Marine predation injuries in yellow-eyed penguins (*Megadyptes antipodes*)

A thesis presented in partial fulfilment of the requirements

for the degree of

Master of Veterinary Science

in

Wildlife Health

at Massey University, Manawatū,

New Zealand

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2023

Abstract

Yellow-eyed penguins (*Megadyptes antipodes*), endemic to New Zealand and its subantarctic islands, are one of the rarest penguin species in the world, with many factors threatening their survival. Marine predation is one such threat to this species, affecting a large proportion of adult and juvenile yellow-eyed penguins each year across their mainland range.

New Zealand sea lions (*Phocarctos hookeri*) have been witnessed attacking yellow-eyed penguins, however other predators have not been definitively determined. Sharks are suspected based on the characteristics of some wounds. Barracouta (*Thyrsites atun*) have been implicated in some of the attacks, but evidence supporting this hypothesis is lacking. A broken fragment of tooth found in a yellow-eyed penguin leg laceration, provides evidence of a shortfin mako (*Isurus oxyrinchus*) or porbeagle shark (*Lamna nasus*) attack, but with the tooth incomplete and molecular testing inconclusive, an exact identification could not be determined.

An increasing frequency of yellow-eyed penguins admitted to veterinary hospitals for treatment of marine predator injuries has been observed in the last decade. Necropsy data shows a similar trend. Assessment of individuals admitted to wildlife hospitals revealed that 84% had moderate to severe injuries. Two thirds (66%) of treated individuals survived to release, and of these 49.1% were not resighted after release, 22.4% were resighted as non-breeders and 28.4% subsequently made breeding attempts post release. Injury recrudescence occurred in 5% of cases. More than 15% of yellow-eyed penguins were hospitalised for multiple predation attacks during the study. Prognosis was largely attributed to the presence of severe injury complications. Hospital survival was reduced by nine times with the presence of a tendon or ligament injury, seven times with nerve damage and three times with osteomyelitis. Complications secondary to captivity were observed in 51.5% of necropsied yellow-eyed penguins which died or were euthanised in veterinary or rehabilitation centres, with fungal and bacterial pneumonia and air sacculitis, and tracheal stenosis, identified as the most common diseases.

Acknowledgements

Kāhore taku toa i te toa takitahi, he toa takitini (Māori proverb).
We cannot succeed without the support of those around us.

Firstly, I wanted to express my upmost thanks and appreciation for my supervising team, Professor Brett Gartrell, Dr Stuart Hunter and Dr Wendi Roe. Brett, as a research supervisor, you always seemed to know what to say to get me past every roadblock I came across. I'm so grateful for your positivity throughout this project, pulling me out of all the metaphorical rabbit holes I fell into, helping me to see the bigger picture, and for your friendship in the residency and beyond. Stu, you have a masterful eye for photography and have contributed in so many ways to this project through diligently taking measurements and photographs of penguin wounds and teeth, as well as necropsying hundreds of yellow-eyed penguins. Thank you for your banter, sense of humour and listening ear which helped me through these years of my residency. Wendi, thank you for your honest advice to keep me on track for this research project and your detailed feedback which improved this manuscript greatly.

I'd like to thank everyone who allowed me to join their fieldwork expeditions with yellow-eyed penguins. In particular, Melanie Young, who has been very generous in sharing her enormous knowledge of all things yellow-eyed penguin, letting me sleep on her couch for six weeks during this project, allowing me to keep barracouta in her bathroom overnight, introducing me to many penguin colleagues, and delivering incredibly well-organised field trips. Thanks also extends to Sandy King, Trudi Webster and the rest of the Yellow-eyed Penguin Trust for including me on the 2018 nesting surveys on Stewart Island and the Bravo Islands, and Phil Crutchley from the Christchurch City Council who facilitated my fieldwork on the Banks Peninsula during the nesting survey in the same year. I am also appreciative of Rachel Hickcox, for taking the time to collect yellow-eyed penguin foot photos on my behalf, whilst undertaking her PhD research.

I'm extremely grateful for the willingness of all participating veterinary clinics and wildlife hospitals to be part of this project and share their clinical records and diagnostic test results. In particular the Wildlife Hospital Dunedin, who contributed a large amount of data, provided the tooth discovered in a penguin leg wound and included me as part of the team while I was in Dunedin. Thanks also to the Wildbase Hospital, Wellington Zoo, St Kilda Veterinary Centre, Oamaru Veterinary Centre, Vetlife Oamaru and Hornby Veterinary Centre for providing hospital records. Likewise, I'd like to thank the Department of Conservation, Penguin Rescue, Penguin Place and Christchurch Penguin Rehabilitation, for sharing data.

This thesis represents a journey that started in my first veterinary job in Oamaru, where I was first introduced to yellow-eyed penguins by Rosalie Goldsworthy. She welcomed me into her rehabilitation centre as a budding wildlife veterinarian keen to provide veterinary assistance to the penguins in care, where I first learnt of the concerns of yellow-eyed penguin marine predation injuries, which helped inspire this project. Thanks for being a big supporter of mine professionally and personally. Your dedication to saving the yellow-eyed penguins is inspirational, and they are lucky to have a martyr like you on their side. Thanks to wildlife pathologist Maurice Alley for your assistance in measuring and photographing fish teeth, as I delivered on a promise I made to you at the 2015 International WDA Conference in

Maroochydore about sourcing barracouta for examination at Massey, bringing this project full-circle.

A huge extension of thanks to others with very specific skillsets without which the broad reach of this project would not have been possible. Kristene Gedye, thank you for your enthusiasm and persistence with the molecular testing involved in this project, and for the laughs along the way despite our failed attempts to definitively identify the tooth. I'd also like to thank Foss Leach for his assistance in the morphological identification of the tooth, sourcing fish and shark samples for the molecular investigation and engaging fellow fish and shark colleagues around the world for their expert opinions.

To my Wildbase and Wellington Zoo whānau, thank you for the friendship, encouragement, quiz nights, rock climbing, hits of tennis and laughs which helped keep me sane during an intense but thoroughly rewarding three-year program. To my New Zealand friends outside the residency, thank you for the in-person support you provided me through a challenging year of 2020, and to my Australian friends for your continued friendship facilitated by phone calls and Zoom. As these projects don't always get completed in the originally planned timeframe, I also wanted to extend thanks to my ZoosSA family, for the genuine encouragement, guidance and offering of time that I needed to get this project over the line.

Thanks to my family for always being a constant source of love and support, and for visiting me in New Zealand, so I could share my adventures with you in-person. Tristan, thank you for inspiring me to be a better person every day, always being there for me without fail and helping me to see the lighter side of life. Your unwavering support has provided me with the means to strive for my aspirations, and I will always be grateful for this.

Preface

This thesis is structured as four chapters; an introduction, literature review detailing relevant background information, the current state of knowledge of marine predator injuries in yellow-eyed penguins, and research aims (Chapter 1); a temporospatial analysis and investigation of potential marine predators (Chapter 2); a clinical, pathological and treatment review of hospitalised yellow-eyed penguins with respect to outcome and prognosis (Chapter 3); as well as a general discussion including key findings of this research, scientific questions raised and conservation implications and field applications (Chapter 4). A single reference list has been compiled at the end of the document. Several appendices with additional data are also included.

