins in the Port River and Barker Inlet. Common prey groups were southern calamari (*Sepioteuthis australis*), *Octopus* spp., cardinal fish (Family Apogonidae), gobies (Gobiidae), whiting (Sillaginidae), silverbellies (Gerreidae), herring/sardines (Clupiidae), jacks/trevallies

(Carangidae), grunters (Tetrapodidae) and garfish (Hemiramphidae) (Gibbs & Kemper 2018). These comprise both pelagic and benthic dwelling species.

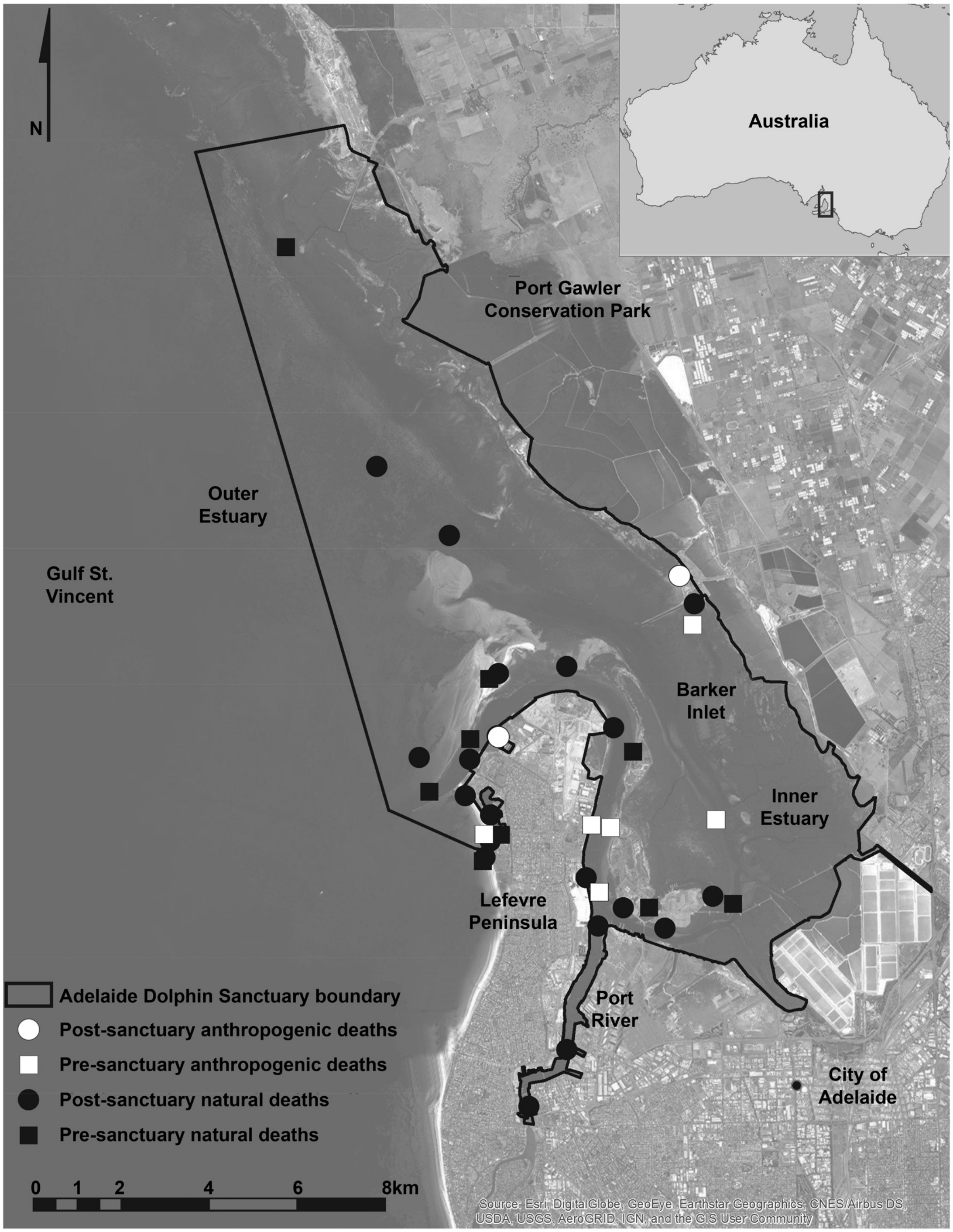


Figure 3. Map of Adelaide Dolphin Sanctuary and locations of recoveries of Indo-Pacific bottlenose dolphin bodies, pre-sanctuary establishment (1987-2005) and post-sanctuary establishment (2005-2013) (figure from Adamczak et al. 2018).

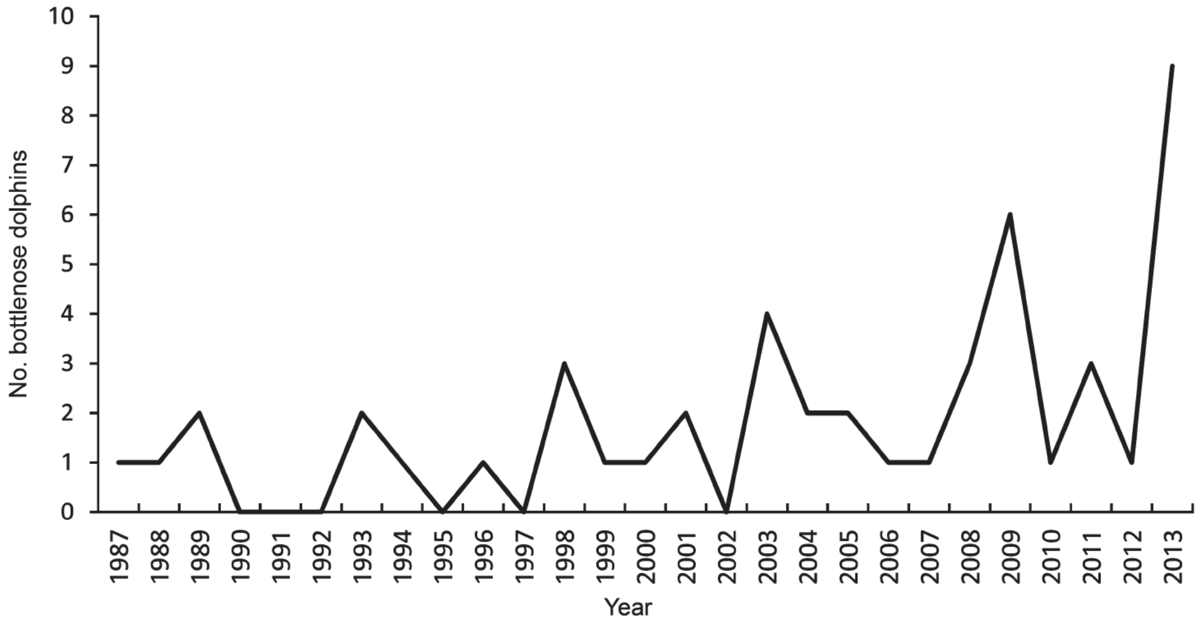
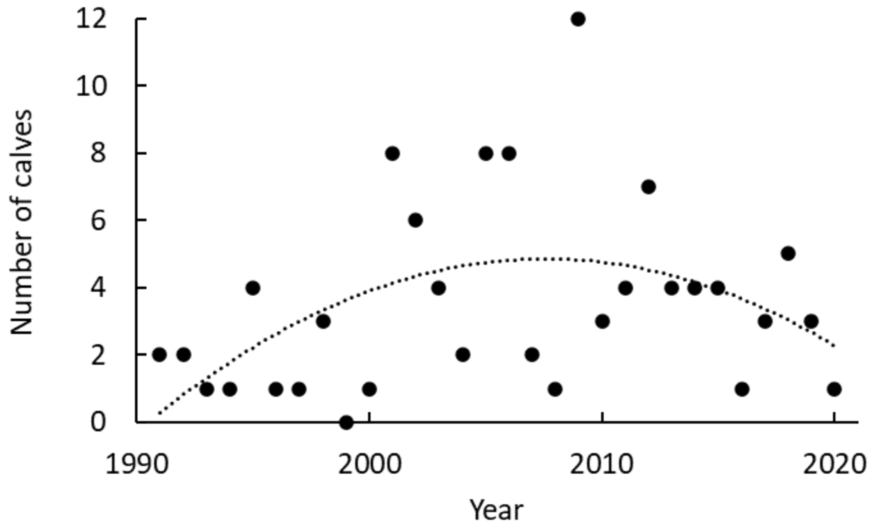


Figure 4. Number of Indo-Pacific bottlenose dolphins that have stranded (mostly stranded dead but includes two live stranded that then died) each year between 1987 and 2013 in the Adelaide Dolphin Sanctuary, South Australia (figure from Adamczak et al. 2018).

Table 1. Circumstance of death of Indo-Pacific bottlenose dolphins in the Adelaide Dolphin Sanctuary, pre and post establishment of the Adelaide Dolphin Sanctuary (Adamczak et al. 2018).

Cause	**Pre-ADS (1987-2005)**	**Post-ADS (2005-13)**
Direct		
Boat/ propellor	2	2
Intentional (shot)	2	0
Entanglement	1	0
Fishing hook	1	0
Other		
Disease	4	17
Natural	4	2
Live stranding	1	1
Unknown	5	5
TOTAL	**20**	**27**
Rate/year	1.1	3.4



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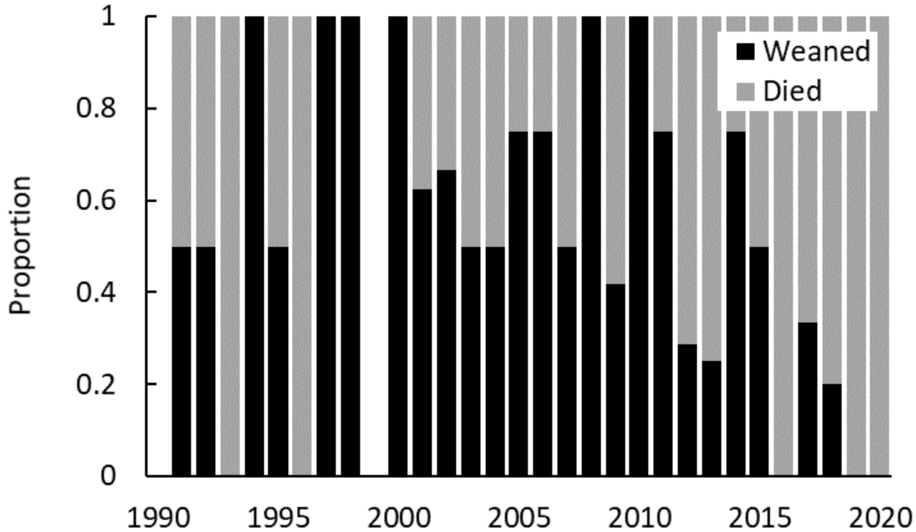

b)

Figure 5. Indo-Pacific bottlenose dolphin calves per year in the Adelaide Dolphin Sanctuary, a) number born, b) proportion that survived to weaning or died (Mike Bossley, unpublished data).

Table 2. Mortalities of Indo-Pacific bottlenose dolphins during 2021 in and around the Port River and Barker Inlet (data from DEW and ADS dolphin mortality investigation team).

Name	Date	Sex	Age	Cause of death	Other conditions	Tested & Absent
Doc	25-Jun	M	8	Death not confirmed	Severe entanglement (removed) *Brucella* antibodies Blood results showed stress leukogram and mild anaemia only Emaciation/ Starvation	
Twinkle	7-Jul	M	adult	Death not confirmed	Begging from boats Swimming in circles and listing Emaciation	
Unknown (Semaphore)	19-Jul	F	adult	Septic peritonitis	Trematode infection of sinus and ear Blunt force trauma to head Toxoplasmosis (sero-positive, high titre) Internal injuries, suppurative periotinitis, stomach abscess (likely due to penetrating foreign object that had passed) – growth of *Pseudomonas ludensis* Poor condition/ Starvation	*Morbillivirus*
Tallula	21-Aug	M	12	Unknown	Historic skin lesions (sunburn?) 2010, resolved at PM Fishing hook in stomach Adrenal enlargement Toxoplasmosis (seropositive but low titre) Oral mucosa and blow hole: hyperplastic mixed dermatitis, ulcerative, hyperkeratotic Spleen: lymphoid follicular lymphocytolysis/ depletion Emaciation/ Starvation	*Morbillivirus*
Hunter	22-Oct	M	6	Euthanised	Deformed jaw (birth defect) Left eye: chronic focal corneal scarring Severe bacterial infection of ear Multiple skin abscesses – multifocal chronic-active ulcerative & necrosuppurative dermatitis with intralesional Gram negative bacteria & invasive ciliate protozoa. Haemorrhagic gastritis, empty stomach Enteritis with villous blunting (no formed faeces) Lung: bronchiolar epithelial degeneration & mineralisation, with attenuation & reepithelialisation Emaciation/ Starvation	*Brucella* Influenza *Morbillivirus* *Toxoplasma* seronegative
Squeak (Hunter's brother)	21-Nov	M	4	Trauma	Historic entanglement (2019) Slight enteritis Multiple skin abscesses Emaciation but stomach contained shrimps & other material	*Morbillivirus* *Toxoplasma* seronegative

5. THREATS TO COASTAL DOLPHINS

This review does not attempt to document all threats to coastal dolphins, but to focus on threats that are likely to impact Indo-Pacific bottlenose dolphins in the Port River and Barker Inlet. For example, bycatch in commercial fisheries is a significant threat to coastal dolphins elsewhere (Mannocci et al. 2012, Reeves et al. 2013, Peltier et al. 2016), but not in the ADS.

Threats to dolphins in the ADS include:

1. Intrinsic traits – related to the species' biology and behaviour that predisposes it to higher risk of population decline, if exposed to additional risks.
2. Disease – microbial (bacteria, viruses) or metazoan (fungi, helminth, crustacean) parasitic pathogens.
3. Algal blooms – a specific form of marine toxicity caused by phytoplankton that can produce toxins which bioaccumulate. Algal blooms may be stimulated by anthropogenic eutrophication.
4. Pollutants – substances that may occur naturally or be manufactured and can be enhanced in the environment through anthropogenic activity, for example, metals, hydrocarbons and polychlorinated biphenyls. Many of these substances bioaccumulate, some have marked negative effects on endocrine systems, and others suppress immune function. Underwater noise is included here as a type of pollutant.
5. Environmental change – specifically referring to current anthropogenically-induced change, such as ocean warming.
6. Direct human interaction – including tourism, boating, fishing, entanglement in marine debris, and habitat modification and loss.

Each threat is discussed in more detail below, including a general description of the threat and reports from other studies and dolphin species, followed by specific reports from ADS dolphins.

5.1. Intrinsic traits

The life-history traits of Indo-Pacific bottlenose dolphins increase their susceptibility to novel pressures. These traits include being long-lived, having slow growth, maturing late, exhibiting low reproductive rates, residing in small groups with relatively small home ranges, and fast metabolism that requires daily intake of large quantities of food relative to their body size (Culik

2004, Anonymous 2012a). They also occupy coastal environments, which exposes them to habitats of high variability and anthropogenic inputs.

A further intrinsic threat comes from the dolphin's highly social behaviour. This results in diseases being highly transmissible between individuals. It also means heightened stress levels, for example, on witnessing distress in conspecifics or losing contact with group members (Kuczaj et al. 2015). In such social animals, the loss of individuals can interfere with demographically important social processes such as mating and information transfer (Davidson et al. 2009). Also, like many cetacean species, Indo-Pacific bottlenose dolphins frequently conflict with each other and can inflict wounds that may become infected. Tooth rake marks are reliable indicators of conspecific conflict in bottlenose dolphins and are present on all age classes and both sexes, being most prevalent in juveniles (Lee et al. 2019). Furthermore, infanticide can occur in bottlenose dolphin populations, being inflicted by males to increase reproductive fitness or by females when resources are limited (Patterson et al. 1998, Robinson 2014, Perrtree et al. 2016).

Threats and risks to health are different for male and female bottlenose dolphins. Males tend to have larger ranges than females and thus may be exposed to a wider number of threats (Scott et al. 1990). Males also tend to be more aggressive and more likely to retaliate following aggression, they herd and compete for access to females, and generally take greater risks than females: consequently, males can have higher mortality rates (Scott et al. 2005). Due to the higher costs associated with enhancing reproductive opportunities, it is normal amongst mammal species for males to have shorter longevity than females (Lemaître et al. 2020).

Modelling has shown that for marine mammals, intrinsic traits can be more important than extrinsic variables in driving a population or species toward extinction (Davidson et al. 2009). Accordingly, the interaction of both intrinsic and extrinsic threats is also important in predicting the risk of a population's extinction (Davidson et al. 2009, Davidson et al. 2012).

Intrinsic traits and ADS dolphins

Using cases (non-survivors) and controls (putative survivors) from a cetacean morbillivirus (CeMV) outbreak in South Australia, Batley et al. (2019) carried out a genome-wide association study to identify candidate genes for resistance and susceptibility to CeMV. Five candidate genes with functions associated with stress, pain and immune responses were identified to differ between the groups, suggesting there could be a genetic basis for host defence against CeMV.

Further studies characterised genomic regions and pathways that may contribute to CeMV immune responses in dolphins (Batley et al. 2021) (see also the section on Morbillivirus).

One example of social learning amongst Indo-Pacific bottlenose dolphins in the ADS was the adoption of tail-walking by wild dolphins following release of a rehabilitated dolphin that had been exposed to tail-walking by trained dolphins (Bossley et al. 2018). While not a threatening behaviour, this observation provides a local example highlighting the dolphin's highly social behaviour. Aggression between dolphins in the ADS (potentially over feeding or reproductive opportunities, or calf defence) has been observed and tooth rake marks on dolphins demonstrate aggressive interactions are frequent (Mike Bossley, pers. obs.). The degree to which aggression or other social behaviours could impact on dolphin numbers present in the ADS, however, has not been quantified.