This thesis was co-supervised by Professor Brett Gartrell, Dr Stuart Hunter and Dr Wendi Roe, and accordingly benefited from the inputs of various individuals. Professor Brett Gartrell provided advice and guidance on study design, research methods, statistical analyses, data interpretation and manuscript feedback. Dr Stuart Hunter provided input into study design, contributed a large quantity of necropsy information and photographs and offered manuscript feedback. Dr Wendi Roe provided input regarding study design and provided manuscript feedback. Dr Kristene Gedye offered guidance on molecular methodology and undertook the molecular testing of the tooth for this project.

Funding Acknowledgement

This research was funded through the Post-graduate Student Research Grant from the School of Veterinary Science (Massey University), Marion Cunningham Memorial Fund (Wildlife Society of the New Zealand Veterinary Association) and the Wildbase Research Trust Fund (Massey University). Whilst undertaking this research, the author was supported by a Masterate Scholarship in Zoo Animal and Wildlife Health, courtesy of Massey University and Wellington Zoo.

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CHAPTER ONE

Introduction, literature review and research aims

1.1 Introduction

New Zealand is listed as one of the world's 36 biodiversity hotspots (Critical Ecosystem Partnership Fund, 2016), due to its high proportion of endemic species, coupled with a substantial loss of habitat (Myers et al., 2000). Fifteen of the world's 21 species of penguins have been reported in New Zealand waters (Cole, 2019; Pan et al., 2019); including six endemic and nine vagrant species (Marchant & Higgins, 1990; Robertson & Heather, 2005; New Zealand Birds Online, 2013). New Zealand's rarest endemic species, the yellow-eyed penguin (*Megadyptes antipodes*), or 'hoiho', is endangered (BirdLife International, 2020), and faces significant challenges in the upcoming decades (Mattern et al., 2017).

The yellow-eyed penguin is an important species to New Zealand in social, cultural, economic, and ecological contexts. The species won New Zealand's 'Bird of the Year' competition in 2019 (Forest and Bird, 2021) and is regarded as a 'taoka' or 'taonga' species in Māori culture (Hoiho Governance Group, 2019). It is estimated that the species contributes at least NZ$100 million annually to the nation's economy directly and indirectly predominantly through tourism; a hefty sum which equates to a value of NZ$200,000 per bird every year (Tisdell, 2007). Penguins are regarded as indicator species providing insight into ecological changes and threats in both marine and terrestrial environments, and declines in population may be indicative of wider ecological degradation (Crawford & Shelton, 1978; Lynnes et al., 2004; Chilvers et al., 2014; Bestley et al., 2020).

With the New Zealand mainland population of yellow-eyed penguins in substantial decline, various organisations are collaborating to understand their most important threats, to take action to conserve the species. Marine predation is a threat which has been largely unstudied (Webster, 2018), despite it being one of the main reasons for admission into veterinary hospitals and rehabilitation centres. Main marine predators of yellow-eyed penguins are likely to include New Zealand sea lions (*Phocarctos hookeri*), various shark species, and potentially barracouta (*Thrysites atun*) (Webster, 2018). This research aims to understand marine predation of yellow-eyed penguins both from an ecological and medical perspective, to provide meaningful information to assist ecologists, wildlife veterinarians and wildlife rehabilitators in the conservation of this important species.

1.2 Yellow-eyed penguins

1.2.1 Geographical Distribution

Yellow-eyed penguins reside on the southern and eastern coasts of the South Island of New Zealand, and the subantarctic islands of Maungahuka (Auckland Islands) and Motu Ihupuka (Campbell Island) (Seddon et al., 2013). These are delineated as the 'northern' and 'southern' populations respectively. The northern population is further characterised by five main breeding areas: Banks Peninsula; North Otago; Otago; Catlins; and Rakiura (Stewart Island), Whenua Hou (Codfish Island) and surrounding islands (Seddon et al., 2013). The northern population originated subsequent to the colonisation of yellow-eyed penguins from the subantarctic, after the extinction of the Waitaha penguin (*Megadyptes waitaha*), from the 15th century onwards (Boessenkool et al., 2009; Rawlence et al., 2015). Due to significant genetic differences between the New Zealand mainland and subantarctic populations, they are now regarded as distinct populations, with very little genetic drift occurring between the two (Boessenkool et al., 2009).

Distribution within the terrestrial environment

Yellow-eyed penguins rely on both the terrestrial and marine environment for different functions. To understand the vulnerability of yellow-eyed penguins to marine predators, it is important to understand the location of penguins at various times. Yellow-eyed penguins reside within coastal forests, and usually nest within 700m from the sea (Ellenberg & Mattern, 2012). They tend to only be found resting on land during the late afternoon and evening, unless they are incubating eggs, brooding young chicks, or moulting where they remain on land continuously (Marchant & Higgins, 1990). Unlike most penguin species, yellow-eyed penguins do not exhibit strong colonial behaviour, with nest sites widely distributed (Darby & Seddon, 1990), with each pair preferring to nest out of visual range of other pairs (Marchant & Higgins, 1990), resulting in a low nesting density of approximately 1-5 nests/hectare (Darby & Seddon, 1990).

Distribution within the marine environment

Yellow-eyed penguins depend on the marine environment for food acquisition. Adults and juveniles spend the majority of the day at sea (Chilvers et al., 2014). Most birds make daily foraging trips, leaving in the morning and returning during the afternoon or evening (Richdale, 1941), however this varies with age, time of year and stage of the breeding cycle (Moore, 1999). Multi-day foraging trips are most common during incubation (Moore, 1999), pre-moult and in winter (M. Young, personal communication, 2021). Conversely breeding adults tending to chicks close to fledging can make two to three foraging trips each day (Moore, 1999).

Mainland yellow-eyed penguins disperse to different geographical locations at various life-stages. Adult penguins are central place foragers, foraging within close proximity of their nesting sites, reflective of their daily returns to shore. Post-fledge juveniles swim in a northerly direction along the coast of mainland New Zealand (Marchant & Higgins, 1990; Young et al., 2019; Young et al., 2022), rarely landing on shore, until they are resighted again near their natal breeding beaches from July onwards (Marchant & Higgins, 1990).

Yellow-eyed penguin foraging is limited by the continental shelf for both the northern and southern populations (Moore, 1999; Mattern et al., 2007; Muller et al. 2020a; Muller et al., 2021). Most mainland birds feed within 25km of the coastline (Moore, 1999; Mattern et al., 2007) and subantarctic birds 30-40km offshore (Muller et al., 2021), with a maximum recorded foraging distance of 57km offshore from the natal beach (Moore, 1999). Typical diving depths are 20-40m in Otago waters (Mattern et al., 2007), 50-70m off Stewart Island (Chilvers et al., 2014) and 50-100m around the subantarctics (Muller et al., 2021). They are restricted by the 150m contour line, with only a couple of individuals surpassing this depth (Young, 2017; Mattern & Wilson, 2018). This distribution is important when considering the predators that the birds are exposed to.