5.2. Diseases

A disease is a condition that negatively affects the structure or function of an organism. It may occur as a process over time or an internal dysfunction (for example, hereditary diseases or deficiency diseases) or be stimulated by an external stressor (for example, by an infection). Disease can target individual organs or spread through multiple organs. For example, pneumonia, caused by bacterial, viral or fungal infection of the lung, is one of the most common causes of morbidity in bottlenose dolphins (Venn-Watson et al. 2012).

Dolphins are subject to a range of hereditary and deficiency diseases, including cancers, heart conditions, and malnutrition (Newman & Smith 2006). In this report, however, we focus on the infectious diseases caused by parasites, bacteria, viruses, and fungi. Skin lesions, which can be caused by physical damage rather than a pathogen and are easily identifiable on dolphins while they are free ranging, are included as a specific condition.

Individual habitat specialisation, spatial use and local geography interact to influence exposure risk to socially transmitted diseases (Cloyed et al. 2021). Also, males and females of a species have different inherited immune systems, related to genetic and hormonal differences, which can result in sex differences in vulnerability to diseases (Venn-Watson et al. 2007, Klein & Flanagan 2016). In captive bottlenose dolphins, for example, males appear to be more vulnerable to a tattoo skin disease (associated with a poxviruses of the subfamily Chordopoxvirinae) than females (Van Bressem et al. 2018) but a sex bias in tattoo skin disease expression is not been evident in free-ranging bottlenose dolphins (Van Bressem et al. 2009). The sex-bias in captive dolphins is probably related to males in captivity being more vulnerable to stress (Van Bressem et al. 2018). Similarly, there is a male-biased prevalence of papillomavirus seropositivity in captive dolphins, which is not evident in free-ranging dolphins (Rehtanz et al. 2010).

All wild cetaceans are host to parasites. These generally do not cause illness or injury, however, during periods of ill-health, immune suppression, or senescence, parasitic infections can intensify and compromise health and survival. The presence of a parasite does not necessarily indicate a health concern, but when high parasite abundances are recorded in patently compromised individuals, they may combine with other stressors and threaten the individual's survival.

This review of bottlenose dolphin diseases is representative but not exhaustive. Some diseases undoubtedly have yet to be linked to infection in dolphins. For example, the bacterial pathogen *Coxiella burnetii* (the causative agent of Q fever and linked to increased abortions) has been detected in many mammals, including seals (Minor et al. 2013, Gardner et al. 2022), but not yet in cetaceans – although it may be present.

5.2.1. Helminths

Helminths are parasitic worms whose life cycles may comprise several hosts that interact through food-chains or habitats. Numerous helminth species live in the gastrointestinal tract and other organs of dolphins including nematodes (round worms), cestodes (tape worms) and trematodes (flukes) (Woodard et al. 1969).

Most bottlenose dolphins carry intestinal parasites, many of which have life cycles that include prey of the dolphins. Individual dolphins may carry many helminth species at once. For example, eight species were identified from the stomachs of 15 bottlenose dolphins that stranded in the western Mediterranean (Quiñones et al. 2013), and seven species (6477 individual helminths) were found in the gastrointestinal tracts of six bottlenose dolphins from Patagonia (Romero et al. 2014). Common gastrointestinal helminths in dusky dolphins (*Lagenorynchus obscurus*) include *Anisakis* spp., *Braunina cordiformis* and *Poleter gastrophylus* (Van Waerebeek et al. 1993). Sinusitis due to severe infestations by the nematode *Crassicauda grampicola* appeared to be the primary cause of the death of adult Risso's dolphins in the western Mediterranean (Cuvertoret-Sanz et al. 2020). Lungworms (*Stenurus ovatus*, *Halocercus lagenorhynchi*, *Skrjabinalius cryptocephalus*) are commonly recorded during autopsies of stranded bottlenose dolphins (Kuwamura et al. 2007, Fauquier et al. 2009, McFee & Lipscomb 2009, Pool et al. 2021).

Trematodes infect many body tissues. Hepatic and pancreatic infection with *Campula palliata* frequently induces interstitial fibrosis in bottlenose dolphins (Woodard et al. 1969). *Pholeter gastrophilus* causes the proliferation of fibrous tissue in the wall of the stomach into a tumorous mass (Woodard et al. 1969). *Nasitrema* spp. infect the middle ear, inner ear and brain, and are a suspected cause of death of many cetaceans (Ebert & Valentere 2013, Lim et al. 2016, Díaz-Delgado et al. 2018, Sierra et al. 2020). Severe pterygoid sinusitis with middle and inner ear involvement and extension to the central nervous system caused by a *Nasitrema* sp. was observed in bottlenose dolphins that stranded in the Canary Islands (Díaz-Delgado et al. 2018), and severe inflammation in the dorsal and mid-thalamus of striped dolphin (*Stenella coeruleoalba*) that stranded in Florida was associated with *Nasitrema* sp. adults and eggs (O'Shea et al. 1991).

Helminths and ADS dolphins

In South Australia, lung infections of *Halocercus lagenorhynchi* and *Stenurus ovatus* (Nematoda) were identified in 19 of 167 (11%) stranded Indo-Pacific bottlenose dolphins (Tomo et al. 2010).

5.2.2. Crustacean parasites

Marine crustaceans including amphipods, brachiurans and copepods colonise the skin of dolphins. The amphipod *Scutocyamus antipodensis* is an ectoparasite of Hector's dolphin in New Zealand (Lincoln & Hurley 1980), and the copepod *Harpacticus pulex* has been found in large cutaneous ulcerations on an aquarium held bottlenose dolphin in Florida (Humes 1964). Two sessile crustaceans, the barnacle *Xenobalanus globicipitis* and the copepod *Pennella balaenopterae* are specific parasites of cetaceans in the Mediterranean (Hogans 1987, Aznar et al. 1994).

These crustacean parasites typically occur at low abundances on dolphins and do not cause gross pathology. If the host is subjected to other stressors or environmental conditions that favour infection, however, more intense infestations can occur (Aznar et al. 2005). Thus, the abundance of skin parasites can provide an indication of an individual’s health (Vecchione & Aznar 2014).

Crustaceans and ADS dolphins

There are no published data on the presence or prevalence of skin crustaceans in bottlenose dolphins in South Australia.

5.2.3. Protozoa

5.2.3.1. *Toxoplasma*

Toxoplasmosis is an infection caused by the protozoan, *Toxoplasma gondii*. The parasite can persist for long periods in a host, with the host's immune system preventing the parasite from proliferating and causing disease. *Toxoplasma gondii*, however, can encyst in many tissues, including brain, lungs, heart, liver, spleen and adrenal glands, where it undergoes asexual reproduction, causing toxoplasmosis which, among other effects, can weaken the immune system (Bowater et al. 2003). *Toxoplasma gondii* has been isolated from numerous stranded and free-living cetaceans (Cabezón et al. 2004, Santos et al. 2011, Roe et al. 2013), including bottlenose dolphins (Inskeep et al. 1990, Dubey et al. 2008). It was also detected in many tissues of a still-born foetus of a captive Indo-Pacific bottlenose dolphin in Western Australia (Jardine & Dubey 2002).

When a host is challenged by injury or other illness, toxoplasmosis often manifests as a coinfection. For example, coinfection by *T. gondii* was observed in brain tissue of a striped dolphin from the Mediterranean with *Brucella* infection (Alba et al. 2013). A coinfection of *T. gondii* with a herpesvirus was also recorded in common dolphins in the Mediterranean (Bigal et al. 2018), and *T. gondii* coinfection with Morbillivirus was observed in a stranded fin whale (Mazzariol et al. 2012).

Although *T. gondii* passes between species via contact or consumption, the only known definitive hosts are felines (domestic cats and their relatives). *Toxoplasma gondii* detection in oceanic cetaceans has led to speculation of an alternative life-cycle independent of land, which warrants further investigation (Di Guardo & Mazzariol 2013).

<u>Toxoplasma and ADS dolphins</u>

Toxoplasma gondii has been detected in tissues of several Indo-Pacific bottlenose dolphins found dead in the ADS (SA Museum data). In 2013, *T. gondii* was detected in brain tissue of a dolphin that had other complications, including *Morbillivirus* infection and evidence of head and neck trauma (Kemper et al. 2016). *Toxoplasma gondii* was also detected in a known 16-year-old dolphin from the ADS that was emaciated, had injuries to its dorsal fin and a foreign body in its gastro-intestinal tract (C. Kemper unpublished data). In 2016, *Toxoplasma* cysts were also detected during post-mortem in the brain of an ADS dolphin (Lucy Woolford, pers. comm.).

Two dolphins that died in 2021 were found to be seropositive for *Toxoplasma*, along with other conditions, but had no evidence of fulminant disease due to toxoplasmosis (Table 2). The significance of sero-detection without evidence of pathology is uncertain.

5.2.3.2. *Other protozoa*

Several protozoa have been identified in dolphins (Miller et al. 2018). For example, the ciliated protozoan *Kyaroikeus cetarius* has been identified as part of the normal microbial community in the upper respiratory tract of bottlenose dolphins (Arkush et al. 1998).

<u>Other protozoa and ADS dolphins</u>

There are no published data on the presence or prevalence of other protozoa in bottlenose dolphins in South Australia.

5.2.4. Bacteria

5.2.4.1. *Brucella*

Brucella ceti infections have been increasingly reported in cetaceans since original detection in the aborted foetus of a bottlenose dolphin (Ewalt et al. 1994). *Brucella* spp. infections in humans, and domestic and wild animals, can be associated with gross organ failure, including neural failure and abortion. *Brucella ceti* was isolated from brain, lung and intestinal lymph nodes of a dead adult male striped dolphin found stranded in Tuscany, Italy and was associated with moderate to severe lesions of meningo-encephalitis (Alba et al. 2013). From 2012 through 2017, 90 of 282 (32%) stranded bottlenose dolphins in South Carolina tested positive for *B. ceti* in at least one tissue (brain, lung, blowhole swab) (McFee et al. 2020).

Dolphins suffering *B. ceti* infection can display an inability to maintain equilibrium, lateralised swimming, floating and lethargy, (Isidoro-Ayza et al. 2014b). *Brucella* spp. infection appears to be prevalent in dolphins but may be difficult to detect and not always associated with pathology. A difficult to detect *Brucella* sp. infection in the vertebrae was implicated as a pre-condition to a chronic *Staphylococcus* sp. infection in a captive bottlenose dolphin (Goertz et al. 2011).

Brucella and ADS dolphins

Antibodies to *Brucella* spp. were detected in a sample taken from an ADS dolphin that was captured to remove entangled fishing line (Table 1). This dolphin was last sighted in poor condition in June 2021, and is presumed to have died.

5.2.4.2. *Other bacteria*

All animals have communities of bacteria living in their tissues and digestive systems and healthy bacteria loads are critical to dolphins. For example, *Lactobacillus* strains with probiotic features have been identified in the gastrointestinal tract of bottlenose dolphins (Diaz et al. 2013). Potentially pathogenic bacteria may also reside in tissues and have no health consequences, so presence alone does not signal a cause for poor health.

Bacteria that may impact on the health of coastal dolphins can be endemic in the population, introduced from within their natural ecosystem, or introduced by human activities, such as via wastewater (Jaing et al. 2015). Many bacterial species identified on the skin of bottlenose dolphins off southern California were likely associated with terrestrial runoff (Russo et al. 2017).

A survey of pathogens in bottlenose dolphins off the USA Atlantic coast detected a range of terrestrial and human derived bacteria: *Clostridium perfringens*, *Campylobacter* sp., *Staphylococcus* sp., *Erwinia amylovora*, *Helicobacter pylori*, and *Frankia* sp. (Jaing et al. 2015). A survey of antibiotic-resistant bacteria isolated from bottlenose dolphins in the southeastern USA similarly identified bacteria associated with human illness, including *Staphylococcus aureus*, *Escherichia coli* and potentially pathogenic environmental bacteria such as *Vibrio* spp., *Shewanella putrefaciens*, and *Pseudomonas* sp. (Stewart et al. 2014).

Streptocossus iniae infection was the apparent cause of death of a common dolphin found deceased on the Adelaide metropolitan coast in 2019 (Souter et al. 2021). *Streptocossus iniae* is a significant pathogen of farmed fish species and has been identified in skin abscesses in captive Amazon River and bottlenose dolphins – known as 'golf ball disease' (Pier & Madin 1976, Song et al. 2017).

Exhalant respiratory bacterial communities of wild Indo-Pacific bottlenose dolphins in Shark Bay, Western Australia, identified by DNA analysis included genera that are infectious to marine mammals (Nelson et al. 2019). Fecal microbiota of captive Indo-Pacific bottlenose dolphins at a facility in Japan included the potentially pathogenic bacteria *Morganella* and *Mycoplasma* (see Suzuki et al. 2021).

Mycobacterium is a genus of bacteria that includes pathogens that cause disease in marine mammals: several species of pathogenic mycobacteria have been detected in individual dolphins (Wünschmann et al. 2008). For example, *M. abscessus* related pneumonia was detected in the lung of a 23-year old, aquarium-kept bottlenose dolphin in Maryland (Clayton et al. 2012), *M. chelonae* was isolated from pooled tissue of another captive bottlenose dolphin (Wünschmann et al. 2008) and *M. marinum* infection was passed on to a trainer by a bite from a captive bottlenose dolphin (Flowers 1970). Antibodies to *M. marinum*, *M. fortuitum*, and *M. chelonae* were detected in sera from free-living bottlenose dolphins in west-coast USA (Beck & Rice 2003). Moreover, *M. tuberculosis* was reported in a bottlenose dolphin from Tasmania in 2006 (Nugent & Cousins 2014, Knowles et al. 2021).

Numerous other potentially pathogenic bacteria have been isolated from dolphins. For example, *Staphylococcus* is a genus of bacteria, some of which produce toxins causing skin wounds, immune-suppression, and even cardiac arrest. Captive dolphins have succumbed to infection by a highly pathogenic, enterotoxin-secreting *Staphylococcus* sp. (Goertz et al. 2011). *Staphylococcus* sp. antibodies have also been detected in wild dolphins, although not routinely (Anderson 2021).

Leptospira interrogans, which causes renal infection in many wild animals, was isolated from the kidney of a stranded bottlenose dolphin in the Mediterranean Sea (Piredda et al. 2020).

Serological evidence of exposure to *Chlamydia abortus* (formerly *Chlamydophilia psittaci*), which can infect through the lungs and has been responsible for abortion in many mammals, including humans, was recorded in bottlenose dolphins from South Carolina (Schaefer et al. 2009). A pathway through migratory birds was proposed as a means for the dolphins' contact with these bacteria. A suspected fatal *C. abortus* infection was reported for a single, stranded, female striped dolphin in southern Italy (Santoro et al. 2019).

Helicobacter spp. are linked to gastritis with and without the presence of ulcers and were found in dolphins in a collection in Tenerife (Bernal-Guadarrama et al. 2015). In Japan, a *Helicobacter* infection of captive dolphins was identified as a new species, *H. delphinicola*, probably resident in dolphin populations, and likely linked to gastritis and gastric ulcers (Segawa et al. 2020). Clinical signs of *Helicobacter* presence included a lack of appetite, anorexia, abdominal tenderness, depression, and occasional unresponsiveness.

Clostridium perfringens infection of muscle, heart, blood, and body fluids was identified as the cause of death of a captive Atlantic bottlenose dolphin (Buck et al. 1987) and *Clostridium* sp. were identified in the cerebrum of a stranded bottlenose dolphin at the Canary Islands (Díaz-Delgado et al. 2018).

A suppurative necrotising bronchopneumonia caused by *Nocardia cyriacigeorgica* infection was recorded in a stranded striped dolphin in Japan (Ito et al. 2021). Bacteria identified by Venn-Watson et al. (2012) to be causes of pneumonia in bottlenose dolphins have been *Staphylococcus aureus*, *Streptococcus zooepidemicus*, *Erysipelothrix rhusiopathiae*, *Proteus* spp., and *Pseudomonas aeruginosa*.

Bacteria and ADS dolphins

Single dolphins that stranded as part of the 2013 unusual mortality event in Gulf St Vincent had bacterial infections in skin lesion (*Pasteurella* spp.), blowhole (*Salmonella* spp.) and liver (*Streptococcus* spp.) (Kemper et al. 2016). Microbial assessment of skin lesions on a dolphin that died from entanglement in fishing hooks and fishing line identified *Streptococcus dysgalactiae*, *Staphlococcus aureus* and *Pseudomonas* sp. (Byard et al. 2020). *Streptococcus iniae* infection was identified in skin lesions and heart blood of a common dolphin that stranded on the Adelaide metropolitan coast (Souter et al. 2021).

A wide range of opportunistic infections by bacteria with pathogenic potential have been detected in skin lesions, ear infections, and as part of enteric infections during current investigations into ADS mortalities, including *Edwardisella tarda*, *Vagococcus fluvialis*, *Erysipelothirx rhusiopathiae*, and various *Vibrio* spp. (*V. harveyii*, *V. parahaemolyticus*, *V. alginolyticus*).

5.2.5. Viruses

5.2.5.1. *Morbillivirus*

Dolphin morbillivirus (DMV) is a virulent pathogen that can cause high mortality outbreaks in delphinids globally and is spread via contact among individuals (Domingo et al. 1990, Cloyed et al. 2021).

Morbillivirus infection has been recognised in stranded carcases of several cetaceans, including bottlenose dolphins in Europe (Van Bressem et al. 2001, Di Guardo et al. 2013) and Indo-Pacific bottlenose dolphins in Western Australia (Stephens et al. 2014) and in South Australia (Kemper et al. 2016). Active morbillivirus infection and antibodies indicating recovery from infection have also been detected in bottlenose dolphins in south-eastern USA (Bossart et al. 2010, Bossart et al. 2017).

Morbillivirus and ADS dolphins

A morbillivirus-associated unusual mortality event of dolphins occurred in South Australia in 2013 (Kemper et al. 2016) in association with a marine heatwave and widespread fish and marine animal kills (Roberts et al. 2019). All three resident dolphin species were involved, with most deaths recorded for Indo-Pacific bottlenose dolphins, including individuals from within the ADS. Genomic studies of Indo-Pacific bottlenose dolphins involved in the event and control dolphins in the region revealed differences in susceptibility and resistance between individuals (Batley et al. 2019), and genomic pathways for immune response to disease (Batley et al. 2021).

5.2.5.2. *Other viruses*

Numerous other viruses have been identified in cetaceans, although few with associated diseases or marked pathology (Birkun Jr 1996). Viruses identified in bottlenose dolphins that have been associated with disease include *Gammaherpesvirinae* sp. (Van Bressem et al. 1994, van Elk et al. 2009), papillomaviruses (Rehtanz et al. 2012), equine encephalitis viruses (Schaefer et al. 2009), and enteric coronaviruses (Wang et al. 2020). A possible pathway for the equine encephalitis virus to infect dolphins was via mosquitoes (Schaefer et al. 2009). Viral pneumonia can be caused by parainfluenza virus infection (Venn-Watson et al. 2012). Herpesviruses have been detected in several dolphin species (Exposto Novoselecki H et al. 2021).

Other viruses and ADS dolphins

Apart from poxviruses (tattoo skin disease) which is seen commonly on ADS dolphins and is not fatal (Lucy Woolford, pers. comm.), other viral pathogens have not been reported in South Australian bottlenose dolphins.

5.2.6. Fungi

Mycoses (fungal diseases) are documented in both captive and free-ranging cetaceans, and may be a cause of stranding in bottlenose dolphins (Wünschmann et al. 1999, Abdo et al. 2012, Isidoro-Ayza et al. 2014a). Common sites of infection are the skin, the respiratory system, and occasionally the central nervous system. For example, a bottlenose dolphin that stranded in the Spanish Mediterranean was found to have pyogranulomatous, and necrotising meningo-encephalomyelitis and radiculitis, caused by the fungus *Cunninghamella bertholletiae* (see Isidoro-Ayza et al. 2014a).

Paracoccidioido-mycosis ceti (PC) is a disease caused by the fungus *Paracoccidioides brasiliensis*, which can enter into and cause lesions in the skin and subcutaneous tissue of dolphins (Vilela & Mendoza 2018). Historically, this disease in dolphins was attributed to the fungus *Lacazia loboi* and named lobomycosis (Lobo's disease). It has been recorded in bottlenose dolphins in Asia, Europe and the Americas (Migaki et al. 1971, Kiszka et al. 2009, Tajima et al. 2015). A survey of dolphin health in southeastern USA found PC to be one of the most prevalent infections in coastal bottlenose dolphins (Bossart et al. 2017). Conditions that promote PC infection in coastal bottlenose dolphins are higher than average water temperatures, runoff from agricultural watersheds, and freshwater intrusion (Bossart et al. 2017).

Other fungal pathogens of dolphins include *Candida glabrata* and *Ajellomyces dermatitidis* in the upper respiratory tracts, and *Aspergillus* spp. and *Cryptococcus* spp. in the lungs (Stellick-Seepaulsingh 2014). Venn-Watson et al. (2012) reported pneumonia in bottlenose dolphins caused by *Cryptococcus neoformans* and *Histoplasma capsulatum* infections, and disseminated Cryptococcosis has been observed in dolphins in the Swan River in Western Australia (Nahiid Stephens, pers. comm. to Lucy Woolford).

Fungi and ADS dolphins

The pathogenic fungus *Aspergillus fumigatus* was identified in the lungs and brains associated with pneumonia and meningoencephalitis, respectively, in Indo-Pacific bottlenose dolphins that stranded during the 2013 unusual mortality event in Gulf St Vincent (Kemper et al. 2016).

5.2.7. Syndromic lesions, skin disease

Skin lesions in dolphins can be associated with immunologic disturbance, anthropogenic contaminants, collisions, interactions with conspecifics or predators, or exposure to air, heat or hypo-salinities. They may be colonised by a range of parasites: viruses, bacteria, protozoans, fungi and crustaceans. For example, in Indian River, Florida, pathologic diagnoses of skin lesions included viral (papillomavirus/ herpesvirus) associated with orogenital sessile papilloma (39.7%), cutaneous lobomycosis (16.7%), poxvirus associated tattoo skin disease (15.4%), nonspecific chronic to chronic-active dermatitis (15.4%), and epidermal hyperplasia (12.8%) (Bossart et al. 2015).

Ulcerative dermatitis has been associated with an unnamed ciliated protozoa in dolphins in the Northern Hemisphere (Schulman & Lipscomb 1999), and is seen sporadically in skin lesions in dolphins from the ADS (Lucy Woolford pers. comm. e.g., Hunter in Table 2, and Byard et al. 2020)

A further cause of skin lesions in coastal dolphins can be prolonged exposure to freshwater. Extended hyposaline conditions and single flood events can correlate with an increased prevalence of skin lesions in bottlenose dolphins (Duignan et al. 2020, Toms et al. 2021).

Skin lesions and ADS dolphins

In a review of marine mammal strandings in South Australia up to 2010, skin lesions were noted on the carcasses of 11 Indo-Pacific bottlenose dolphin that had lived in the ADS but only one bottlenose dolphin from elsewhere in South Australia (Kemper & Tomo 2011). It was speculated that some of the lesions could have been related to pollutants in the waters of the ADS.

A single dolphin that stranded as part of the 2013 unusual mortality event in Gulf St Vincent had a bacterial infection (*Pasteurella* sp.) in skin lesions (Kemper et al. 2016). Also, two dolphins, a mother and calf, with extensive skin injuries thought to be associated with a tidal stranding and sun-burn were observed in the Port River in April 2011 (Bossley & Woolfall 2014). The wounds were documented to heal over several months without apparent infection. In 2013, a third dolphin presented with similar injuries that also healed rapidly.

Recently, as mentioned in the above section on bacteria, multiple cutaneous abscesses and sepsis due to *Streptococcus iniae* infection were reported as the probable cause of death of a common dolphin outside of the ADS (found near Aldinga Beach, South Australia) (Souter et al. 2021).

5.3. Algal blooms

Algal blooms occur naturally but can also be caused or enhanced by anthropogenically activity. Some algal blooms are localised, occurring in bays or estuaries, others are massive and can cover thousands of square kilometres. Some blooms are seasonal and occur at the same time and place each year, while others are random; they can last from a few weeks to years; some can be toxic and others benign (Anderson 1997). Products from harmful algal blooms 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 (Fire et al. 2008, Hinton & Ramsdell 2008). Algal blooms do not necessarily associate immediately with the introduction of high nutrient levels. If conditions are not appropriate, algal cysts can be dormant for many years in sediments and hatch when disturbed by heavy water runoff or dredging.

Harmful or toxic algal blooms occur world-wide (Anderson et al. 2002) and are caused by changed nutrient conditions, such as lowered N:P ratios (Hodgkiss & Ho 1997). The density of algae can cause mechanical damage to the gills of fish, causing fish deaths and, thereby, reducing prey availability to higher predators such as dolphins (Roberts et al. 2019). Of greater concern, though, is that the algae can produce toxins which can kill marine organisms directly, or bioaccumulate in them then biomagnify up the food-chain and be toxic to higher predators (Gaydos 2006, Fire et al. 2008).

The algae *Karenia brevis*, *Dinophysis* spp. and *Pyrodinium bahamense* in the Gulf of Mexico and Atlantic Coast of Florida have resulted in persistent and chronic toxin (e.g., brevetoxins) exposure and occasional mass mortalities of bottlenose dolphins (Fire et al. 2007, Cammen 2014, Cammen et al. 2015, Fire et al. 2020b). In Florida again, diatoms of the *Pseudo-nitzschia* genus produce the toxin domoic acid which has been linked to eosinophilia in bottlenose dolphins, (Schwacke et al. 2010). Dolphins in Florida can carry high levels of toxins between algal bloom events, making them more prone to mortality in future bloom events (Twiner et al. 2011, Twiner et al. 2012, Fire et al. 2020a). Neurotoxic metabolites from cyanobacteria have also been associated with intoxication of captive bottlenose dolphins in Florida Keys (Lydon et al. 2021), and toxic algae-induced immunomodulation may increase an animal's susceptibility to bacterial, viral, or fungal infections (Gebhard et al. 2015).

<u>Algal blooms and ADS dolphins</u>

Algal blooms have been a persistent environmental concern in the Port River and Barker Inlet (Thomas et al. 2001). *Alexandrium minutum*, a dinoflagellate that blooms in the Port River, produces a neurotoxin which accumulates in shellfish and can cause Paralytic Shellfish Poisoning (Parker et al. 2002). However, these blooms do not appear to have a direct impact on Indo-Pacific bottlenose dolphins in the ADS.

As part of the investigation into the 2013 unusual mortality event of dolphins in Gulf St Vincent, the stomach contents of one juvenile Indo-Pacific bottlenose dolphin (collected 1 April 2013 in

Gulf St Vincent) were analysed for 17 toxins that could be produced during harmful algal blooms (Kemper et al. 2016). Results were negative. Concurrent with the mortality of dolphins, however, there was a prolonged and widespread marine mortality event of abalone and at least 29 fish species (Roberts et al. 2019). This was linked to a marine heatwave, high nutrient concentrations, high concentrations of the harmful diatom *Chaetoceros coarctatus*, which causes gill damage in fish, and lethal bacterial septicaemia (Roberts et al. 2019).

Initial investigations into toxins associated with harmful algal blooms in livers from ADS dolphin mortalities in 2021 have not revealed the presence of shellfish neurotoxin (brevetoxin), amnesic shellfish toxin (domoic acid) or diarrhetic shellfish toxin (Lucy Woolford pers. comm.).

5.4. Pollutants

Most anthropogenically produced pollutants known to impact on the health of coastal dolphins are slow to degrade and continue to bioaccumulate. Thus, they can be problematic for years after their introduction into the environment has ceased. Many will be deposited in marine sediments and can be returned to food-chains over time through bioturbation, storm-induced turbidity and dredging (Pandit et al. 2006).

Reviews on toxicology specific to marine mammals, relative to the range of toxic anthropogenic-derived chemicals introduced into the marine environment, include those of Vos (2003) and Fossi and Panti (2018). In part because of their ability to bioaccumulate pollutants, bottlenose dolphins that occupy discrete coastal habitats, have been proposed as useful bioindicators of environmental disturbance and sentinels of health risks for humans who frequently consume seafood (Irwin 2005, Bossart et al. 2017).

5.4.1. Metals

Potentially toxic levels of trace elements, including heavy metals, have been recorded in numerous cetaceans (Wood & Van Vleet 1996, Evans 2003). This has included species inhabiting open water environments such as around the Hawaiian Islands (Hansen et al. 2015), and isolated regions such as subantarctic waters (Cáceres-Saez et al. 2012). When heavy metals enter marine environments, they usually deposit into sediments where they may be buried or can be ingested by benthic organisms and accumulate through food chains. Some metal ions/ compounds remain in the water column sufficiently long to be absorbed by plankton and enter food chains that way.

Certain metals, such as zinc and copper, are essential for metabolic processes but become toxic at high concentrations, while other metals, such as cadmium, lead, mercury and arsenic (a metalloid), are non-essential and can be toxic even at low concentrations. Part of their toxicity is to replace essential metals and minerals and block their function. The highest concentrations of toxic metals are typically found in liver and kidney tissues.

High mercury (Hg) levels have been recorded frequently in tissues of coastal bottlenose dolphins (Schaefer et al. 2015, Bellante et al. 2017, Alava et al. 2020). A range of heavy metals (and other toxins) have long been detected in cetaceans from Australian waters, including those in South Australia (Kemper et al. 1994, Long et al. 1997, Evans 2003).

Using combined field and laboratory data, threshold levels for suppression of lymphocyte proliferation are 0.002–1.3 ppm for Hg, 0.009–0.06 for methyl-mercury (MeHg), and 0.1–2.4 for cadmium; thresholds for suppression of phagocytosis are 0.08–1.9 ppm for Hg (Desforges et al. 2016).

In Victoria, Australia, Hg levels in bottlenose dolphin tissues were high compared with studies elsewhere, with levels in stranded individuals being almost three-times higher than in live animals (Monk et al. 2014). Arsenic and lead, and poly-chlorinated biphenyls (PCBs, see later section) were also detected, although at levels lower than those known to cause health effects.

<u>Metals and ADS dolphins</u>

High levels of cadmium, lead and copper have also been recorded in several fish species from Barker Inlet (Edwards et al. 2001). These fish represent a prey sources for dolphins, and the metals likely have been passed on to them from the fish and bioaccumulated in the dolphins.

Since the 1990s, tissue samples from stranded and bycaught bottlenose dolphins in South Australia have been examined for cadmium, lead, zinc and mercury (Kemper et al. 1994, Long et al. 1997, Butterfield 2003, Butterfield & Gaylard 2005, Lavery et al. 2008, Lavery et al. 2009). Mean concentrations have been predominantly classified as low, however, individuals had high concentrations in some tissues. Indo-Pacific bottlenose dolphins in South Australia have had the greatest mean burdens in the liver (lead 0.45 mg/kg, cadmium 6.45 mg/kg, mercury 475.78 mg/kg, zinc 93.88 mg/kg) and bone (lead 2.78 mg/kg), probably reflecting their coastal habitat and benthic prey (Lavery et al. 2008). Identified impacts on the dolphins included elevated metallothionine (a protein that sequesters heavy metals and has a detoxifying function), renal damage, and bone malformation, suggesting long-term exposure (Lavery et al. 2009). However, lesions typical of lead toxicity, such as tubular degeneration and necrosis of kidneys with eosinophilic acid-fast intranuclear inclusions and persistence of mineralised cartilage trabeculae in bone metaphyses with acid fast intranuclear inclusions, were not described, leaving a diagnosis of lead toxicity unclear (Lucy Woolford, pers. comm.).

5.4.2. Persistent organic pollutants (POPs)

POPs comprise a range of synthetic organic compounds that derive from use in agriculture and industry (Fossi & Panti 2018). Their use has declined since the 1960s largely due to recognition of their persistence in the environment, capacity to bioaccumulate and magnify through the food chain, and their negative outcomes on health (Naso et al. 2005, Asaoka et al. 2019, Alava et al. 2020). Specific health effects of POPs include allergies and hypersensitivity, damage to the central and peripheral nervous systems, cancer, endocrine and reproductive disruption, and immune dysregulation (Siciliano et al. 2018, Sonne et al. 2020).

While the use of many POPs has been banned or severely restricted, they remain in marine ecosystems and may increase through terrestrial runoff or resuspension of contaminated sediments (Marsili et al. 2018, Asaoka et al. 2019, Cagnazzi et al. 2020b). In places, their use continues, for example in flame retardants and surfactants. Also, compounds once considered to be safe are being recognised as containing legacy contaminants. For instance, perfluoroalkyl substances (PFASs) were in use in numerous products for over 50 years before their detection and recognition of adverse effects to wildlife (Giesy & Kannan 2001). There are numerous types of POPs. In this report, we focus on organohalogen compounds (containing chlorine, bromine or fluorine) as they have frequently been detected in cetacean tissues (Vetter et al. 2001, Mwevura et al. 2010, Barón et al. 2015, Siciliano et al. 2018).

Organohalogen compounds can derive biogenically or from human sources. Due to their extreme persistence in the environment, bioaccumulative nature, and long term health effects, some organohalogen compounds are particularly well-known marine contaminants that bioaccumulate in dolphins (Zanuttini et al. 2019, Yu et al. 2020). Examples included in this review are organochlorines, such as the pesticide dichloro-diphenyl-trichloroethane (DDT), polychlorinated biphenyls (PCBs), and the industrial byproducts, poly-brominated diphenyl ethers (PBDEs) and PFASs.

5.4.2.1. *DDT*

Agricultural pesticides that persist as organic pollutants in the marine environment include dieldrin, chlordane, endrin, heptachlor, aldrin, mirex and DDT (Butler 1966, Shaw et al. 2010). Although use of these pesticides has been restricted globally since the 1970s and 1980s, their impacts persist (Zhang et al. 2020).

Records of DDT in dolphins was common during the late 1900s (Cockcroft et al. 1989, Lahvis et al. 1995, Law et al. 1995). In the Mediterranean, declines in DDT levels in striped dolphins were evident between 1987 and 2002 (Aguilar & Borrell 2005), and levels did not appear to enhance mortality during a morbillivirus epizootic outbreak in 2007 (Castrillon et al. 2010). DDT-related compounds continue to bioaccumulate in dolphins (Mackintosh et al. 2016, Alonso et al. 2017, Yu et al. 2020), however, including in Queensland coastal waters, where pulses in prevalence are likely related to flooding events (Cagnazzi et al. 2013, Cagnazzi et al. 2020a).

While our focus is on persistent and highly toxic pesticides such as DDT, pesticides with current regulatory authorities for use are also detected in marine environments (Mai et al. 2013).

DDTs and ADS dolphins

There are no reports of DDT or other agricultural pesticides in ADS dolphins.

5.4.2.2. *PCBs*

Use of PCBs declined following the realisation of their toxic qualities in the 1970s-80s, however, they have remained as POPs (Borja et al. 2005). PCBs were used in a range of industrial products including sealing and caulking compounds, inks and paint additives, coolants and lubricants. Accumulation of PCBs is higher in male dolphins. Females can pass PCB residues through the placenta to their young, which reduces their own levels (Marsili & Focardi 1997).

Elevated levels of PCBs are correlated with reduced health in marine mammals (Pulster et al. 2009, Schwacke et al. 2012, Jepson et al. 2016, Cagnazzi et al. 2020a). In the Mediterranean, a majority of bottlenose dolphins tested between 2011-2017 had PCB concentrations in their tissues that were above toxicity thresholds (Genov et al. 2019). In southern Brazil, total PCB levels in coastal bottlenose dolphins were above threshold levels known to have physiological effects (Righetti et al. 2019). PCB thresholds for suppression of lymphocyte proliferation are 0.001–10 ppm; thresholds for suppression of phagocytosis are 0.6–1.4 ppm (Desforges et al. 2016).