1.2.2 Foraging

Yellow-eyed penguins primarily adopt a benthic foraging strategy (Moore, 1999; Mattern et al., 2007; Mattern et al., 2013; Chilvers et al., 2014) and will navigate using sea floor landmarks (Mattern et al., 2007; Mattern et al., 2013). Subantarctic yellow-eyed penguins have been noted to switch to a pelagic feeding strategy in certain years (Muller et al. 2020a; Muller et al., 2021). It is suspected that mainland yellow-eyed penguins only adopt a pelagic strategy when visibility along the seabed is poor, prohibiting effective feeding (Mattern & Wilson, 2018). Birds mostly feed during the day, and hunt alone (Marchant & Higgins, 1990). This solo foraging strategy increases the risk of predation, due to lower vigilance and less individuals for predators to target, however reduces the requirement for resource sharing when hunting (Lett et al., 2004; Lima, 2009). In other species, it is speculated that lone prey strategies are adopted when the risk of predation is low (Lett et al., 2004).

1.2.3 Population and threat classification status

Yellow-eyed penguins are the second rarest penguin species in the world, with a population of 2600-3000 mature individuals (BirdLife International, 2020). They are classified as endangered, both globally (BirdLife International, 2020), and nationally (Robertson et al., 2017). It is estimated that roughly three quarters of the population occur in the subantarctic islands, based on recent population surveys on the Auckland Islands (Muller et al., 2020b) although population data for Campbell Island has not been updated since the 1990s (Moore, 1992). Whilst the subantarctic population is believed to be stable, the mainland population is in substantial decline (Mattern et al., 2017).

The mainland population has been intensively monitored since the 1980s. Yellow-eyed penguin populations have been known to fluctuate quite substantially on an annual basis, both on the mainland (Ellenberg & Mattern, 2012) and in the subantarctics (Muller et al. 2020b), a common phenomenon in seabirds, where the main driver of their population size is food availability (Morrison et al., 2017). In recent years, however, the mainland population has been showing a declining trend due to a range of threats, such that yellow-eyed penguins may be extinct on the South Island as early as 2043, only to

remain in the subantarctic islands (Mattern et al., 2017). Since this prediction, there has been an accelerated decline of the population at many mainland breeding sites (Department of Conservation, unpublished data).

The effective population size of the mainland population is well below the critical threshold to maintain substantial genetic diversity to adapt to various threats, such as disease or toxin exposure (Boessenkool et al., 2010). A significant sex bias also exists within the population, due to higher female mortality. The sex ratio at hatching is 1:1 male to female. By 10-12 years of age, this ratio is typically 2:1 (Marchant & Higgins, 1990), resulting in many non-breeding males unable to find mates. With the mainland population in severe decline, every bird is considered important for the conservation of the species, hence interventions such as veterinary treatment and rehabilitation are deemed necessary for injured, unwell or emaciated birds, to circumvent further declines (Webster, 2018).

1.2.4 Biology of yellow-eyed penguins

The life history of yellow-eyed penguins has been extensively studied within the terrestrial environment. Yellow-eyed penguins breed annually, during spring and summer, in relative synchrony. They exhibit strong natal philopatry, with 81% returning to their natal sites to breed (Richdale, 1957), and have very high nest site fidelity (Ratz et al., 2004). Most females begin breeding from 2-3 years of age; males from 2-4 years (Richdale, 1957). Similar to most penguin species, yellow-eyed penguins are regarded as monogamous, pairing up with the same partner from previous seasons (Richdale, 1957), although they are capable of re-pairing after the death or disappearance of a mate (Marchant & Higgins, 1990).

Breeding begins in late September, usually with two eggs laid, three to five days apart (Marchant & Higgins, 1990). Eggs are incubated for between 39-51 days, hatching in November and December (Richdale, 1957). Initially one of the parent birds remains with the chicks constantly (guard stage), but as the chicks develop both adults will leave the chicks during the day to feed (post-guard stage) (Marchant & Higgins, 1990). Both adults are involved in the parental care, and are required for chicks to fledge successfully, except once the chick reaches post-guard stage (Marchant & Higgins, 1990); an important consideration when uplifting injured adult birds during breeding. Fledging occurs at 3.5 months of age, between February and April (Marchant & Higgins, 1990).

The life stages of yellow-eyed penguins can be distinguished by differences in plumage. Young chicks initially have a light grey-brown, downy feather covering, prior to the emergence of contour feathers through the skin. After this time, they have grey-blue feathers covering the dorsal aspect of their head, body and flippers, and white feathers ventrally on the body and neck. Pre-fledged chicks (pullets) are distinguished by a region of pale-yellow feathering interspersed with grey feathers around the head and a green-grey iris. Juvenile birds (1-2 years) have a similar pattern of feathers on the head but have a yellow iris. Adult birds retain the yellow iris but have a distinct band of bright yellow feathers around the back of the head (Marchant & Higgins, 1990).

All adult and juvenile birds undergo an annual catastrophic moult, during which time they remain on land for 24 days until a complete replacement with new feathers occurs (Richdale, 1951). Moulting may commence as early as January in juveniles, non-breeders and unsuccessful breeders. For successful breeding birds, moulting occurs approximately three weeks after chicks have fledged (Marchant & Higgins, 1990), with the timing ranging from January to June, partially dependent on food availability (Marchant & Higgins, 1990). Individuals must rapidly increase their body weight in the weeks prior to moulting to survive, reaching a maximum weight of approximately 8.5kg (males) and 7.5kg (females), compared to a usual weight of 4.4-5.7kg (males) and 4.2-5.3kg (females) at other times of year (Marchant & Higgins, 1990).

Yellow-eyed penguins have not been extensively studied during the non-breeding season, although it is believed that they undertake longer foraging trips (M. Young, personal communication, 2021), as well as courtship and nest building behaviours during this time, prior to the commencement of the subsequent breeding season (Seddon et al., 2013). The average lifespan of yellow-eyed penguins is 9 years, although some individuals can live for over 20 years (Stein et al., 2017).

1.2.5 Population monitoring and conservation efforts

Research and monitoring

Population monitoring of yellow-eyed penguins began in the 1930s (Marchant & Higgins, 1990). Observations were limited to mainland birds and were made by very few independent researchers. Monitoring efforts have substantially increased over time, assisted by the unique identification of individual penguins. Currently yellow-eyed penguins are monitored at all major mainland breeding sites at three main time points in the breeding season: incubation, guard-stage and post-guard stage (Webster, 2018). Monitoring of the subantarctic island populations occurs less frequently, on an opportunistic basis. Penguins have been more extensively monitored on the Auckland Islands compared to Campbell Island. Extreme weather conditions and limited accessibility restrict the majority of the monitoring to summer, during the breeding season (November-February) in these locations (Muller et al. 2020a; Muller et al., 2021).

Ecological surveys of yellow-eyed penguins are coordinated by the Department of Conservation in conjunction with various groups and contractors. These include the Yellow-eyed Penguin Trust, Christchurch City Council, Otago University post-graduate students and researchers, and other private contractors. In addition, certain nesting sites are closely monitored by local penguin rehabilitation groups or ecotourism operators, including Penguin Rescue, Penguin Place and Elm Wildlife Tours.