Reproductive failure was correlated with high levels of PCBs in harbour porpoise (*Phocoena phocoena*) in the UK (Murphy et al. 2015). Another study in the UK showed that, between 1990 and 2017, blubber PCB concentrations in stranded harbour porpoises fell to below the proposed thresholds for toxic effects (Williams et al. 2020).

Low PCB concentrations can still combine with other stressors to influence survival. In harbour porpoise, an increase in PCB blubber concentrations of 1 mg/kg lipid corresponded with a 5% increase in risk of infectious disease mortality (Williams et al. 2020). Guo et al. (2021), using measured PCB accumulation rates of 0.29 ± 0.07 mg/kg lipid per year and individual-based model simulations, determined that Indo-Pacific humpback dolphins in the Pearl River Estuary, China, would incur a 0.9% decline in calf survival based on PCB exposure alone. When combined with other stressors, namely underwater noise and prey depletion, an 8.1% decline in calf survival was predicted.

<u>*PCBs and ADS dolphins*</u>

A single bottlenose dolphin that stranded in Port Adelaide was found to have higher concentrations of PCBs in its blubber than 12 other marine mammals, mostly oceanic whales from Tasmania, from around Australia (Gaus et al. 2005). It was noted the PCB levels in the Port Adelaide dolphin were comparable to levels in two bottlenose dolphins previously sampled from the Port Adelaide (M. Ruchel, unpublished data, cited in Gaus et al. 2005) and to marine mammals from areas that are considered to be relatively polluted, such as Risso's (*Grampus griseus*) and bottlenose dolphins in the Mediterranean Sea (Jimenez et al. 2000)

Weijs et al. (2020) reported that PCB residues in multiple tissues (blubber, liver, kidney, muscle) in stranded and bycaught Indo-Pacific bottlenose dolphins from South Australia's gulfs increased between 1989 and 2014. The PCB residues were high by Australian standards, with several exceeding toxicity thresholds, but were low compared with marine mammals from severely polluted areas, such as the Mediterranean Sea.

5.4.2.3. *PBDEs*

Polybrominated diphenyl ethers (PBDEs) are a class of halogenated organic brominated flame retardants (BFRs) that were used for flame protection in products such as electronic equipment and textiles. They are highly persistent in the marine environment (Hu & Hu 2014). Effects on mammalian health include endocrine disruption, carcinogenesis, and reproductive and developmental toxicity by retarding steroidogenesis, including hormone production by ovarian cells for the maintenance of reproductive tissues (Song et al. 2008, Lavandier et al. 2016). PBDEs are commonly detected in tissues of stranded dolphins along with a suite of other organohalogens and heavy metals, such that their individual impact is difficult to isolate (Ko et al. 2014, Weijs et al. 2016).

Methoxylated polybrominated diphenyl ethers (MeO-PBDEs), recognised as both a natural product and novel pollutant, were detected in the blubber of Indo-Pacific bottlenose dolphins in Tanzania and, like organochlorines, transferred readily from mother to calf (Mwevura et al. 2010). While structurally similar to PBDEs, the biological consequences of MeO-PBDEs accumulation are unknown. Baron et al (2015) found bioaccumulation of total halogenated flame retardants in brain and blubber tissue of dolphins in the Mediterranean Sea potentially had neurotoxic effects.

PBDEs and ADS dolphins

As with PCBs, levels of PBDEs in stranded and bycaught Indo-Pacific bottlenose dolphins from South Australia's gulfs increased between 1989 and 2014 (Weijs et al. 2020). Levels in some individuals were amongst the highest in the sourced literature. The results indicated that legacy pollutants may play a role in the long-term health of Indo-Pacific bottlenose dolphins in South Australia (Weijs et al. 2020).

5.4.2.4. *PFASs*

Per- and poly-fluoroalkyl substances (PFASs) include >3000 highly stable compounds that were extensively used in fire-fighting foams, pesticides, and household products, such as protective coatings on furniture and non-stick surfaces on cookware (Wang et al. 2017b, Kirk et al. 2018). They have subsequently been found to compromise reproduction, metabolism, brain and nerve function, heart and blood vessels, airways and lungs, thyroid gland and immune function in humans and wild animals (Kirk et al. 2018). Immunotoxicity is one of the more sensitive and notable effects of PFASs affecting both cell-mediated and humoral immunity (Corsini et al. 2014).

PFAS compounds can enter the marine environment through long-range atmospheric transport and runoff from coastal sites, particularly where fire-fighting foam has been used over extended periods (Berger et al. 2004, White et al. 2015, Lin et al. 2020).

Dolphins are likely exposed to PFASs from their food. The PFASs can then accumulate in blood plasma and liver tissues, and can pass on to young through the placenta and milk (Lynch et al. 2019, Sciancalepore et al. 2021, Stockin et al. 2021). PFASs have been detected in most marine mammals screened for these compounds (Law et al. 2003, Dorneles et al. 2008, Quinete et al. 2009, Moon et al. 2010, Galatius et al. 2013).

While literature on the prevalence of PFAS in marine mammals grows, there are few studies on impacts to marine mammals (Fair & Houde 2018). In bottlenose dolphins in South Carolina, USA, however, associations between perfluoroalkyl compounds and abnormal immune and clinical chemistry parameters suggested impacts on immune, hematopoietic, kidney, and liver function (Fair et al. 2013). Perfluoro-octane sulfonate (PFOS) is the most prevalent PFAS detected within the environment (López-Berenguer et al. 2020). Elevated PFOS levels in bottlenose dolphins in South Carolina directly dysregulated the dolphin's cellular immune system (Soloff et al. 2017).

PFASs and ADS dolphins

A preliminary investigation by the EPA compared PFAS levels in dolphins from Barker Inlet and metropolitan Adelaide with dolphins elsewhere in Australia (Gaylard 2017). Levels of PFAS, predominantly PFOS, in Indo-Pacific bottlenose dolphins in the Swan River (2,800–14,000 ng/g) and Barker Inlet (510–5,000 ng/g) are amongst the highest so far recorded (Table 3). PFAS compounds (PFOS, perfluoro-octanoic acid PFOA and perfluoro-nonanoic acid PFNA) have also been detected in livers of autopsied Australian sea lion pups (*Neophoca cinerea*) from Kangaroo Island (Taylor et al. 2021).

5.4.1. Hydrocarbons

Hydrocarbons comprise thousands of compounds of hydrogen and carbon. They may enter the marine environment through natural releases of underground oil and gas, oil spills, terrestrial runoff and the degradation of manufactured products and textiles (Godard-Codding & Collier 2018). Over time, hydrocarbons in the marine environment will break down. Cetaceans may inhale, ingest or be exposed to hydrocarbons through skin contact (Godard-Codding & Collier 2018). These hydrocarbons can have a range of health consequences, including reduced body condition, calcium imbalance, inflammation, reproductive failure, organ damage, altered liver function, immune changes, and susceptibility to infection (Helm et al. 2015).

Table 3. Levels of PFOS recorded in tissues of dead (liver) and live (plasma) bottlenose dolphins.

Species	Tissue	Country	Location	n	Median PFOS (ng/g)	Max. PFOS (ng/g)	Reference
T. aduncas	liver	Aust.	Port River, SA	9	1986	5000	(Gaylard 2017)
T. aduncas	liver	Aust.	Adelaide metro, SA	5	436	690	(Gaylard 2017)
T. aduncas	liver	Aust.	West Coast, SA	6	7	13	(Gaylard 2017)
T. aduncas	liver	Aust.	Swan River, WA	4	6975	14000	(Gaylard 2017)
T. aduncas	liver	Aust.	Mandurah, WA	2	227	420	(Gaylard 2017)
T. aduncas	liver	Aust.	Bunbury, WA	8	37	97	(Gaylard 2017)
T. aduncas	liver	China	Pearl River	52	1180		(Gui et al. 2019)
T. truncatus	liver	Aust.	Offshore, NSW	7	705	1800	(Gaylard 2017)
T. truncatus	liver	Aust.	Tasmania	3	46	71	(Gaylard 2017)
T. truncatus	liver	US	Sarasota Bay, Fl	20	489	1520	(Kannan et al. 2001)
T. truncatus	liver	Italy	North Adriatic	20	194	630	(Sciancalepore et al. 2021)
T. truncatus	liver	Spain	W Mediterranean	5	211		(López-Berenguer et al. 2020)
T. truncatus	liver	Croatia	North Adriatic	1	43		(Kannan et al. 2002)
T. truncatus	plasma	US	Charleston, SC	76	1246	6260	(Fair et al. 2012)
T. truncatus	plasma	US	Charleston, SC	19	571	1833	(Soloff et al. 2017)
T. truncatus	plasma	US	Indian River, Fl	81	598	3620	(Fair et al. 2012)
T. truncatus	plasma	US	Sarasota Bay, Fl	12	340		(Houde et al. 2006)

Most of the information on hydrocarbon impacts on cetaceans comes from the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico in 2010. This oil spill had far-reaching consequences for cetaceans in the region. There were over 1140 cetacean strandings following the event (Litz et al. 2014) and >50% declines in nearby bottlenose dolphin populations (Schwacke et al. 2017). The spill contributed to adrenal disease, lung disease, and poor health in dolphins throughout the Gulf of Mexico (Venn-Watson et al. 2015). There was also evidence of long-term immune suppression in bottlenose dolphins (Schwacke et al. 2013, De Guise et al. 2021). Eight years on, responses included sustained T-lymphocyte proliferation and a shift in cytokine expression toward a T-helper 2 response through the modulation of regulatory T-cells. Evidence points to regulatory T-cells as a target for the immunomodulatory effects of oil exposure. There is also potential multigenerational immune health effects, as immunological trends appeared exaggerated in dolphins born after the spill (De Guise et al. 2021).

Impacts of long-term exposure to hydrocarbons, such as might be experienced by coastal dolphins through stormwater runoff from urban areas, are poorly known. Pulses in hydrocarbon concentration from industry and road runoff likely occur through drought and rain cycles.

Hydrocarbons and ADS dolphins

Hydrocarbon levels have not been investigated in bottlenose dolphins in South Australia.

5.4.2. Wastewater contaminants

A principal impact of wastewater outflow has been increased nutrient loads into coastal waters. In addition, chemicals such as pharmaceutical and personal care products, and endocrine disrupting compounds, such as steroid estrogens, are pollutants that escape wastewater treatment and are discharged with effluents into coastal environments (Braga et al. 2005a, Bertin et al. 2011, Green et al. 2013). They can be resistant to degradation in seawater and marine sediments (Ying & Kookana 2003). Although they may be detected distant from the source (Harries et al. 1997), many will accumulate in marine sediments adjacent to the outflow site (Braga et al. 2005b).

The toxicological impacts of chemicals from wastewater discharge on marine ecosystems is poorly understood (Fernandes et al. 2010, Dhillon et al. 2015, Yueh & Tukey 2016). One example, however, is that synthetic estrogen was linked to collapse in freshwater fish populations in Canadian rivers (Kidd et al. 2007). Several potentially pathogenic bacteria of humans and domestic animals, including *Clostridium perfringens*, *Campylobacter* spp., *Staphylococcus* spp., *Erwinia amylovora*, and *Helicobacter* spp. and the parasite *Toxoplasma* gondii have also passed through wastewater outflows and been detected in tissues and digestive tracts of coastal bottlenose dolphins (Stewart et al. 2014, Bernal-Guadarrama et al. 2015, Jaing et al. 2015, Anderson 2021).

Wastewater contaminants and ADS dolphins

Wastewater-derived endocrine disrupting chemicals and triclosan have been detected in sediments in Barker Inlet, in association with out-flow from the Bolivar plant (Fernandes et al. 2008, Fernandes et al. 2010). Levels recorded were amongst the highest reported globally. Their effects, if any, on dolphins in the estuary are unknown.

5.4.3. Plastics

Plastics are the main form of marine litter to impact on marine mammals, through ingestion and entanglement (Fossi et al. 2018). Macro-plastics are often noted in the stomach contents of stranded marine mammals (Lusher et al. 2018). Large pieces of plastic may be inadvertently consumed during foraging and play while smaller pieces may be consumed via prey (Lusher et al. 2018). These macro-plastics can cause immediate distress, reduced gastric capacity and intestinal obstruction or perforation, leading to starvation (Panti et al. 2019, Byard et al. 2020).

Micro-plastics may also biomagnify up food chains. Plastics absorb and thereby concentrate POPs from seawater (Bakir et al. 2012, Van et al. 2012), thereby representing a pathway for POPs to concentrate in higher marine predators such as dolphins (Fossi et al. 2018).

Plastic additives may be toxic to cetaceans (Fossi et al. 2018). Phthalate esters (PAEs) are chemical additives to common consumer goods including cleaning products, cosmetics, personal care products, and plastic, that have been associated with endocrine disruption and reproductive impairment (Hart et al. 2018). Because they are not chemically bound to these products and are widely used, their potential for environmental contamination is significant. Diethyl phthalate (DEP) and di-2-ethylhexyl phthalate (DEHP) were detected in bottlenose dolphin urine, in Sarasota Bay, Florida, but with undetermined health consequences (Hart et al. 2018).

Plastics and ADS dolphins

An Indo-Pacific bottlenose dolphin carcass recovered from the ADS in December 2019 contained in its stomach two heavy-duty work gloves, parts of a plastic squid lure and two unattached fishing-hooks (Byard et al. 2020). The dolphin had died from injuries related to fishing hook impalement and fishing line entanglement (see section 5.6.1).

5.4.4. Water quality

As marine species that often occupy estuarine environments, bottlenose dolphins can tolerate a range of salinities, temperatures, turbidities and pH, however, they avoid environmental extremes (Fury & Harrison 2011a).

5.4.4.1. *Salinity*

Extended exposure to hyposaline conditions below 10 ppm can disrupt skin permeability causing skin lesions, known as freshwater skin disease; lesions can be colonised by fungi, bacteria and algae, and can ultimately lead to mortality (Colbert et al. 1999, Mullin et al. 2015, Duignan et al. 2020). Extended hyposaline conditions and single flood events have been correlated with an increased prevalence of skin lesions in bottlenose dolphins (Toms et al. 2021), including Indo-Pacific bottlenose dolphins in Australia (Duignan et al. 2020). Significant increases in lesions may be delayed and become apparent several months after the flood.

Dolphins abandon estuaries at times of lower salinity, high turbidity, or low levels of pH and dissolved oxygen, associated with floods (Fury & Harrison 2011a). The time until dolphins return to the estuary post flood is variable and likely related to need (to forage for example) as well as the return of higher salinity levels, for example to above 29 ppm (Fury & Harrison 2011a).

Salinity and ADS dolphins

Through tidal circulation and flow of seawater that is pumped into West Lakes out through the Port River, salinity in the Port River and Barker Inlet generally remains near that of normal seawater. Outflow of wastewater and occasional runoff from storm events may lower local salinity in some areas of the Inlet.

Salinity may increase above seawater levels during summer, when the whole of Gulf St Vincent can experience hypersaline conditions (Bye & Kampf 2008). A major factor influencing hypersaline conditions is flushing of salt-extraction ponds that line the coast of Barker Inlet. Brief periods of excessive hypersalinity have damaged vegetation (seagrass, mangrove and saltmarsh) in the Port River and Barker Inlet (South Australian Environment Protection Authority 2021[2]) however, the impacts of changing salinity levels on dolphins and the availability of their prey have not been investigated.

5.4.4.2. *Temperature*

Subcutaneous blubber performs a key function in regulating thermal tolerance in dolphins. It insulates them from both cold and hot water temperatures (Heath & Ridgway 1999). In response to entering warm water, dolphins redistribute body heat such that core temperature is reduced

[2] https://www.epa.sa.gov.au/community/stay-informed/dry-creek-saltfields

and blubber and appendage temperatures are increased (Heath & Ridgway 1999). Extended exposure to thermal extremes may be managed through movement to more thermo-neutral waters, at depth, offshore or migration to different latitudes, and managing activity to periods of the day or night when ambient temperatures can be more optimum (Noren et al. 1999).

Body size greatly influences tolerance to temperature extremes. Experimental studies on captive bottlenose dolphins weighting >187 kg determined a lower critical water temperature to be 5.5-5.7 °C (Yeates & Houser 2008). There are no data on upper critical temperatures for dolphins.

The importance of skin and subcutaneous blubber to thermal tolerance highlights that damage to these body parts may reduce thermal tolerance. Co-occurrence of skin lesions and thermal extremes can increase stress on individuals.

Temperature and ADS dolphins

Water temperature in Barker Inlet generally ranges between 15 and 25 °C (Jones et al. 2008). Thermal effluent from Torrens Island Power-station has been recorded to increase summer water temperatures in Angus Inlet, in the Port River and Barker Inlet, to over 30 °C (Thomas et al. 2001). Pulses of high temperature such as this could influence the distribution and abundances of fish and benthic species nearby (Jones et al. 1996). The impact of this thermal effluent on the distribution of dolphins in the ADS has not been investigated. Dolphins do frequent Angus Inlet, however, suggesting they can tolerate the higher temperatures in these waters.

The heatwave induced algal bloom and fish mortality in Gulf St Vincent in 2013 (Roberts et al. 2019), which coincided with the unusual mortality event of dolphins (Kemper et al. 2016), may indicate a link between extreme high temperature and dolphin health. The dolphins could have been heat stressed or nutritionally compromised by loss of prey, increasing their susceptibility to disease such as that caused by *Morbillivirus*.

5.4.4.3. *pH*

pH is a measure of the relative amount of free hydrogen and hydroxyl ions in a solution, a low availability is termed acidic and a high availability is termed basic or alkaline. The pH level of aqueous solutions affects rates of chemical reactions, equilibrium conditions, biological processes, and toxicant availability (Knutzen 1981, Marion et al. 2011). For example, heavy metals such as Cd, Zn, Pb and Cu precipitate (or co-precipitate) at higher pH levels, but are released into solution at lower pH levels (Hamdan 2009). Many chemical reactions that are essential for life are sensitive to small changes in pH. Changes to pH can influence growth rates of organisms (Harris et al. 1999, Hansen 2002, Ringwood & Keppler 2002) and at extremes, out of the range pH 5.0 to 9.0, many species do not survive (Ohrel & Register 2006). Estuarine pH levels generally average from 7.0-7.5 in fresher sections, to 8.0-8.6 in more saline areas (Ohrel & Register 2006).

Studies have not documented impacts or tolerance of dolphins to change in pH. pH is included in this review as its documented impacts on other marine organisms signal it to be a potential concern to coastal ecosystems where dolphins live and anthropogenic influences can rapidly change sea water pH.

pH and ADS dolphins

A major factor influencing seawater pH in the ADS is the highly acidic (pH levels <3.5) outflow from the former salt extraction ponds (Thomas et al. 2003). Impacts on dolphins in the ADS of rapid changes in pH caused by this outflow have not been investigated.

5.4.4.1. *Nutrients*

Nutrient enrichment and eutrophication are major concerns in many estuarine and wetland ecosystems (Smith et al. 2006, Day et al. 2013). Excess nutrients negatively impact on mangroves and seagrass (Edyvane 1999), challenging ecosystems and reducing prey availability to dolphins. Nutrient enrichment can also stimulate blooms of toxic algae that also impact on dolphin health (see above). Primary sources for anthropogenic nutrient enhancement in coastal ecosystems are through effluent outflows and runoff from agricultural land (Ito et al. 2021).

Nutrients and ADS dolphins

The main sources of anthropogenic nutrient enhancement in the Port River and Barker Inlet have been point source discharges from the wastewater treatment plants and the Penrice Soda Products plant. Extensive loss of seagrass at the outflow from the largest wastewater treatment plant, at Bolivar, has led to a changed wave climate and contributed to a die-off of mangroves (Mifsud et al. 2004). Nutrient enriched discharges have also originated from other industries, stormwater, vessels and atmospheric fallout (Anonymous 2008).

Impacts of nutrient enrichment and eutrophication in the Port River and Barker Inlet on the local bottlenose dolphin population, either directly, through stimulation of algal blooms, or through degradation of their food chains, have not been investigated.

5.4.5. Air pollution

Air pollution can impact on the health of coastal dolphins. Following extensive forest fires around San Diego, USA, in 2003 and 2007, bottlenose dolphins in a coastal naval facility had higher serum carbon dioxide and chloride, and lower calcium and neutrophil levels, than before the fires (Venn-Watson et al. 2013). It was concluded that fire smoke inhalation has effects on dolphin physiology, including calcium homeostasis, lung function and immune response.

Increased nutrients from the air, derived from agriculture and coastal vegetation, can also influence nutrient availability in coastal ecosystems.

Air pollution and ADS dolphins

Air pollution influences on dolphin health in the Port River and Barker Inlet have not been investigated. Nearby sources of air pollution include trace products released from the gas fired power-stations and dust from Adelaide Brighton Cement.

5.4.6. Noise pollution

Dolphins rely on echolocation to hunt prey and audible sounds to communicate and detect threats (Tyack 1997). Many coastal industries and on-water activities produce sound which is transmitted underwater and could be detected by dolphins. While dolphins adjust call frequency parameters to compensate for increased background noise (Papale et al. 2015), if sufficiently loud, anthropogenic noise can alter the behaviour of dolphins, masking their ability to detect prey or avoid danger (such as approaching vessels), leading to reduced health and injury (Southall et al. 2007). Examples of anthropogenic noises that have been linked to avoidance and potential injury of dolphins include shipping, seismic exploration, naval sonar operations, underwater electrical currents, underwater explosions, and pile-driving (Richardson & Würsig 1997, Weilgart 2007, Thompson et al. 2010, Aarts et al. 2016).

To reduce noise exposure to cetaceans, industries have trialed mitigation measures including exclusion zones, vessel speed limits, acoustic decoupling of noisy equipment (e.g., mounting engines on rubber blocks that separate engine noise from the ship's hull), altering propellor shape to reduce cavitation, bubble curtains/jackets, gradual ramping up of piling hammers, no-dumping policies and silt curtains (Jefferson et al. 2009). All methods have varying levels of success depending on the species of marine mammal being shielded, along with compliance, enforcement, and noise propagation qualities of the location (depth, sediment, coastal features etc.). The biological significance of observed responses to vessel noise is mostly unknown (Erbe et al. 2019).

Bottlenose dolphins are sensitive to coastal construction noise, such as sheet and pile-driving (David 2006). During pile-driving activities in the Swan River, Perth, there was reduced detection of bottlenose dolphins (Paiva et al. 2015).

Noise pollution and ADS dolphins

Noise pollution in the Port River and Barker Inlet are poorly understood but are likely to be predominantly from shipping, including commercial (e.g., dredging, container ships and fishing vessels) and recreational vessels (e.g., boats and jet skis). For example, as recognised in the Swan River (Paiva et al. 2015), dolphins in the Port River would move out of and avoid areas of pile driving during construction and infrastructure maintenance.

There are guidelines for piling noise mitigation in South Australian waters (Anonymous 2012b). These include having observers spot for cetaceans during piling procedures, stopping activities if cetaceans are nearby, preferentially using low-noise procedures such as vibro-piling rather than impact piling, and using cofferdams where possible (solid casing around the pile and draining water from the casing). There is no on-going monitoring of underwater noise levels in the Port River and Barker Inlet, nor understanding of the tolerance of Indo-Pacific bottlenose dolphins to underwater noise.

5.5. Environmental change

Impacts of environmental change on animal health are most influenced by the rate of change. At the scale of thousands of years, as has occurred through sea-level rise since the last ice-age, generations of a species could evolve with the change. At the rate of current anthropogenic enhanced climate change, species may need to alter their distribution (Perry et al. 2005). Migration and re-distribution can help some species survive if they detect gradual changes in water quality that impact on their health, such as seasonal cycles in prey availability or water temperature. Sudden events, such as floods and heatwaves, come with insufficient time to avoid them, so may have the greatest impacts on health.

Current rates of climate change, and associated increased frequency of extreme events, have the potential to add multiple stressors to coastal dolphins. These include changes to water flow from increased flooding and longer drought events, changes to water and air temperature, along with increased frequency and intensity of heat waves.

Indo-Pacific bottlenose dolphins already reside in tropical and temperate coastal waters in wide-ranging temperatures, 10 to 35°C, therefore, they are likely to retain a broad latitudinal range during currently predicted increases in sea temperature. During heatwaves, however, dolphins may move toward known cooler waters, offshore and at depth. At greatest risk will be calves, which have less experience, and lactating females that may remain to support their calves.

Warmer water temperatures tend to be associated with changes in other water quality indicators, such as reduced dissolved oxygen, increasing bacterial activity and more frequent algal blooms (Paerl & Huisman 2008, Scofield et al. 2015). Thus, warmer waters and increased frequency of heatwaves may be associated with multiple stressors on coastal dolphins that are currently adapted to temperate conditions.

Environmental change may alter prey availability, lead to increases in competition, stress, movement, and mortality. If known prey availability reduces, for example, dolphins may consume novel prey that may be detrimental, such as porcupine fish and stingrays that can give puncture wounds in the digestive tract, or toxic species, like toadfish (Byard et al. 2010).

Environmental change and ADS dolphins

Potential impacts of environmental change on dolphins in the ADS have not been investigated.

5.6. Direct human interaction

5.6.1. Dolphin watching

Presence of tour boats can influence the behaviour of dolphins, including feeding, suckling, and rest behaviours (Bejder et al. 2006a, Allen et al. 2007, Arcangeli et al. 2009, Christiansen et al. 2010, Dans et al. 2012). In Doubtful Sound, New Zealand, male bottlenose dolphins started to avoid tour vessels as they approached whereas females switched to avoidance strategies only when interactions became intrusive (Lusseau 2003). In Port Stephens, Australia, dolphin-watching vessels frequently contravened cetacean watching regulations and caused measurable changes to Indo-Pacific bottlenose dolphin behaviour, including 66.5% less time feeding, 44.2% less time socialising and four times more milling. Additionally, dolphins were never observed to rest in the presence of dolphin-watching boats (Steckenreuter et al. 2012b). Moreover, dolphin groups were more cohesive during dolphin-watching boat encounters and dolphins tended to avoid tour boats. These effects were exacerbated as the number of boats increased and the distance from boats decreased (Steckenreuter et al. 2012b).

A retrospective analysis of 30 years of cetacean tourism management in New Zealand recommended that cetacean tourism be acknowledged as a sub-lethal anthropogenic stressor, that management be site specific, and that management should engage collaboratively with operators and the community (Fumagalli et al. 2021). Establishment of sanctuary zones is a common means for attempting to protect coastal dolphins from dolphin-watching (and other vessel) approaches, however, they can be ineffective unless there is sufficient monitoring and implementation of consequences should regulations be contravened (Howes et al. 2012).

Dolphin watching and ADS dolphins

In 2005 the Australian Commonwealth and state governments jointly developed the '*Australian National Guidelines for Whale and Dolphin Watching*' to provide codes of conduct for all human activities involving wild cetaceans: these have been updated several times (see Appendix 10.4) (Anonymous 2017).

In Gulf St Vincent, South Australia, swim with dolphins tourism was noticed to cause increased milling behaviour and attraction of some groups of bottlenose dolphins which potentially delayed their feeding (Peters et al. 2013). A tour company offering dolphin sightings in the Port River from a passenger vessel operated between the early 1990s and 2021. There was no monitoring of potential impacts on dolphins of the vessels approach. Kayak tours within Barker Inlet, that advertise the potential to see dolphins, are based out of Garden Island and operate mainly through warmer months. There has been no monitoring of potential impacts on dolphins of the kayak approaches.

5.6.2. Provisioning (feeding) & unintentional harm

Provisioning of wildlife is usually undertaken without malice and without recognising that it may cause harm. Rather, the intent is to gain a closer wildlife experience or to assist the wildlife. Food provisioning can increase the risk of injury to dolphins from fishing gear entanglement, external hooking or ingestion of hooks, ingestion of debris and boat strikes (Christiansen et al. 2016). In some cases, but not always (Neil & Holmes 2008), provisioning has led to females paying less attention to calves, potentially reducing calf survival (Mann & Kemps 2006, Foroughirad & Mann 2013).

Attraction to fishing vessels that discard a part of the catch, such as prawn vessels, or recreational fishers discarding unwanted catch and bait, may inadvertently acclimatise dolphins to vessels (Durden 2005). Acclimatised dolphins will be less vigilant for dangers such as entanglement and collision, less vigilant of young in their care and distracted from rest and foraging behaviours (Durden 2005).

Provisioning and ADS dolphins

In South Australia, it is an offence to feed marine mammals or dispose of materials into the water if a marine mammal is present and likely to eat the material. In the ADS, deliberate attempted feeding of dolphins is observed occasionally (ADS Annual Reports[3]).

5.6.3. Harassment by vessels, kayakers, swimmers, surfers

Changes in movement, diving behaviour and elevated stress levels in bottlenose dolphins have been linked to increases in boat activity (Seuront & Cribb 2011). For example, in Jervis Bay, New South Wales, powerboat approaches influenced the direction of travel and surfacing behaviour of bottlenose dolphins (Lemon et al. 2006), and in Milford Sound, New Zealand, residency time of bottlenose dolphins is reduced by elevated boat traffic (Lusseau 2005). Behavioural changes could indicate masking of the dolphins' acoustic communication, disturbance of prey, increased dolphin transition times, and/or induced stress and changes to group structure (including increased mate guarding) (Puszka et al. 2021).

Bottlenose dolphins can discriminate between different vessels and respond according to perceived benefits or impacts. Some close approaches by humans, such as by surfers, swimmers

[3] https://www.environment.sa.gov.au/about-us/our-reports/annual-reports

and kayakers, can be relatively benign (Fandel et al. 2015), while others elicit a response. In Lampedusa Island, Italy, bottlenose dolphins preferred to leave an area in response to increased disturbance from motorboats, which had no benefits, but remained and changed their acoustic behaviour to compensate for the masking noise of trawlers, which could enhance feeding opportunities (La Manna et al. 2013).

Voluntary codes of conduct may mitigate but not negate vessel traffic around dolphins in coastal environments (Duprey et al. 2008). In Port Stephens, New South Wales, a comparison between protected area classes found control zones (no boat access) were effective and speed restriction zones were not effective at limiting vessel impacts on dolphins (Steckenreuter et al. 2012a). Likely having both zone types was beneficial overall, providing an area where humans could view dolphins that tolerated the close approach and a zone where dolphins could avoid vessels.

Disturbance of dolphins, from vessel activity for example, can be measured but it is difficult to relate this to population level consequences (New et al. 2020). Moreover, behavioural change does not automatically correlate with biological significance (New et al. 2013). To conceptualise how disturbance-induced changes in individual behaviour and physiology affect population dynamics via changes in individual health and vital rates, a population consequences of disturbance (PCoD) framework has been developed (National Research Council 2005, Keen et al. 2021). This could be applied to assess likely disturbance to groups of coastal dolphins.

Harassment and ADS dolphins

One study has recorded that Indo-Pacific bottlenose dolphins in the ADS did not appear to respond to kayakers but displayed increased stress in the presence of fishing boats, motorised inflatable boats and powerboats (Seuront & Cribb 2011). Additionally, a study of mortalities and strandings of cetaceans in South Australia between 1985 and 2000 identified that 5% (including about 17 bottlenose dolphins) had died due to intentional killings, mostly stabbings and shootings (Kemper et al. 2005). This included two Indo-Pacific bottlenose dolphins in the Port River and Barker Inlet that were shot in 1998 (Adamczak et al. 2018). In 2013-14, two more bottlenose dolphins found dead in the ADS had shotgun pellet wounds (South Australian Museum, unpublished data).

In 2010, the South Australian Government gazetted regulations to enhance cetacean protection[4].

[4]https://www.legislation.sa.gov.au/lz?path=%2FC%2FR%2FNational%20Parks%20and%20Wildlife%20(Protected%20Animals%20-%20Marine%20Mammals)%20Regulations%202010

<u>Regulations include:</u>

- Recreational vessels (including motorised and sailing vessels) must not approach dolphins closer than 50 m, with a speed restriction of 4 knots if approaching / departing the area from a marine mammal.
- Jet skis and other jet-propelled vessels must not move closer than 300 m from dolphins
- Swimmers and surfers must not approach dolphins closer than 30 m.
- If approached by a marine mammal: Put your engine in neutral, do not engage propellers until they move off.
- Fines and penalties of up to $100,000 are enforceable for breaches of the regulations.

Annual reports for the ADS (op. cit.) document instances of potential breaches of these regulations. Levels of deliberate harassment of dolphins in the ADS have reduced over time as the public have become more familiar with the presence of the dolphins and their legislative protection.

5.6.4. Boat approach/strike

Approaches by vessels can alter the behaviour of dolphins, causing them to move away from preferred feeding or resting areas and inducing additional metabolic costs. If vessels are not detected, they may strike and injure individuals. Dolphins, along with other cetacean species, are particularly susceptible to vessel strike injuries as they must regularly return to the surface to breath. Vessel impacts and propeller injuries can cause instantaneous death or external and internal injuries that lead to death (Wells et al. 2008). Nonetheless, bottlenose dolphins may recover within weeks from large wounds to the skin and underlying blubber (Zasloff 2011) and have survived and continued to breed following amputations of the distal ends of fins (Wells et al. 2008). As an example of the frequency of impacts and dolphin survival, 6% of bottlenose dolphins in Indian River Lagoon, Florida, had injuries related to vessel impact (Bechdel et al. 2009).

<u>Boat strike and ADS dolphins</u>

In the ADS, dolphin deaths associated with vessel collisions is a known cause of anthropogenic-attributed mortalities, with many dolphin carcasses examined having suffered gross trauma (Adamczak et al. 2018). Between 1987 and 2013, anthropogenic-attributed mortalities included four associated with propellor injuries. Individual dolphins that suffer from health issues, such as high toxin or parasite loads, or auditory damage, may not detect approaching vessels and therefore have an increased susceptibility to boat strikes.

As a mitigation approach, vessel speeds in the ADS are limited to under 4 knots in some areas and under 7 knots in others, in part to reduce the chance of collisions with dolphins. Furthermore, it is an offence for vessels to approach to within 50 m of dolphins, or within 150 m of a distressed dolphin or a calf.

5.6.5. Reduced fish availability

Dolphins are predominantly carnivorous and cannot survive for more than several days without eating. When healthy, adult bottlenose dolphins could consume 35 ± 5 kg/kg body weight/year (Bejarano et al. 2017). For adult Indo-Pacific bottlenose dolphins weighing 100 to 200 kg (Kemper et al. 2014), this equates with 10 to 20 kg/day. If prey availability is reduced in a dolphin's normal foraging range, it must seek resources elsewhere or suffer a rapid deterioration in body condition.

Fish availability may be reduced by removal of potential prey or alterations to food chains. Fishing may not necessarily reduce overall fish biomass but still alter the availability of prey for dolphins. Bottlenose dolphins likely consume smaller fish species and different age classes of the same species than would be targeted by fishers. Hence, removal of larger fish could increase the availability of smaller fish for the dolphins. However, removal of larger fish by fishing could equally reduce the spawning biomass of important prey for dolphins. Understanding flow-on ecosystem effects of removal of larger fish by fishing is complicated.

Ecosystem changes can also reduce prey availability for dolphins. Reduced or rapidly changing water quality can reduce fish abundance in an area. These changes could be short term, lasting days, if the fish can detect and move away from a source of altered conditions (such as increased turbidity or lowered salinity resulting from increased stormwater discharge). Or they may also for years, particularly if the breeding habitat of the fish is removed.