Most mainland yellow-eyed penguins have been individually identified. Historically flat, metal flipper bands with a unique number engraved were placed around the flipper at the level of the humerus just proximal to the elbow joint, as described by Ratz et al. (2004). In recent years, these flipper bands have been removed and identification is achieved through passive integrated transponder (PIT) tags. Morphometric measurements are routinely collected, including head length and foot length, and can

be used to approximate sex (Setiawan et al., 2004). Information is collated and entered into the Yellow-eyed Penguin Database, a central database managed by the Department of Conservation, allowing a large dataset to be accessible to different monitoring groups and researchers.

Different monitoring modalities have been adopted to study yellow-eyed penguins within the marine environment. Specific individuals have been closely monitored using GPS trackers, satellite transmitters, time depth recorders and Go Pro© cameras to better understand foraging and habitat use at sea (Seddon & Vanheezik, 1990; Moore, 1999; Mattern et al., 2007; Mattern et al., 2013; Chilvers et al., 2014; Mattern et al., 2018; Seed et al., 2018; Muller et al. 2020a; Muller et al., 2021; Hickcox, 2022; Young et al., 2022). During these studies, more frequent visitation to yellow-eyed penguin breeding sites occurs in a short period of time, for device deployment and retrieval.

Interventions for sick or injured birds

Management interventions for sick or injured birds varies with geographical region, usually determined by proximity to veterinary or rehabilitation services. Almost all unwell, emaciated or injured yellow-eyed penguins found on the mainland of the South Island are uplifted and admitted into various veterinary hospitals or rehabilitation centres. Birds found with similar presentations on Stewart Island, Whenua Hou, The Bravo Islands and the subantarctic islands are not generally provided with veterinary treatment.

Historically rehabilitation centres were the principal stabilisation and treatment sites for sick or injured hoiho on the South Island. Rehabilitation at Penguin Rescue (Moeraki) began in 1984 and at Penguin Place (Dunedin) in the early 1990's. A third rehabilitation centre, New Friends of Bushy Beach (Oamaru), was operational between 2008 and 2016. The majority of birds received veterinary treatment from local general practice veterinarians until the mid-2010s. Several wildlife hospitals opened in the North Island of New Zealand in the 2000's, with the formation of Wildbase Hospital (Massey University) and The Nest Te Kōhanga (Wellington Zoo), at which time the most severely affected birds were flown by airplane to these facilities for veterinary treatment by wildlife veterinarians.

In 2015, a veterinary team from Massey's University's Wildbase Hospital ventured to the South Island to assess injured yellow-eyed penguins in rehabilitation centres during March and April. Many birds were radiographed at local veterinary clinics, and birds with more severe injuries were either euthanised or referred to the Wildbase Hospital for more intensive treatment. In 2016 and 2017, a temporary penguin hospital was established in Dunedin over summer by an experienced wildlife veterinarian to treat birds. This led onto the establishment of the Wildlife Hospital Dunedin in early 2018, ensuring dedicated veterinary care was available for local wildlife year-round, and hence the majority of injured yellow-eyed penguins receive assessment at wildlife-specific veterinary hospitals, prior to being sent to rehabilitation centres.

Assessment of deceased birds

Necropsies are performed on most deceased yellow-eyed penguins from the mainland population. These were initially performed by a local human doctor in Oamaru in the 1990s and early 2000s (Hocken, 2005). Since 2000, the majority of deceased yellow-eyed penguins are necropsied by specialist veterinary pathologists and wildlife veterinary residents at Wildbase Pathology (Massey University).

1.3 Threatening processes

Yellow-eyed penguins contend with a multitude of threatening processes contributing to their population decline. Some of these threats are emerging or potentially increasing in frequency or severity, placing more pressure on the species to survive. Most of these threats are isolated to the mainland population, affecting individuals at the most northern extent of their range; and include disease, habitat loss, change of prey availability, human disturbance, climate change, fishery by-catch, terrestrial predation and marine predation (Webster, 2018). It is important to be aware of the context of challenges threatening yellow-eyed penguin survival, when considering post-release outcomes after veterinary treatment, and to distinguish between injuries caused by terrestrial or marine predation.

Disease

Several diseases have caused significant mortalities of mainland yellow-eyed penguins, including avian malaria and diphtheritic stomatitis. Avian malaria, caused by *Plasmodium elongatum* and *Plasmodium relictum* (Hunter et al., 2019; Kay, 2021) has been detected in yellow-eyed penguins on multiple occasions (Fantham & Porter, 1944) and has been linked to large mortalities within the northern population of birds in certain years (Alley & Webster, 2019; Hunter et al., 2019). Diphtheritic stomatitis, a syndrome affecting young chicks, is characterised by stomatitis and caseous plaques within the oral cavity. It reduces the chick's ability to feed, resulting in emaciation and dehydration, and can cause asphyxiation from inhalation of plaques (Alley et al., 2017). It is a common cause of chick mortality, present in 50% of yellow-eyed penguin chicks submitted to Massey University for necropsy between 1994-2014, but doesn't appear to affect birds post-fledge (Alley et al., 2017).

In addition, there have been several reported mass mortality events of unknown origin or novel causes. One event spanning a two-month period in 2013 involved the death of 67 individual yellow-eyed penguins. There were no abnormalities identified on necropsy examination and all were in good body condition. Consistent findings between cases included erythrophagocytosis and haemosiderosis in the liver, spleen and lungs on histopathological examination (Gartrell et al., 2017). Extensive testing has not yielded a causative agent, and so an environmental toxin is suspected (Gill & Darby, 1993; Gartrell et al., 2017). A recent discovery of a novel gyrovirus has been linked with a 'respiratory distress syndrome', in chicks (predominantly 2-10 days of age), which was first recognised in 2019, but historical review of records suggest it may have been present in the population since 2015. The disease causes proteinaceous fluid accumulation and haemorrhage in the parabronchi, collapse of air capillaries, as well as lymphoid

depletion and lympholysis in the spleen and bursa, leading to a high mortality rate despite treatment attempts (Wierenga et al., 2023).

Climate change

Climate change is likely having both direct and indirect impacts on yellow-eyed penguins. Rising average temperatures and more extreme maximum summer temperatures threaten yellow-eyed penguins, as they are particularly susceptible to heat stress, being predominantly adapted to subantarctic conditions. Heat stress behaviours have been observed during hot conditions, including panting and sitting upright on nests rather than prone, and overheating can cause mortality in chicks (Seddon & Davis, 1989) and adults. It is believed their geographical range is limited in-part by climate, therefore changing climate may potentially reduce the geographic range of the species (Seddon & Davis, 1989). Similarly, change in sea surface temperatures is believed to be one of the most important factors for yellow-eyed penguin population decline, with higher sea surface temperatures linked to reduced abundance and quality of prey items (Mattern et al., 2017). Penguins are also more likely to be at risk of disease due to indirect effects of climate change. Changes in environmental conditions may place animals under additional physiological stress, leading to immunosuppression. Climate change is also affecting the distribution of many species, leading to novel interactions between species, and increased exposure to vectors (Hoegh-Guldberg & Bruno, 2010), such as mosquitoes.

Change of prey abundance

Predominant prey items of yellow-eyed penguins have changed over recent decades. Diet studies in the 1980s reveal larval red cod (*Pseudophycis bachus*) and opalfish (*Hemerocoetes monopterygius*) to be the most commonly consumed species, in addition to slender sprat (*Sprattus antipodum*), arrow squid (*Nototodarus sp.*) and ahuru (*Auchenoceros punctatus*) (van Heezik, 1990). Recent studies suggest a prey switch, such that penguins are now predominantly consuming juvenile and adult blue cod (*Parapercis colias*), in place of young red cod (Mattern & Wilson, 2018; Young et al., 2019; Young et al., 2020). Go Pro© camera footage of devices deployed on the backs of yellow-eyed penguins supports these studies, both in terms of relative abundance of the species and feeding attempts (Mattern et al., 2019). Starvation events have occurred in recent years, believed to be linked to the change of prey abundance (Webster et al., 2019) due to the lack of appropriate size fish offered to chicks and increased energy demands required to catch blue cod compared to opalfish (Mattern et al., 2018; Mattern et al., 2019). It is presumed that the change in prey consumed may be directly related to the abundance of certain prey items, with a predominance of blue cod being seen in areas of oyster dredging, creating disturbed benthic environments preferred by blue cod (Mattern & Ellenberg, 2018).

Terrestrial predators

The impact of terrestrial predation on yellow-eyed penguin populations is unclear. Chicks and eggs are most vulnerable to attack by terrestrial predators. Ferrets (*Mustela furo*) (Darby & Seddon, 1990; Hocken, 2005) and stoats (*Mustela erminea*) (Ratz, 1999; Ratz & Murphy, 1999) are the most significant predators of eggs and chicks on the South Island. Weka (*Gallirallus australis*) are believed to predate eggs and small chicks on

various offshore islands in Southland where they are abundant (Richdale, 1942; King et al., 2012) Domestic cats (*Felis catus)*, brushtail possums (*Trichosurus vulpecula*), rats (*Rattus spp.*) (Massaro & Blair, 2003), mice (*Mus musculus*) and European hedgehogs (*Erinaceus europaeus*) may predate chicks, however direct evidence is lacking (Taylor, 2000; Webster, 2018).

Adult birds are more likely to be targeted by larger predators, such as domestic dogs (*Canis familiaris*) or feral pigs (*Sus scrofa*). Dogs are common on mainland beaches near Dunedin. Necropsy reports are highly suggestive of domestic dogs as a predator of yellow-eyed penguins, based on intercanine distance and characteristic pathological findings. Bite wound features including extensive pectoral muscle damage, haemorrhage, bruising or organ damage from shaking, along with large wound spacing (Alley et al., 2004; Hocken, 2005). There is evidence to suggest feral pigs eat yellow-eyed penguins on the Auckland Islands (Challies, 1975; Beer, 2010), and it is highly likely they kill adult birds, based on their behaviour towards other nesting seabirds (Taylor, 2000). Whilst pigs are one of the few terrestrial predators capable of killing adult yellow-eyed penguins, there have been no reports of pigs predating yellow-eyed penguins on the New Zealand mainland, and with their numbers controlled due to active hunting in these regions, their impact on this population is likely minimal.

Other threats

Habitat degradation, fisheries by-catch and human disturbance are all believed to have a negative effect on yellow-eyed penguin populations, although the impacts of these are difficult to quantify. Extensive habitat degradation and native vegetation clearance has occurred across the mainland range of yellow-eyed penguins, particularly with large-scale redevelopment of land for dairy farming in recent years. They display an ability to adapt to changing landscapes, and will inhabit artificial nest sites in preference to poor quality habitat when available (Lalas, 1999) although nesting success may still be affected by suboptimal surrounding habitat. Reduced density of remnant coastal vegetation also makes them more prone to heat stress (Seddon & Davis, 1989). Intensive farming and associated run-off may contribute to eutrophication, sedimentation and algal blooms, affecting water quality and turbidity, and could potentially be linked to toxicity events (Webster, 2018).

The current impact of fisheries by-catch on yellow-eyed penguin populations is unknown. Anecdotal and published reports exist demonstrating that yellow-eyed penguins have drowned in set-nets. A report published in 2000 suggested 72 individuals died from gillnets in the 18-year period between 1979 and 1997 (Darby & Dawson, 2000). More recent reports indicate numbers are no longer as high (Hocken, 2005; Ellenberg & Mattern, 2012; Crawford et al., 2017), which could be a result of under-reporting, rather than an absolute drop of numbers despite banning gillnet fisheries within 2-4 nautical miles of the coast to protect hectors dolphins (*Cephalorhynchus hectori*) (Crawford et al., 2017).

Yellow-eyed penguins are highly sensitive to human disturbance. Not only does the presence of humans impact normal behaviour displayed including affecting their transits

to and from the ocean (French et al., 2018), but breeding success has been shown to be significantly impacted by unregulated tourism (Ellenberg et al., 2007).

1.4 Marine predation

Yellow eyed penguins have been observed with injuries caused by marine predators since scientific study of yellow-eyed penguins commenced in the first half of twentieth century (Richdale, 1942), but definitive confirmation of the predator species involved has proven difficult. New Zealand sea lions are known predators of yellow-eyed penguins (Moore & Moffat, 1992; Schweigman & Darby, 1997; Lalas et al., 2007). Additional suspected marine predators include sharks and barracouta (Richdale, 1942; Marchant & Higgins, 1990; Webster, 2018). There have not been any confirmed reports of leopard seals (*Hydrurga leptonyx*), New Zealand fur seals (*Arctocephalus forsteri*) or orca (*Orcinus orca*) attacking or consuming yellow-eyed penguins, although these species could be sporadic predators that have not yet been documented.

1.4.1 Incidence of marine predator attacks

There is some contention as to whether the incidence of marine predation of yellow-eyed penguins is increasing. A report from Wildbase Pathology (Massey University) suggests an increase in submissions of deceased yellow-eyed penguins fatally injured by marine predators, with a peak incidence in 2015 (Hunter et al., 2015), the final year data was reported. This could indicate marine predator attacks are becoming more frequent over time. A review of necropsies performed locally by a human physician in Oamaru between 1996 and 2003 proposed the death of 10/114 adult and fledged juvenile yellow-eyed penguins were attributable to marine predators (Hocken, 2005).

There are only rare reports of yellow-eyed penguins injured by marine predators in the subantarctic. An individual bird with a healed disarticulated stump at the level of the intertarsal joint of one leg has been filmed in a documentary (Scollay, 2016). There is a single report of a witnessed attack of a male New Zealand sea lion killing two yellow-eyed penguins and pursing others on Campbell Island (Moore & Moffat, 1992). Typical foot lacerations seen commonly on the mainland have not been reported in the literature or observed by researchers (C. Muller personal communication 2020; R. French personal communication, 2020; M. Young personal communication, 2023) in the subantarctic population.

1.4.2 Known and suspected marine predators of yellow-eyed penguins

1.4.2.1 New Zealand sea lions (Phocarctos hookeri)

There is substantial evidence that New Zealand sea lions predate yellow-eyed penguins in the marine environment. Their similar geographic distributions increase the feasibility of frequent interactions. There have also been several accounts of witnessed attacks, in addition to remnant body parts from deceased animals in sea lion colonies.