A further threat to individual dolphins is they may choke or suffocate on prey items or natural debris (for examples, see Krzyszczyk et al. 2013, Stephens et al. 2017). Individuals would be more likely to choke while attempting to consume unfamiliar prey, which may be more likely when recognised prey are scarce.

Reduced fish and ADS dolphins

Prey availabilities for dolphins in the Adelaide Dolphin Sanctuary have not been monitored. Known reductions in seagrass habitat due to pollution has probably reduced the abundances of many fish species in Barker Inlet and Gulf St Vincent (Jones et al. 1996, Blandon & zu Ermgassen 2014). The invasive alga *Caulerpa taxifolia*, which was introduced via the aquarium trade (Deveney et al. 2008), now covers large areas in the Port River and Barker Inlet (Wiltshire & Deveney 2017). *Caulerpa taxifolia* can outcompete seagrasses (Westphalen 2008). It does appear to provide habitat that is suitable for local invertebrate species, but with a different faunal composition to local seagrass (Deveney et al. 2008, Lavery et al. 2008).

5.6.6. Entanglement

Entanglement and drowning in set nets for shark control or fishing, such as gillnets, purse-seine nets, and trawler nets, is a common anthropogenic cause of mortality of dolphins (Archer et al. 2010, Reeves et al. 2013, Tulloch et al. 2019, Fruet et al. 2021). For example, in the past 30 years, >1000 bottlenose dolphins have entangled and drowned in bather protection nets off KwaZulu-Natal, South Africa (Plon et al. 2020).

Fishing has other impacts which would be difficult to quantify. For example, dolphins may take baits or lures, or entangle in snagged fishing line, resulting in injury and death (Byard et al. 2020). In coastal areas, dolphins frequently take bait or fish caught on hooks set by recreational fishermen. Recreational fishing gear interactions caused a 2% population decline of bottlenose dolphins in Sarasota Bay, Florida, in 2006, and needs to be considered along with other cumulative human impacts in the development of conservation measures (Powell & Wells 2011). A study in coastal Florida found that fishing hooks embedded in the beak, throat, esophagus or stomach of bottlenose dolphins generally led to death (Wells et al. 2008). Fishing line wrapped around fins could lead to blood loss, infection, impaired mobility, amputation, and death, while line wrapped around the beak, generally results in death (Wells et al. 2008). The type of fishing line used can influence the severity of the entanglement (Barco et al. 2010).

Entanglement and ADS dolphins

Between 1990 and 2010, at least three Indo-Pacific bottlenose dolphins from the Port River and Barker Inlet died from entanglement in fishing line and a further two carcasses had fishing hooks and/or lines in their stomachs (Kemper & Tomo 2011). Since 2010, at least one additional dolphin has died from fishing line entanglement (Byard et al. 2020). Death was caused by septic complications of fishing hook impalement in the blowhole, line entanglement around the rostrum preventing effective feeding, and associated inanition (Byard et al. 2020). At least three dolphins have been caught in the ADS and released from fishing line that otherwise could have resulted in their deaths (Mike Bossley, unpublished data).

5.6.7. Dredging

Dredging activities can displace bottlenose dolphins from foraging patches both through underwater noise and increased turbidity (Pirotta et al. 2013). Dredging can also resuspend toxins that accumulate in sediments, making them available for bioaccumulation (van den Berg et al. 2001, Nayar et al. 2004, Cappuyns et al. 2006), and alters flow rates and water turn-over rates within an estuarine system.

Dredging and ADS dolphins

Substantial dredging of the Port River has increased the depth and volume of water present in the estuary, and has removed/replaced benthic seagrass, algal and infauna communities (Edyvane 1999). Spoils from dredging in the Port River are dumped in a designated site offshore from Outer Harbor. Impacts of dredging activities on dolphin behaviour, distribution and health in the ADS and adjacent waters of Gulf St Vincent has not been investigated.

5.6.8. Coastal change

Most coastal change in the last century has been undertaken to enhance human occupation (Valiela 2006). Forms of anthropogenic coastal change include restructuring of waterways, drainage of coastal swamps and intertidal areas, reclamation of shallow sea environments, fortification against wave action and storm events, and removal of coastal vegetation (Griggs & Tait 1988, Wu et al. 2018). Coastal change alters long established routes of water flow, erosion and sedimentation processes, and coastal marine ecosystems (Louters et al. 1991, Verdiell-Cubedo et al. 2012). Accordingly, higher predators including coastal dolphins have changed their distribution and abundances following coastal modifications (Wang et al. 2017a).

Modification of surrounding coastal habitats can also influence ecosystems within a marine embayment. Vegetation surrounding estuaries are important as habitat for different life stages of numerous marine species, and as filtering mechanisms for sediments and potential toxins that may otherwise enter the estuaries (Van Santen et al. 2007, Chen et al. 2018). Severe reductions in the extent of intertidal and adjacent vegetation can degrade estuarine communities (Mifsud et al. 2004, Svensson et al. 2007).

Coastal change and ADS dolphins

Coastal modifications since European settlement around the Port River and Barker Inlet have influenced water flow, water quality, vegetation and sedimentation processes. Water flow has been altered through dredging activities and barriers. The latter include the construction of training walls at Outer Harbor, to maintain the navigation channel, separation of West Lakes from the Port River to prevent tidal incursions into West Lakes, and the closure of the southwestern end of Angas Inlet, to prevent re-intake of heated discharge water from the Torrens Island Power-station (Jones 2008).

Another critical change in the Port River and Barker Inlet has been the reduction of surrounding mangrove and saltmarsh. This reduction has included die-off through pollution and removal to create access to the water, drainage and elevation of areas to provide space for urban and industrial development (Edyvane 1999, Thomas et al. 2001).

Coastal change around the Port River and Barker Inlet has changed the habitats available for coastal dolphins. Likely, this has influenced the density of dolphins that may occupy the embayment. Actual impacts of individual or cumulative habitat modifications on dolphins, however, have not been investigated.

5.6.9. Research

Research on wildlife has the potential to negatively impact on the individuals' behaviour or health, hence, all research on wildlife in Australia needs to be vetted and approved by an animal ethics committee. Even standard focal follows of individual dolphins have the potential to alter the dolphins' behaviour, in a similar way that tour vessels targeting dolphins may alter the dolphins' behaviour. For example, if distracted by the observer or their vessel, female dolphins may pay less attention to calves, spend time attempting to avoid detection rather than resting or foraging, or move out of preferred foraging areas. It is important that researchers can measure their impacts.

Invasive research procedures can have greater impacts on individuals, but often it is easier to quantify and document these impacts than those of less invasive (or less individually directed) research, such as approaches for behavioural studies. Carrying instruments (such as location and dive-recording devices, has known consequences on hydrodynamic abilities of marine mammals (van der Hoop et al. 2014). Tissue sampling using biopsy darts rarely will cause a long-term injury, but can elevate stress levels in dolphins, both immediately and for future vessel approaches. Although rare, deaths have been recorded as a result of biopsy dart tissue sampling (Bearzi 2000). The measurable increased energy metabolism, stress and trauma related to capture, for example, highlights the importance of weighing up the value of data obtained from studies against the potential impacts of the study (Mancia et al. 2008).

Research and ADS dolphins

Research undertaken to date of live dolphins in the ADS has been minimally invasive, involving observations (Bossley & Rankin 2015, Bossley et al. 2017)., although, several dolphins have been captured to remove fishing line entanglements or for health checks. Biopsies of skin or capture for other research purposes have not been permitted. In adjacent Gulf St Vincent, skin biopsies of Indo-Pacific bottlenose dolphins have been taken for research purposes, such as genetics studies (Pratt et al. 2018). As dolphins in the ADS can frequent the Gulf St Vincent, it is possible that dolphins sampled in Gulf St Vincent have also resided in the ADS.

6. SOURCES OF POTENTIAL THREATS TO DOLPHINS IN THE ADS

This section presents several of the sources of potential threats. It is not a comprehensive list.

6.1. Stormwater/ groundwater

Stormwater runoff from urban areas flushes potential contaminants from roofs, paths, roads, septic sewage systems, agricultural land, industrial sites and stagnant ponds into coastal environments (Ahmed et al. 2019) (Figure 6). Contaminants recorded in stormwater runoff include plastics, heavy metals, PCBs, PBDEs, polycyclic aromatic hydrocarbons, halogenated aliphatics, halogenated ethers, monocyclic aromatics, phenols and cresols, phthalate esters, nitrosamines, pesticides, and other organics (Makepeace et al. 1995, Ahmed et al. 2005, Huber et al. 2016, Maruya et al. 2016). Pathogenic bacteria, viruses and protozoa can also be found in stormwater runoff (Rajal et al. 2007). Due to its range of potential sources from human, livestock and domestic animals, and reduced level of treatment, stormwater is likely to contain a different pathogen profile than sewage (Ahmed et al. 2019).

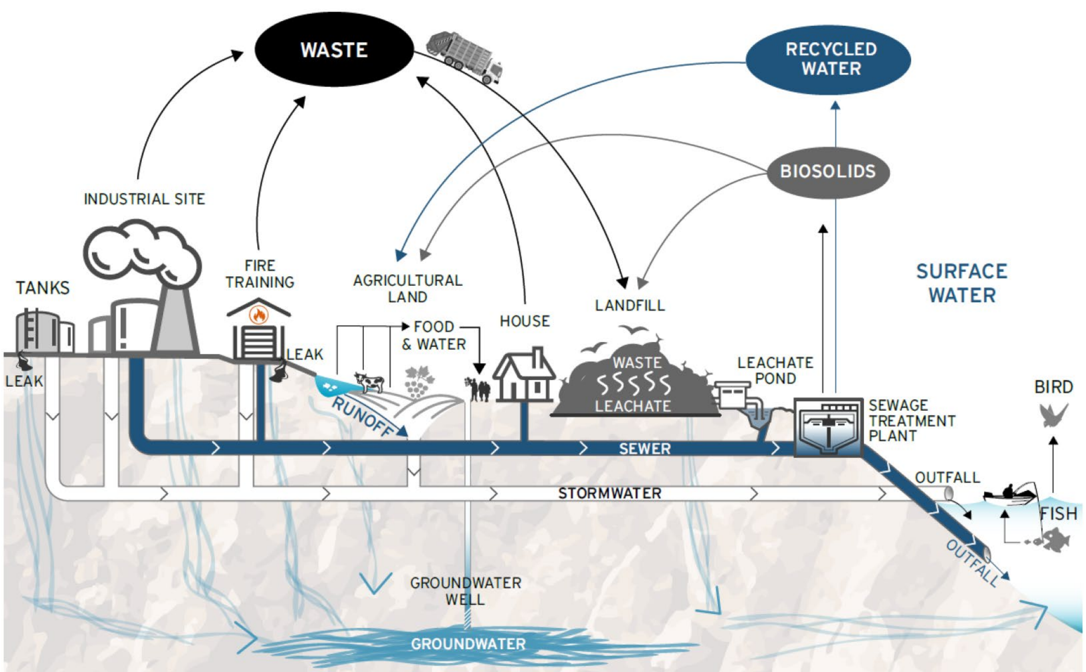

Figure 6. Pathways for pollutants of anthropogenic origin into coastal environments (source, Victorian Environment Protection Authority).

A 2011 review of marine debris along the metropolitan coast of Adelaide recorded a dominance of plastic food packaging (wrappers) (Peters & Flaherty 2011). Much of this debris would have derived from stormwater runoff.

Storm water runoff has the potential to introduce many chemicals into the Port River and Barker Inlet that may by hazardous or toxic to marine organisms and to biomagnify up the food chain, resulting in toxic levels accumulating in dolphin tissues. In addition, storm water can temporarily lower the salinity of waters in the ADS causing injury to dolphins and encouraging them to move out of the area, at least temporarily.

6.2. Wastewater

In the past, raw sewage once was fed into North Arm, within Barker Inlet, from the Islington Sewage Treatment Works (Thomas et al. 2003). Then, in the mid-1900s, a wastewater treatment plant at Port Adelaide opened and discharged chlorinated effluent into the Port River. Among other impacts, this plant contributed to an algal bloom problem in the river. In 1966, a new treatment plant was established at Bolivar: treated waste was discharged to the north of Barker Inlet (within the ADS, see Figure 1). The Port Adelaide treatment plant was upgraded in 2004, with much of its discharge transferred to the Bolivar Treatment plant (Fernandes et al. 2010). Bolivar has become Adelaide's largest wastewater treatment plant, and in 2021 it treated approximately 70% of metropolitan Adelaide's wastewater.

Pollution from the Bolivar outflow was implicated in reductions of seagrass communities in the immediate area of the discharge in the early 1990s (Overton 1993). The loss of seagrass caused de-stabilisation of sediments and increased the energy of wave action on the coast. This along with direct pollution from the sewage outflow were thought to play a role in the decline of mangrove communities in the vicinity of the outflow (Overton 1993). Loss of seagrass and decline of mangrove communities would have had flow on effects through the coastal ecosystems, ultimately reducing prey availability to dolphins in the ADS.

While much of the outflow from the Bolivar wastewater treatment plant flows through channels to the north and out of Barker Inlet, under certain hydrodynamic conditions, wind and tide drive pulses of the discharge directly south to southern Barker Inlet (Cox et al. 2013). Wastewater derived endocrine disrupting chemicals and triclosan have been detected in sediments in Barker Inlet, in association with out-flow from the Bolivar plant (Fernandes et al. 2008, Fernandes et al.

2010) at concentrations among the highest reported globally. Their effects, if any, on dolphins in the estuary are unknown.

6.3. Other industries

A range of industries that have operated adjacent to and within the stormwater catchment of the Port River and Barker Inlet have introduced pollutants into the estuary. Many pollutants would have flushed from the estuary through saltwater flow from West Lakes, stormwater runoff and tidal action, while others would have entered the sediments or entered food chains through which they accumulate and biomagnify over time.

Historically, industries such as tanneries, foundries, gas works, refineries, fertilizer works, timber treatment, metal plating and rendering plants, and abattoirs have been located near the shores of Port River and Barker Inlet. There is a legacy of contaminated land and contaminated groundwater in places due to these industries (Thomas et al. 2001). Licensing of discharges to the marine environment, including Port River and Barker Inlet began in 1990 (Pfennig 2008). The discharges from industries that have lined the Port River undoubtedly introduced hazardous and toxic products into the coastal waters, degrading coastal ecosystems, reducing dolphin carrying capacity and reducing the health of dolphins that remained.

Several recent industries adjacent to Port River and Baker Inlet have had the potential to substantially alter water quality or introduce toxicants. These include salt extraction, Penrice Soda Products, Torrens Island Power-station, and Wingfield Municipal Landfill site.

6.3.1. Salt fields

Salt extraction from 4000 hectares of ponds established along 30 km of coast north of Adelaide commenced in 1940 and ceased in 2014 (Bell 2014, Size 2020). Construction of the salt fields was predominantly on top of saltmarsh. During operation, the ponds were flooded with seawater that evaporated allowing the salt to be harvested. The ponds acted as a barrier to recolonisation of coastal areas by native vegetation and marine invertebrates. Over time, hypersaline and acidic, sulfide-rich sediments have built up in the ponds posing an environmental hazard, particularly if resuspended (Dittmann et al. 2019). To reduce the environmental hazard, in 2017, tidal cycling was introduced to the ponds to allow for managed flushing.

In mid-September 2020, a dieback of approximately 10 ha of mangroves and 35 ha of saltmarsh occurred at St Kilda adjacent to the Dry Creek salt fields (Environment Protection Authority 2021[5]). While it is not known what caused the dieback, salinity and the management of water in the salt fields is implicated. It is also likely that waterlogging following the introduction of managed flushing has contributed to saltmarsh die-off in some areas.

Construction of the salt fields has therefore reduced vegetation quality around the ADS, thereby reducing ecosystem capacity and ultimately prey availability to the dolphins. Direct impacts of releases of hypersaline and acidic, sulfide-rich outflows from the fields on residence or health of dolphins in the ADS are unknown.

6.3.2. Penrice Soda Products Pty Ltd

Prior to 1999, Penrice Soda Products Pty Ltd was licensed to deposit 75ML/day of processed water containing calcium chloride, ammonia, along with 100,000 tonnes of insoluble residues known as "calsilt" and 540 tonnes of total nitrogen per year into the Port River (Thomas et al. 2001). Every two to three years the river was dredged, and sediments were deposited at a gazetted spoil ground off Outer Harbor. Penrice started using settlement ponds in 2001, which reduced insoluble residue discharge into the river by an estimated 95%. Calsilt from the ponds was stock piled at several locations. Penrice became insolvent in 2013 and ceased operations in 2014.

During its period of operation, residues discharged into the Port River undoubtedly reduced water clarity and smothered benthic communities. Nitrogen discharges would have increased the intensity of algal blooms. Direct impacts on the dolphins are unknown, however, the industry potentially reduced the habitat quality and therefore carrying capacity of dolphins in the river.

6.3.3. Power-stations

There are several natural-gas fired power-stations that discharge thermal effluent into the Port River and Barker Inlet. These include Torrens Island (commissioned 1967), Dry Creek (commissioned 1973), Osbourne (commissioned 1998), Pelican Point (commissioned 2001), and Quarantine (commissioned 2002). Torrens Island, located on Angus Inlet, is the largest, although it has reduced capacity considerably since 2014, due to increasing levels of wind and solar

[5] https://www.epa.sa.gov.au/community/stay-informed/dry-creek-saltfields

generation in the state. Thermal effluent from Torrens Island Power-station has been recorded to increase summer water temperatures in Angas Inlet to over 30 °C (Thomas et al. 2001). The effluent was determined to affect the distribution and abundances of fish species, alter benthic communities and cause seagrass loss in the inner estuary (Jones et al. 1996). The magnitude of the increased temperature of the power-station effluent could alter benthic communities in the vicinity of Angas Inlet (Jones 2008).

Temperature increases in estuarine waters may result in lower oxygen levels in summer. This can be associated with raised hydrogen sulfide levels and the re-dissolution of nutrients and metals (Harbison 1986).