Witnessed attacks and evidence of predation

On numerous occasions, New Zealand sea lions have been sighted attacking yellow-eyed penguins both on the mainland (Schweigman & Darby, 1997; Lalas et al., 2007) and in the subantarctic (Moore & Moffat, 1992). There were seven predation events witnessed on the Otago Peninsula in the 10-year period from 1996-2005; all located within the nearshore zone (Lalas et al., 2007).

Sea lions have been observed ambushing penguins in shallow water. Once captured, they vigorously shake or bite the bird before repeatedly projecting it in the air (Moore & Moffat, 1992; Schweigman & Darby, 1997; Rey et al., 2011; Morrison et al., 2017). It is believed the hunting of penguins by New Zealand sea lions is a learnt behaviour that only a few individuals undertake, and that penguins do not constitute a major portion of their diet (Moore & Moffat, 1992; Lalas et al., 2007). It is not known whether New Zealand sea lions also hunt penguins offshore.

Additionally there was indirect evidence of a further 13 predation events in the form of unconsumed remains (e.g. pelt, degloved body, head, foot) found on Otago beaches where both yellow-eyed penguins and New Zealand sea lions reside (Lalas et al., 2007). Yellow-eyed penguin feathers were also identified in the regurgitation contents of sea lions 10 times in a five-month period in 2003, three of which were found next to one individual (Lalas et al., 2007). It was subsequently estimated that New Zealand sea lions may kill up to 20-30 mainland yellow-eyed penguins annually (Lalas et al., 2007), although this figure may have been an overestimation as all the unconsumed remains were linked to the individual specialisation of one sea lion (Lalas et al., 2007), who is no longer alive (Boren, 2015). Individual specialisation is an ecological term for a predator that specialises in a particular prey type (Araujo et al, 2011). It has some interesting implications for the evolution of novel hunting behaviour and predator-prey dynamics (Araujo & Moura, 2022). In the case of the yellow-eyed penguins, it may mean that predation by sea lions varies between years and is difficult to predict with any accuracy.

Pathological features of sea lion attack

Typical injury features of New Zealand sea lion attacks include fractured bones (Schweigman & Darby, 1997), evisceration or degloving injuries (Schweigman & Darby, 1997; Lalas et al., 2007), such that the pelt may be inverted and stretched backwards over the head of the bird. Generally sea lions consume the majority of the bird, so often there are no remains, or only a piece of pelt, head, flipper or leg recovered (Schweigman & Darby, 1997; Lalas et al., 2007). In other instances where the kill appears to be related to play behaviour, deep puncture wounds may occur to the head and neck, in conjunction with vertebral fractures (Schweigman & Darby, 1997).

1.4.2.2 Sharks (Class Chondrichthyes, sub-class Elasmobranchii)

There is very little information detailing shark predation of yellow-eyed penguins. No reports have been published to date confirming witnessed attacks. Anecdotally sharks are suspected to injure some individuals, based on the wound characteristics of penguins examined at necropsy.

Evidence of predation

There are no reports of witnessed sightings of sharks predating of yellow-eyed penguins. There are very few studies investigating the diets of New Zealand shark species (Yano, 1993; Dunn et al., 2010; Dunn et al., 2013; Horn et al., 2013; Finucci et al., 2016), none of which reveal seabirds as prey items for these selected species. Most shark diets have not been studied, due to the logistical challenges of undertaking this research in the marine environment. Information provided by diet studies is often limited, with many methodologies only indicating recently consumed prey items which are still remnant in the gastrointestinal tract.

Pathological features of shark attacks

There are no reports describing pathological lesions of local shark species on yellow-eyed penguins. There is a single study examining injuries to African penguins (*Spheniscus demersus*), believed to be attacked by sharks, which showed wound characteristics were likely dependent on the species of shark, due to different tooth shapes and arrangements (Randall et al., 1988). More than 90% of necropsied African penguins had puncture wounds involving a single jaw. Injuries varied from cuts across the feathers, lacerations, superficial wounds, deep punctures extending through muscle or into the coelomic cavity and one bird severed in half. Sharks inflicted injuries to the main body, flippers or feet (Randall et al., 1988). Tooth fragments were not found in African penguin wounds (Randall et al., 1988), however have been found in wounds on sea otters (*Enhydra lutris*) (Tinker et al., 2016; Miller et al., 2020) and occasionally in human shark attacks (Woolgar et al., 2001; Ihama et al., 2009a), as sharks shed and replace teeth very frequently (Correia, 1999).

Common wound features seen in forensic investigations of shark attacks in humans include sharp incisions in a linear or circular arrangement, or deep puncture wounds arranged in a regular arrangement, such as an arc, which may penetrate into the chest or abdomen (Ihama et al., 2009a). Triangular or rectangular flaps of skin, sometimes with jagged edges, may be seen (Ihama et al., 2009a). Tissue damage from shark bite wounds in humans can be extensive, involving the transection of muscles, nerves and blood vessels (Byard et al., 2000; Woolgar et al., 2001). Some species of sharks may sever limbs at joints, with fractures mid-diaphysis rarely reported (Işcan & McCabe, 1995; Ihama et al., 2009a). Sharks may leave linear gouges on bone surfaces (Işcan & McCabe, 1995; Woolgar et al., 2001; Ihama et al., 2009a). Many reports focus on autopsy findings, which are biased towards the most severely affected individuals. Injuries in shark attack survivors are less severe, with the majority of injuries (81%) being uncomplicated puncture wounds or lacerations (Woolgar et al., 2001).

1.4.2.3 Barracouta

Barracouta have long been implicated in yellow-eyed penguin injuries, believed to have been ‘damaging the feet and legs of birds’ for decades (Marchant & Higgins, 1990). This was first deduced from conversations with local fishermen, who witnessed the predatory fish’s behaviour whilst at sea, despite there being no reports of observed attacks on yellow-eyed penguins (J. Darby, personal communication, 2021). Barracouta have a reputation as voracious hunters that ‘snap at almost anything that moves’ (Anderson, 1981). Diet studies analysing barracouta stomach contents have never

showed seabirds to be consumed by barracouta (Blackburn, 1957; Mehl, 1969; Crawford & de Villiers, 1985; O'Driscoll, 1998; Griffiths, 2002; McQueen & Griffiths, 2004; Devine et al., 2022). There have been no witnessed sightings of barracouta attacking other species of penguins around the world, despite a widespread distribution in the southern temperate zone, and overlap with other penguin species in this region.

Yellow-eyed penguins consume small barracouta as a minor proportion of their diet (van Heezik, 1990). In a conference presentation, footage from GoPro© cameras placed dorsally on yellow-eyed penguins facing anteriorly revealed the penguins showed no behavioural changes when barracouta were in the same locality (Seed et al., 2018), and hence some researchers suggest they are not a major predator of yellow-eyed penguins (Mattern & Wilson, 2018). Conversely, it has been speculated that an interaction may occur between yellow-eyed penguins and barracouta in a feeding frenzy as an accidental maiming event when hunting fish from bait balls (C. Lalas, personal communication, 2018). This is plausible, as there is considerable overlap between barracouta and yellow-eyed penguin diets, with barracouta reported to consume sprat (*Sprattus sp.*), red cod, blue cod, ahuru, blue warehou (*Seriolella brama*), ling (*Genypterus blacodes*) and southern pigfish (*Congiopodus leucopaecilus*) (Graham, 1939), all reported components within yellow-eyed penguin diets (van Heezik, 1990). Alternatively maiming may occur incidentally when penguins are transiting through shallow coastal waters.