Dolphins in the ADS likely tolerate increased water temperatures produced around the power-station discharges. Impacts on the dolphins would come from reduced prey availability through ecosystem changes driven by plankton extraction at intakes and temperature increases at discharge sites.

6.3.4. Wingfield Municipal Landfill Site

The Wingfield Municipal Landfill Site is located immediately south of Barker Inlet. Numerous former liquid waste disposal ponds are buried beneath the Wingfield Landfill (Belperio and Harbison, 1992, cited in Thomas et al. 2001). Groundwater bores show elevated but not hazardous levels of nitrogen, phenols, cyanide and metals (Zn, Pb, Ni, Al), with higher concentrations where the water is acidic. The contaminants are consistent with leakage of landfill leachate, possibly being spread by surface waters during winter prior to moving into the shallow groundwater. Drainage from Wingfield Municipal Landfill site is currently directed through ponding systems to minimise the chance of contaminants seeping into Port River and Barker Inlet.

Seepage from the Wingfield Site into the Port River and Barker inlet likely has introduced toxic chemicals into adjacent waters and sediments. Increased control of the seepage would now be reducing input. Legacy contaminants may still be present in the environment, however, and could biomagnify into dolphin tissues.

6.4. In-water activities

Numerous factors influence how water activities may impact coastal dolphins. Increases in number and frequency of boating activities, and harbour and marine activities, have the potential to displace bottlenose dolphins from sections of their home ranges through noise pollution and changes to water quality (Allen & Read 2000). Also, dredging is an on-going activity in the Port River to maintain and, at times, increase channel depth. Dredging can exclude dolphins due to underwater noise and turbidity, and release legacy toxins that have accumulated in the sediments. Dumping of dredged spoil in waters adjacent to the coast may also influence those ecosystems, dolphin movement and the spread of the legacy pollutants. Overall, however, removal by dredging of sediments containing legacy pollutants from within the ADS would have the long-term benefit of negating the potential resuspension and gradual bioaccumulation these pollutants.

Irregular boating and rapidly moving vessels have the potential to startle dolphins, increasing the chance of collisions and heightening stress levels. Vessel speeds in much of the Port River and Barker Inlet are limited to <7 knots and <4 knots in marina areas (Figure 7). Exceptions include the main channel into Barker Inlet, an east-west stretch near the entrance to the Port River and a section of North Arm (for the Adelaide Speedboat Club) where limits do not apply. The Port River is South Australia's largest port with approximately 2,000 large vessel movements every year. Recreational vessel use is year-round with a peak in summer months.

Dolphins may habituate to regular vessel traffic that passes rather than approaches them. Unusual, infrequent or rapid vessels, especially if directed toward them, however, likely will induce stress in dolphins, alter their behaviour and potentially to motivate individuals to spend less time in an area. There is also the greater potential for infrequent and rapidly moving vessels to startle and collide with dolphins. Further potential impacts of in-water activities, including fishing, within the ADS on prey availability to dolphins are discussed in Section 5.6.

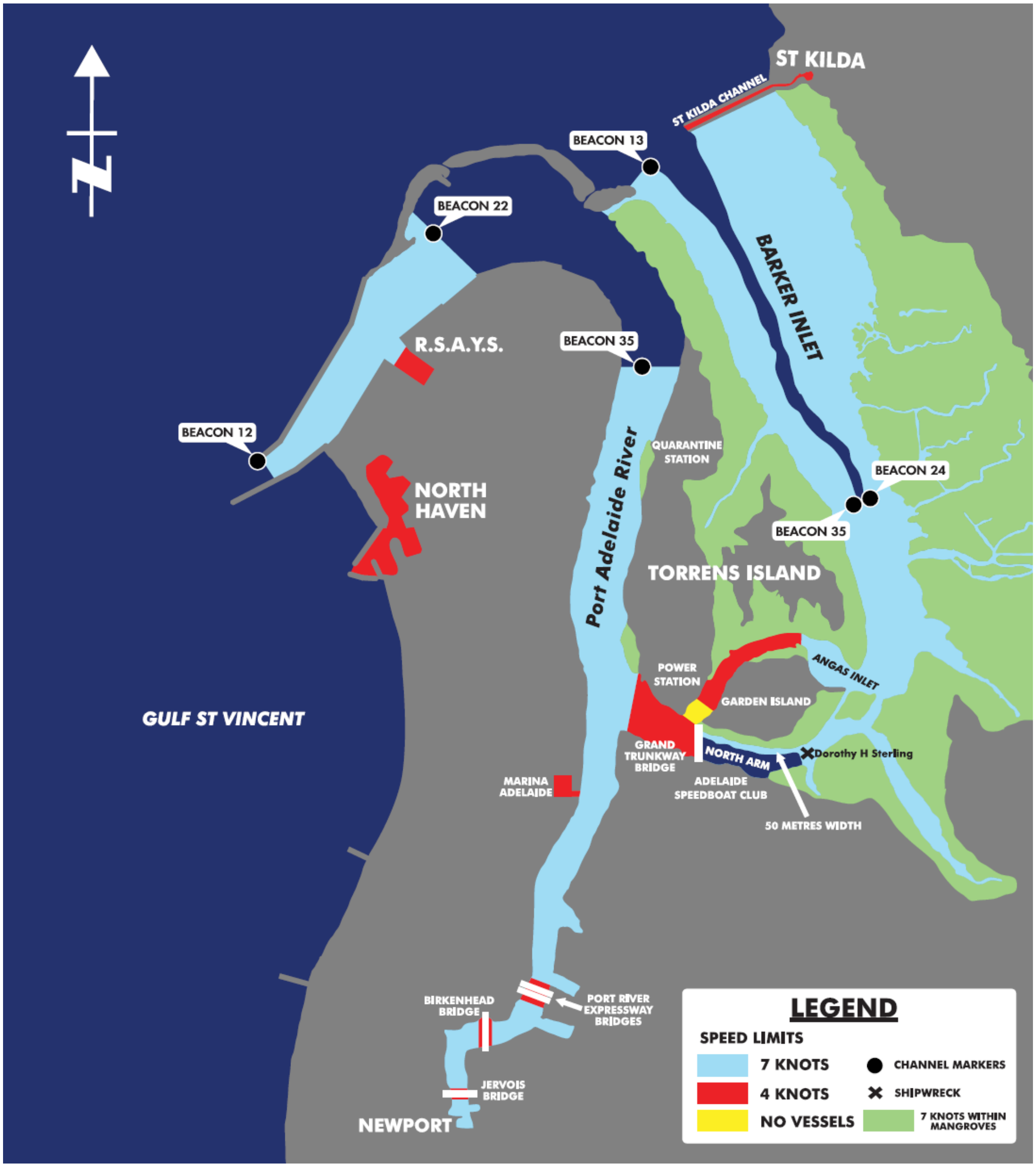

Figure 7. Vessel speed limits in the Port River and Barker Inlet.

7. DISCUSSION

This review focuses on intrinsic, disease, environmental and anthropogenic threats to Indo-Pacific bottlenose dolphins in the ADS. The ADS is situated over the Port River and Barker Inlet, an embayment within the larger Gulf St Vincent, South Australia. It has estuarine qualities, including surrounding mangrove vegetation, tidal regulated water turn-over, and salinity and temperature ranges that fluctuate more than open waters. Compared to other estuarine systems, though, it has a low input of freshwater, so does not always have marked salinity gradients. Nonetheless, during periods of high rainfall, considerable amounts of freshwater runoff do occur, and salinity gradients can establish within the estuary.

There has been considerable industrial use of the coasts and waterways of the ADS. The Port River is the major port for the state of South Australia. Many industries with variable pollution control measures have been and still are located along its shores. Stormwater runoff from a significant proportion of the city of Adelaide drains into the estuary and discharge from Adelaide's largest wastewater treatment plant enters the ADS to the north of Barker Inlet. Pollution levels in the estuary have been high. Since the 1960s, however, pollution input has steadily been reduced due to increased awareness of, and the motivation to reduce, impacts on coastal ecosystems. Still, many legacy contaminants remain in sediments and biological systems, and continue to be discharged into these coastal waters.

Indo-Pacific bottlenose dolphins are a coastal species. Throughout their range, they occur predominantly in bays and inlets, with minimal genetic exchange between populations. The groups of Indo-Pacific bottlenose dolphins that enter the ADS are a component of the Gulf St Vincent bottlenose dolphin population. Most of this population lives in the Gulf, outside of the ADS, but some will enter the ADS from time-to-time. Others are more resident within the ADS, though presumably can leave for periods if given sufficient motivation, for example, an absence of sufficient prey or high levels of disturbance in the ADS. The degree to which con-specific pressure could restrict movement in and out of the ADS is unknown.

Multiple pressures can compound to reduce the health of estuarine-dwelling dolphins (Figure 8). Natural pressures of being a top predator living in a non-static ecosystem, due to the fluctuating environmental conditions, combine with the burdens of parasites that all wild animals live with, and become exacerbated by human induced toxins and activities.

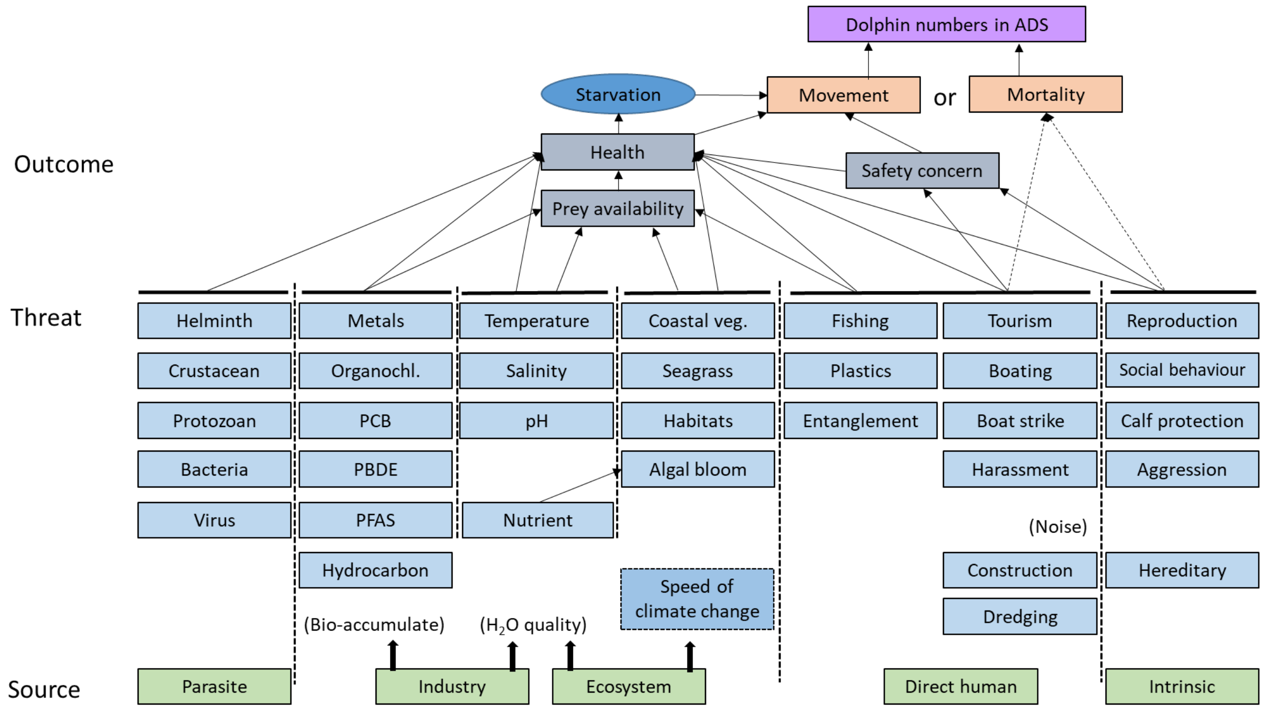

Figure 8. Simplified diagram of sources, threats and outcomes to the number of dolphins in the Adelaide Dolphin Sanctuary.

An initial step to quantify the overall risk to a populations' survival is to assess the abundance, status and trends of the population (Currey et al. 2009b). To do this, the local population needs to be identified (Chabanne et al. 2017). It is important to understand the size of the population of animals that utilise the Port River and Barker Inlet and their connectivity, or lack thereof, with populations in adjacent Gulf St Vincent and further away. This will inform on the vulnerability of dolphins using the Port River and Barker Inlet along with the dolphin population's ability to recover should present levels of usage decrease. At present the population status of Indo-Pacific bottlenose dolphins that frequent the ADS in relation to the broader population within Gulf St Vincent is unclear.

There is a range of potential threats to Indo-Pacific bottlenose dolphin residence in the ADS. Some of the threats, like excessive noise, unusual salinity levels, or reduced prey availability, or aggression by other dolphins may displace dolphins out of the ADS area. To date, movement of individual dolphins in and around the ADS in relation to such variables have not been documented. Unhealthy dolphins may be less likely to detect or capable of avoiding threats, such as approaching vessels or reductions in prey availability, so have their health further compromised. Other threats, such as toxins that gradually bio-accumulate, would be difficult for even healthy dolphins to detect and avoid. Unsuitable habitats may act as 'ecological traps', where sudden environmental changes, including human disturbances, uncouples the cues that individuals use to assess habitat quality from the true quality of the environment (Battin 2004, Robertson & Hutto 2006, Atkins et al. 2016). For example, Indo-Pacific bottlenose dolphins may perceive the ADS as ecologically attractive given certain cues, but the positive outcome normally associated with the given cue becomes negative in terms of survival and reproduction, due to the presence of other conditions (Guido Parra pers. comm.). Increased mortality rates within a reducing population would be a sign that factors the dolphins cannot cue into are in play.

Pollutants, including PCBs, PBDEs, PFASs and heavy metals, likely play a role in the long-term health of dolphins in the ADS and should be included in biomonitoring efforts (Weijs et al. 2020). While pollutants being released into the waters of the estuary undoubtedly have reduced over time, high levels remain in sediments and food chains. Dredging or water turbulence through high levels of water incursion, such as stormwater runoff, could resuspend legacy pollutants in sediments. A gradual accumulation of legacy pollutants from sediments or biomagnification through the food chain could initiate declines in health of dolphins. Amongst these pollutants, PFASs stand out as being present and understudied. Mixture toxicity (i.e., exposure to more than one toxicant) is also poorly understood and its outcomes are unpredictable in aquatic environments (Sárria et al. 2011). Effects of multiple toxicants need to be considered.

Mortality may be instant in the case of a collision or predation, but more commonly it is a transitional process. The ultimate cause of mortality of a dolphin may not be the reason it became unwell (Figure 9). There are multiple pathways to ill health and mortality. Stressors like reduced prey availability, an injury, a rapid change in salinity, exposure to a novel infection, or an accumulation of toxins, can suppress the immune system, allowing other stressors to compound. For example, an unwell or immune-compromised host stimulates parasites to breed rapidly. High parasite loads in the lungs may reduce breath-hold capacity, while parasites in the auditory system could reduce hearing. Both these conditions could increase the chance of vessel collisions. Thus, death by vessel collision could result from a pathway of pre-disposing conditions.

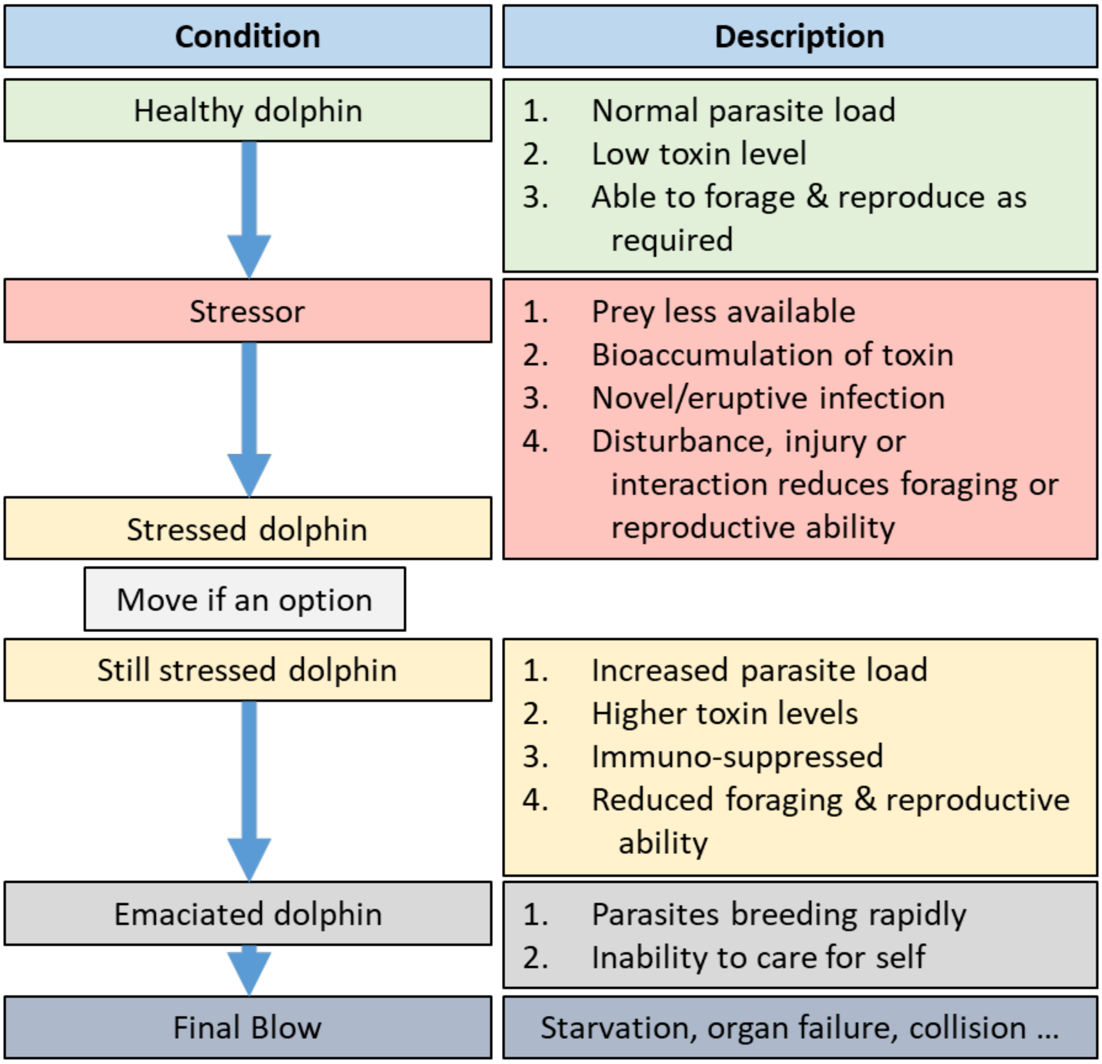

Figure 9. A potential sequential pathway to mortality for a dolphin.