Barracouta are universally found in waters around the New Zealand mainland (McMillan et al., 2011), and infrequently reported in the Auckland Islands (Childerhouse et al., 2001; Devine et al., 2022). They have been an important source of food for Māori people since prehistoric times, particularly for iwi on the east coast of New Zealand (Anderson, 1981), and are therefore not a new predator in New Zealand. There is considerable overlap with barracouta and yellow-eyed penguin distribution in the summer months, consistent with the timing of most predation attacks. In late summer-autumn, barracouta are found in feeding frenzies near the surface of the water column, in shallow coastal waters, aligning with their peak feeding time in February and March (Mehl, 1969). Their feeding declines steadily between April-September, when they are noted to move offshore to deeper waters (up to 400m deep) for spawning, until they return to coastal waters to feed again in December (Mehl, 1969). They are mainly demersal, but can be located at varying points within the water column.

1.4.3 Other theoretical marine predators

New Zealand fur seals, leopard seals and orca could potentially predate yellow-eyed penguins, although there is no current evidence to support these species as major predators.

New Zealand fur seals are the most numerous of these potential predators, with approximately 100,000 individuals in New Zealand waters, distributed across mainland and subantarctic waters, in addition to waters of southern Australia (Chilvers & Goldsworthy, 2015). Yellow-eyed penguins have never been found to be prey items of New Zealand fur seals in diet studies (Boren, 2010; Allum & Maddigan, 2012). There are

no published reports of witnessed attacks of fur seals on yellow-eyed penguins on the New Zealand mainland. In a yellow-eyed penguin necropsy review, pinniped predation could not be distinguished to species level, although the paper concluded that attacks from fur seals were unlikely (Hocken, 2005). A witnessed New Zealand fur seal attack on a little penguin described injuries including puncture wounds, haemorrhage and visceral organ protrusion from an unconsumed individual (Clemens et al., 2011). Similar behaviour to that described in other pinniped species, was observed when killing and dismembering the prey, including thrashing the bird around from side to side, or repeatedly grabbing and throwing the bird (Clemens et al., 2011). The intercanine distances for New Zealand fur seals are 20.8–45.6mm (Colombelli-Négrel & Tomo, 2017), reflecting wound spacing.

Leopard seals, predominantly found in Antarctic waters, are occasional visitors to mainland New Zealand and the subantarctic islands in autumn and winter (Department of Conservation, 2021). Although known to prey on penguin species in Antarctica including gentoo (*Pygoscelis papua*), macaroni (*Eudyptes chrysolophus*), Adélie (*Pygoscelis adeliae*) and chinstrap penguins (*Pygoscelis antarcticus*) (Du Toit et al., 2004; Ainley & Ballard, 2012; Krause et al., 2020), they have never been observed attacking yellow-eyed penguins, nor have diet studies ever demonstrated remains of yellow-eyed penguins (van der Linde et al., 2021). Leopard seal predation could be difficult to distinguish from New Zealand sea lion attacks from the carcass alone. Similar to sea lions, they have been observed degloving seabirds, dismembering them into smaller chunks, and often eating breast muscle and internal organs, but rarely the majority of the pelt. Fractures are common, either as a result of completing the kill, or in the process of preparing the carcass (Edwards et al., 2010; McKinlay et al., 2014).

Orca are known predators of penguins, predating on king (*Aptenodytes patagonicus*), rockhopper (*Eudyptes chrysocome spp.*), gentoo and chinstrap penguins, and likely Magellanic (*Spheniscus magellanicus*), emperor (*Aptenodyptes forsteri*) (Pitman & Durban, 2010) and little penguins (*Eudyptula minor*) (Visser, 2000). Orca will commonly consume the pectoral muscle of penguins, degloving the carcass at this site, removing the pectoral girdle and decapitating the bird in the process. Remnants are commonly the caudal half of the body with the viscera, legs and tail intact (Pitman & Durban, 2010). Such injuries have not been observed in yellow-eyed penguins to date. Although killer whales are found in most oceans worldwide (Reeves et al., 2017), there are less than 200 individual killer whales that inhabit New Zealand waters (Visser & Cooper, 2020). Interactions are likely minimal, and no attacks have ever been witnessed in New Zealand. In New Zealand, little penguins are the only species of penguins to be recorded as prey of orcas in diet studies, although it is possible they also take other species (Visser, 2000).

1.4.4 Marine predator injuries in yellow eyed penguins

A distinctive pattern of injury, unreported in the literature in other penguin species worldwide, has been observed in yellow-eyed penguins. Wounds are primarily to the legs and feet, although they may occasionally be seen on the flippers and rest of the body. Birds often have multiple linear-curvilinear lacerations that are generally 20-40mm apart, 10-40mm in length and ≥5mm wide (Hunter et al., 2015).

The majority of the wounds have been observed to be mainly on the dorsal and lateral aspects of the foot, and involve multiple lacerations parallel, or perpendicular, to one another. Injuries may be uncomplicated, partial-full thickness skin lacerations; however they often extend into deeper structures, including underlying tendons and bone. Most notably the superficial tendons on the dorsal aspect of the leg are affected, specifically cranialis tibialis and the extensor digitorum longus, which if ruptured results in a hyperextension of the hock and subsequent dragging of the dorsal aspect of the foot (Hunter et al., 2015).

Similar wounds have been observed in other penguin species submitted to the Wildbase Pathology service including snares crested penguins (*Eudyptes robustus*), erect crested penguins (*Eudyptes sclateri*) and Fiordland crested penguins (*Eudyptes pachyrhynchus*). Additionally little penguins have presented to various veterinary wildlife hospitals in New Zealand with similar foot lacerations. Wounds in these species have not been investigated in this thesis due to the scope of this study.

1.5 Research aims and thesis structure

There is a paucity of research into the significance of marine predator injuries in yellow-eyed penguins and the impact on their population. With the exception of a few brief reports (Hocken, 2005; Hunter et al., 2015), there has not been a thorough clinical and pathological overview of such injuries in yellow-eyed penguins. With significant interventions being taken to uplift and treat injured birds at wildlife hospitals and rehabilitation centres, follow-up of individuals after treatment is vital to critically analyse the measures that are being undertaken to conserve this endangered species. In addition, determination of prognostic factors for successful treatment and rehabilitation are required to properly evaluate patients and their likelihood of further contribution to the overall population. The focus of this research is on the mainland population, as this is the population that is most closely monitored and where interventions are implemented to treat affected birds.

The primary aim of this research is to investigate marine predator attacks in yellow-eyed penguins, specifically:

1. Epidemiological evaluation of marine predation injuries, including a temporospatial analysis of yellow-eyed penguins injured by marine predators to determine if the predation events are restricted in geographical range, and to investigate a possible increased prevalence over time.
2. Investigation of potential marine predators by morphological and molecular assessment of a tooth found in a penguin leg wound, a pilot study investigating the dentition of several potential predators and an evaluation of bite wound characteristics seen on yellow-eyed penguins.
3. Clinical and pathological review of injuries.
4. Review of the treatment protocols used, with respect to the overall outcome for patients (died/euthanised, released, resighted non-breeding, resighted breeding) and the determination of prognostic factors for hospital survival and post-release outcomes.