Routine monitoring of dolphin presence and survival, which has been on-going in the ADS since the 1990s, represents an enormous asset to understanding dolphin biology and threats to dolphins in the ADS. Investigation of how cyclic and periodic events can influence dolphin abundance, distribution, reproductive success, and survival will enhance understanding of how these dolphins survive.

Currently, dolphin health in the ADS is monitored through records of abundance, visual condition assessments, and autopsies of recovered carcasses. On the east coast of the USA, coastal dolphin health has been monitored through sampling wild dolphins, with or without capture (Beck & Rice 2003, Mancia et al. 2015, Barratclough et al. 2019). A mobile device application has also been developed to aid rapid field assessment of bottlenose dolphin health based on morphometrics (Hart et al. 2017). While health checks are a normal procedure for captive dolphins (Clegg et al. 2015), they are not routinely conducted on wild dolphins.

Reviews of potential threats should be updated periodically as new information and new threats emerge (Nicole & Patricia 2013). Recent model-based assessments of impacts on bottlenose dolphins in South Australia's Gulfs (Spencer and St Vincent) pointed to greatest risks coming from climate change and extreme epizootic events, with pollution and fishing also ranked high (Robbins et al. 2017, Reed et al. 2020). This is likely the case for the broader populations, but different threats are likely to impact on the node of the population that frequents the ADS.

8. CONCLUSION

Indo-Pacific bottlenose dolphin residence in the Port River and Barker Inlet has varied over time. Prior to colonial settlement of Adelaide, there was probably consistent use by dolphins of the Port River and Barker Inlet, with resident dolphins only vacating during periods of heavy flooding. Since colonial settlement, the impacts of habitat modification (vegetation clearance, dredging, industrial pollution, prey depletion) and disturbance (boating and other activities), would have resulted in periods reduced visitation and use. During the 1950s for example, dolphin visitation to the estuary was low (Peter Shaughnessy, pers. comm.). Following remediation measures to improve habitat quality starting to take effect in the late 20^{th} century, and reflecting improvements to the health of the estuary, dolphin residence has again become more consistent.

This report documents the considerable variety of factors and their sources that could threaten coastal bottlenose dolphins, resulting in individual deaths and population declines. The recent apparent decline in numbers and health in the ADS could relate to an intrinsic fluctuation over time or be attributable to anthropogenic influences. Future monitoring and directed research of the dolphins and their habitat requirements will help clarify their status. While the cause of the recent decline remains uncertain, there are several possible contributing factors. There is likely to be a component of stress, a level of immune suppression, and compounding factors including endemic and epizootic diseases that cause deterioration in condition and organ function with subsequent starvation. In many instances, the ultimate cause of death may be distant from the factors leading to ill-health.

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10. APPENDICES

10.1. Conceptual model

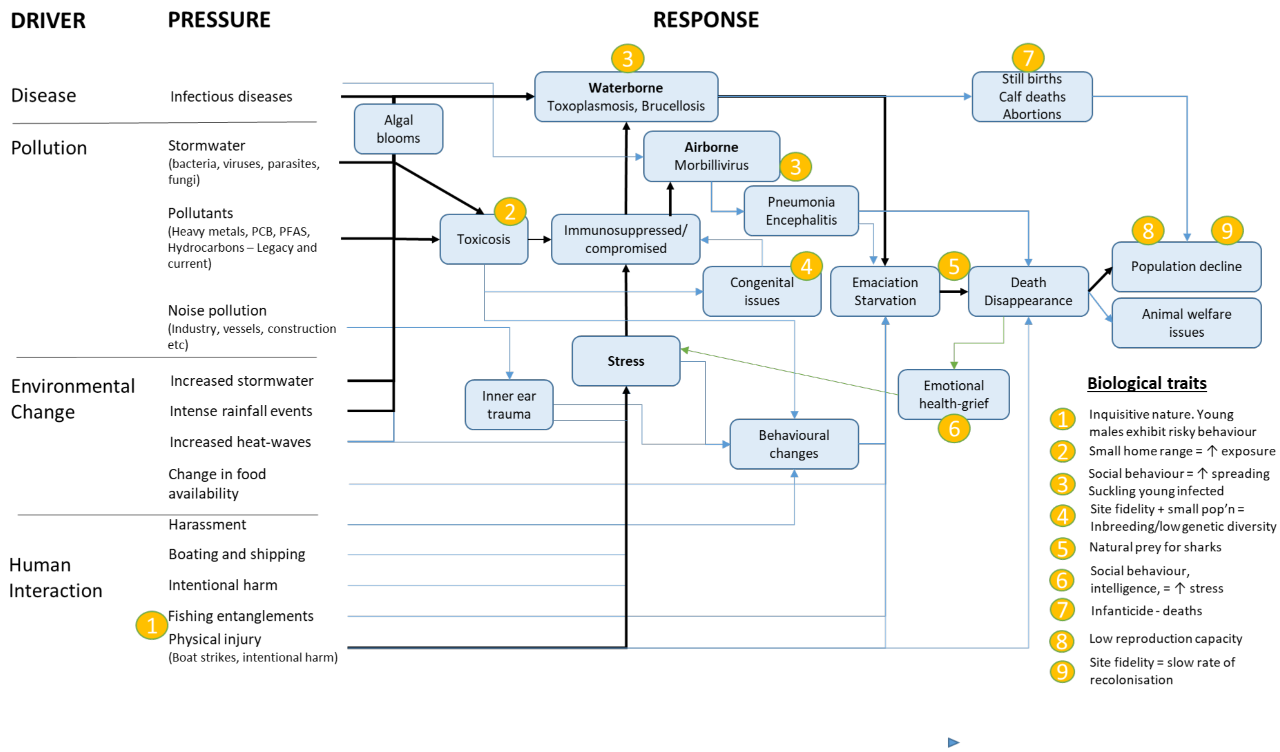

Figure 10. Conceptual model developed by DEW of the potential impacts on Indo-Pacific bottlenose dolphins in the Adelaide Dolphin Sanctuary.

10.2. Legislation of the Adelaide Dolphin Sanctuary – 14 April 2005

Adelaide Dolphin Sanctuary Act 2005

Part 2—Objects of Act and statutory objectives

7—Objects

The objects of this Act are—

(a) to protect the dolphin population of the Port Adelaide River estuary and Barker Inlet; and

(b) to protect the natural habitat of that population.

8—Objectives

(1) The following objectives will apply in connection with the operation of this Act:

(a) the protection of the dolphin population of the Port Adelaide River estuary and Barker Inlet from direct physical harm is to be maintained and improved;

(b) the key habitat features in the Port Adelaide River estuary and Barker Inlet that are necessary to sustain the dolphin population are to be maintained, protected and restored;

(c) water quality within the Port Adelaide River estuary and Barker Inlet should be improved to a level that sustains the ecological processes, environmental values and productive capacity of the Port Adelaide River estuary and Barker Inlet;

(d) the interests of the community are to be taken into account by recognising indigenous and other cultural, and historical, relationships with the Port Adelaide River estuary and Barker Inlet and surrounding areas, and by ensuring appropriate participation in processes associated with the management of the Port Adelaide River estuary and Barker Inlet;

(e) public awareness of the importance of a healthy Port Adelaide River estuary and Barker Inlet to the economic, social and cultural prosperities of the local communities, and the community more generally, is to be promoted;

(f) the principles of ecological sustainable development in relation to the use and management of the Port Adelaide River estuary and Barker Inlet are to be promoted.

10.3. Adelaide Dolphin Sanctuary Management Plan – June 2008

<u>Objectives and issues</u>

1. Protection of dolphins
    1.1. Lack of scientific knowledge about ADS dolphins
    1.2. Vessel strike
    1.3. Entanglement
    1.4. Intentional harm
    1.5. Impacts from human interactions
2. Protection of habitat features
    2.1. Food supply
    2.2. Loss of vegetation: seagrass, mangroves and supporting species
    2.3. New developments
    2.4. Marine pests: *Caulerpa taxifolia*, *C. racemose* and others
    2.5. Recreational fishing
3. Improvement of water quality
    3.1. Discharges – nutrients
    3.2. Discharges – pollutants
    3.3. Discharges of ballast water
    3.4. Turbidity and release of toxins from sediment
4. Community participation
    4.1. Inclusion of all stakeholders
    4.2. Support of recreational users
    4.3. Support of industry interests
    4.4. Protection of indigenous values in the area
5. Promotion of the environmental importance of the ADS
    5.1. Supply of informative, timely and accessible information about the ADS
    5.2. ADS sign strategy
6. Promotion of the principles of ecological sustainable development
    6.1. Promote the implementation of ESD principles with local industries and new developments

10.4. Extracts from National Guidelines for Whale and Dolphin Watching

(Anonymous 2017)

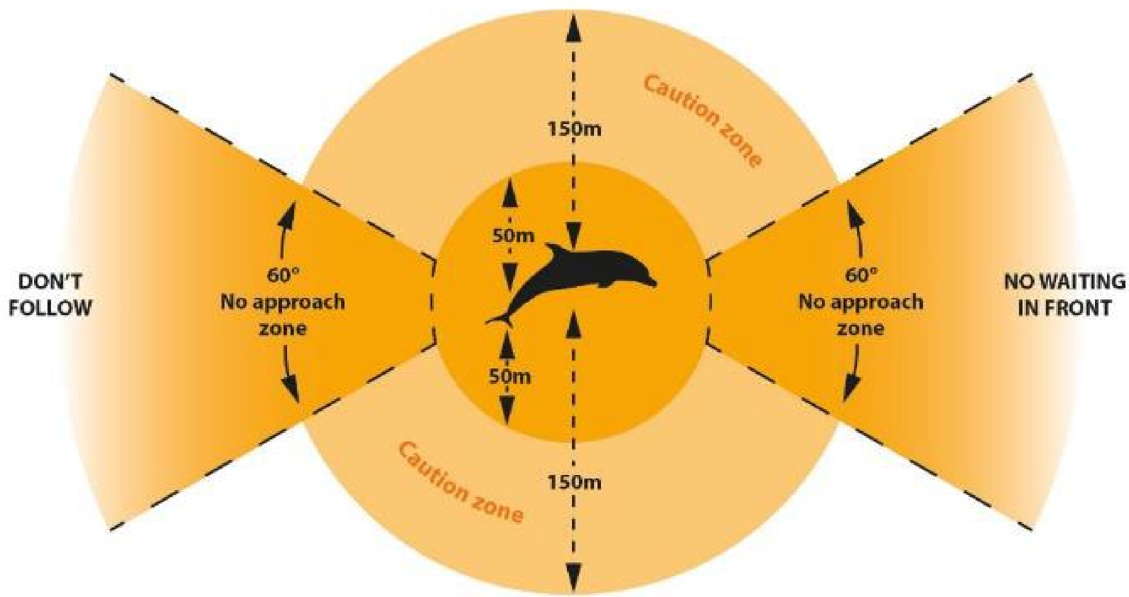

Figure 11. Vessel approach restrictions around adult dolphins (source Anonymous 2017).

- Caution zone: ≤ three vessels, speeds <6 knots: must not enter when a calf is present.
- When dolphin watching in enclosed bays and estuaries, where dolphins are restricted to relatively small areas or where there are many recreational and commercial vessels operating, additional management may be required.
- When fishing in conjunction with dolphin watching, all fishing lines should be reeled in and stowed prior to engaging in dolphin watching activities.

10.5. Key toxicology studies related to dolphin health in the ADS

Sect.	Threat	Key references
5.2.	**Disease**	
5.2.1.	Helminth	(Woodard et al. 1969, Tomo et al. 2010, Díaz-Delgado et al. 2018)
5.2.2.	Crustacean	(Lincoln & Hurley 1980, Vecchione & Aznar 2014)
5.2.3.	Protozoa (eg Toxo.)	(Bowater et al. 2003, Kemper et al. 2016)
5.2.4.	Bacteria	(Jaing et al. 2015, Kemper et al. 2016, Souter et al. 2021)
5.2.4.1.	*Brucella*	(Alba et al. 2013, Isidoro-Ayza et al. 2014b, McFee et al. 2020)
5.2.5.	Viruses	(van Elk et al. 2009, Rehtanz et al. 2012)
5.2.5.1.	*Morbillivirus*	(Kemper et al. 2016, Batley et al. 2021, Cloyed et al. 2021)
5.2.6.	Fungi	(Isidoro-Ayza et al. 2014a, Kemper et al. 2016, Vilela & Mendoza 2018)
5.2.7.	Skin lesions	(Bossley & Woolfall 2014, Bossart et al. 2015, Duignan et al. 2020)
5.3.	Algal blooms	(Parker et al. 2002, Roberts et al. 2019)
5.4.	**Pollutant**	
5.4.1.	Metals	(Butterfield & Gaylard 2005, Lavery et al. 2008, Lavery et al. 2009)
5.4.2.	POPs	(Fossi & Panti 2018, Zanuttini et al. 2019)
5.4.2.1.	DDT	(Mai et al. 2013, Cagnazzi et al. 2020a, Yu et al. 2020)
5.4.2.2.	PCB	(Gaus et al. 2005, Weijs et al. 2020, Williams et al. 2020)
5.4.2.3.	PBDE	(Barón et al. 2015, Lavandier et al. 2016, Weijs et al. 2020)
5.4.2.4.	PFAS	(Gaylard 2017, Sciancalepore et al. 2021, Stockin et al. 2021)
5.4.3.	Hydrocarbons	(Helm et al. 2015, Venn-Watson et al. 2015, De Guise et al. 2021)
5.4.4.	Wastewater	(Fernandes et al. 2008, Fernandes et al. 2010, Green et al. 2013)
5.4.5.	Plastics	(Fossi et al. 2018, Hart et al. 2018, Panti et al. 2019, Byard et al. 2020)
5.4.6.	Water quality	
5.4.6.1.	Salinity	(Fury & Harrison 2011a, b, Duignan et al. 2020)
5.4.6.2.	Temperature	(Noren et al. 1999, Yeates & Houser 2008, Roberts et al. 2019)
5.4.6.3.	pH	(Thomas et al. 2003, Ohrel & Register 2006, Marion et al. 2011)
5.4.6.4.	Nutrients	(Edyvane 1999, Mifsud et al. 2004)

10.6. Coastal dolphin diseases, sources and the tissue most affected

Disease	Source*	Impact site
Helminth - nematode		
Anisakis spp.	I, E	gut
Braunina cordiformis	I, E	gut
Crassicauda grampicola	I, E	sinuses
Halocercus lagenorhynchi	I, E	lung
Poleter gastrophylus	I, E	gut
Stenurus ovatus	I, E	lung
Skrjabinalius cryptocephalus	I, E	lung
Helminth - trematode		
Campula palliata	I, E	liver
Nasitrema spp.	I, E	ear, nervous system
Pholeter gastrophilus	I, E	stomach
Crustacean parasite		
Scutocyamus antipodensis	I, E	skin
Harpacticus pulex	I, E	skin
Pennella balaenopterae	I, E	skin
Xenobalanus globicipitis	I, E	skin
Protozoa		
Toxoplasma gondii	R, W (cat)	brain, lung, liver, adrenal etc.
Bacteria		
Brucella spp.	R, W? (stock)	any organ
Campylobacter spp.	R, W?	gut
Chlamydia abortus (psittaci)	R, E (bird)	lung, systemic, reproductive
Clostridium perfringens	R, W?	gut, muscle, heart, blood, brain etc.
Erydipelothrix rhusiopathiae	R, W?	skin, lung, kidney, systemic
Escherichia coli	R, W?	gut
Helicobacter spp.	I, E, R, W?	gut
Leptospira interrogans	R, W?	liver, kidney
Morganella spp.	R, W?	wound, urinary tract
Mycobacterium spp.	E	lung, skin, lymphoid tissues, soft tissue
Mycoplasma spp.	R, W?	lungs, skin, urinary tract
Nocardia cyriacigeorgica	R, W?	lung
Pasteurella spp.	R, W?	skin, soft tissue
Proteus spp.	R, W?	urinary tract, lung
Pseudomonas spp.	R, W?	ear, eye, skin, lung
Salmonella spp.	R, W?	gut, (skin rarely)
Shewanella putrefaciens	E	ear, gut, liver
Staphylococcus spp.	R, W?	skin, bone, lung

Streptococcus spp.	E, R, W?	skin, throat, lung, liver
Vibrio spp.	E	gut, skin
Virus		
Equine encephalitis virus	R, W?	brain
Gammaherpesvirinae spp	I, R, W?	Lymphatic system
Morbillivirus	I	lung, soft tissue
Papillomaviruses	I, R, W?	skin, reproductive system
Parainfluenza virus	R, W?	lung
Fungi		
Ajellomyces dermatitidis	I, R, W?	skin
Aspergillus fumigatus	E, R	lung
Candida glabrata	I, R, W?	mouth, throat, reproductive system
Cryptococcus spp.	E, W?	lung
Cunninghamella bertholletiae	E, R	lung, nervous system
Histoplasma capsulatum	E, R	lung
Paracoccidioides brasiliensis	E, R	lung
Lacazia loboi	E, R	skin, lesions

* Sources: I = intrinsic, E = environment, R = runoff, W = waste water, ? = unsure