CHAPTER TWO

Temporospatial analysis and investigation of potential marine predators

2.1 Introduction

The significance and impact of marine predation on yellow-eyed penguin populations is poorly understood. Sporadic observations have been made by wildlife rehabilitators, veterinary personnel, pathologists, ecologists and conservationists, who have encountered individuals with injuries suggestive of marine predation events, however this information has not been previously collated.

It has been suggested that there is an increasing frequency of marine predation events within the northern population of yellow-eyed penguins in the last decade (Hunter et al., 2015), although this speculation is yet to be confirmed. It is hoped that by collating available information, a better understanding of the true frequency of these events can be obtained. Further investigation of the occurrence of injury across the mainland range of the species is required to construct a spatial picture of these attacks.

There is a distinct lack of information concerning the potential marine predators of yellow-eyed penguins. New Zealand sea lions are the only species definitively proven as predators. No reports exist to indicate New Zealand fur seals in any attacks, despite the two species commonly cohabiting in both the terrestrial and marine environment. Sharks are thought to attack yellow-eyed penguins, based on the characteristic arrangement of puncture wounds, although evidence examining specific shark species responsible for attacks is lacking. Anecdotally, bites from barracouta have also been implicated in foot injuries, although there is not substantial evidence to support this theory.

In this chapter, a pathological review of marine predation injuries in yellow-eyed penguins was conducted to investigate likely predators of yellow-eyed penguins. This included both an assessment of injuries seen at necropsy, and a pilot study examining dentition and bite impressions from various potential predators. Additionally, morphological and molecular assessment of a predator tooth sourced from a yellow-eyed penguin foot laceration was undertaken to provide direct evidence of interaction with specific predators. Further investigation of the potential predators of yellow-eyed penguins may provide insight into the ecological processes and interactions occurring to cause these injuries, and potentially lead us to understand if there have been recent changes within the local marine ecosystems favouring these encounters.

2.2 Materials and Methods

2.2.1 Temporal investigation of marine predation events in yellow-eyed penguins

Retrospective review of veterinary hospitalisation records

Medical records were reviewed from four general veterinary practices commonly treating injured yellow-eyed penguins across their mainland range. These were the St Kilda Veterinary Centre (Dunedin), Oamaru Veterinary Centre and Vetlife Oamaru (Oamaru) and Hornby Veterinary Centre (Christchurch). All available records were included up to the end of June 2018. The earliest veterinary treatment records for yellow-eyed penguins affected by marine predator attacks was 1996 for the Oamaru Veterinary Centre, 2001 for the St Kilda Veterinary Centre, 2010 for Vetlife Oamaru and 2012 for Hornby Vets. At these practices, penguins received veterinary treatment from general practitioners, who had not undertaken rigorous training in wildlife medicine. Records were located by searching the terms 'yellow-eyed penguin', 'penguin', 'department of conservation' or known rehabilitator names, as clients of the respective clinics. Penguins were usually uplifted and brought to veterinary practices by ecologists, researchers, wildlife rehabilitators, or the general public.

General veterinary practice data was of limited use, due to variability in information recording. Consequently, these records were only used to estimate the total number of yellow-eyed penguins presenting with marine predation wounds each year. For inclusion in this study, records were required to specify the individual to species level (i.e. records were not included if the individual was listed as a 'penguin') and either mention the presence of a wound anywhere on the animal or provide evidence of osteomyelitis or septic arthritis in a limb with radiographic, cytological or culture and sensitivity information. As the majority of individuals did not have a unique identification number recorded, it was assumed that each new record was a new injury, unless it was listed as a revisit. Penguins were excluded if they were lame, or had a fractured limb, without concurrent wounds or osteomyelitis. Suspected dog attacks and bumblefoot cases were excluded, as were unfledged chicks, as they were unlikely to have encountered a marine predator.

Medical records from three veterinary wildlife hospitals that treated the majority of injured yellow-eyed penguins were examined separately; namely the Wildlife Hospital Dunedin, Wellington Zoo's The Nest Te Kōhanga and the Wildbase Hospital at Massey University. Data from these hospitals was collated to determine a total number of yellow-eyed penguins injured in various years, using the same criteria listed above for the general veterinary practices. A more detailed analysis of monthly trends in injury occurrence using this data is presented in Chapter 3. Penguins initially admitted to general veterinary practices before referral to wildlife veterinary hospitals were only included once in the dataset, in the wildlife veterinary hospital category.

Retrospective review of necropsy records

A review of yellow-eyed penguin necropsies in the Massey University Pathology Database, necropsied between January 1st 2000 and January 31st 2019, was performed retrospectively to determine the numbers of deceased yellow-eyed penguins attacked

by marine predators in various years. This included birds found dead in the wild and those that died in human care, such as in veterinary clinics and rehabilitation centres. There were numerous submissions that had been frozen for years in a museum collection, that had been submitted for necropsy many years after the body was collected, which were included if the necropsy was performed between 2000-2019. Archived photographs were reviewed concurrently with reports where available.

Likely marine predator attack was determined by review of the history, wound characteristics and veterinary pathologist interpretation. Birds were included if their injuries were active (defined in Sections 2.2.2 and 2.3.2) but were not included if they only had scars suggestive of previous attack. Examples of recent injuries included mention of a fresh or healing wound on the foot, flipper or body; evidence of osteomyelitis of the appendicular skeleton; septic arthritis (eg. caseous material or turbid fluid within joint); tenosynovitis; amputated limbs; open fracture and dislocations associated with a wound; or a degloving injury. Suspected dog attacks were excluded from the review. Dog attacks were distinguished from New Zealand sea lion attacks based on relevant history information, such as location (e.g. predator free islands excluding dogs) and pathologist interpretation (Alley et al., 2004; Hocken, 2005). Typical features of dog attack injuries included puncture wounds or tears on the body or around neck, with extensive haemorrhage or internal organ damage suggestive of shaking . Dogs rarely degloved carcases, nor did they consume the carcass, unlike New Zealand sea lions (Lalas et al., 2007). Exclusion criteria included carcasses with substantial autolysis or maggot infestation, as the appearance of the wounds was usually significantly altered, and in cases where scavenging was suspected, by the presence of punctures to the skin without associated haemorrhage or bruising, suggesting damage to the carcass occurred post-mortem.

Only fledged penguins were included, as unfledged birds that had never entered the ocean were not likely to be attacked by a marine predator. Age allocation within the Pathology Database was verified for all birds with unique identifiers (flipper bands or microchips) using records in the Yellow-eyed Penguin Database, or in the case of unmarked birds based on plumage and weight. A juvenile bird for the purposes of this research was defined as a fledged bird that had gone to sea (>14 weeks of age) which was younger than 2 years of age, at which point it becomes an adult. Necropsy photographs incorporating the bird's head could be used to determine age class from plumage and eye colour, as outlined in Section 1.2.4. Weight was used to differentiate young chicks from fledged birds. Minimum fledging weight on the mainland is 4.1kg (McClung et al., 2004). To account for potential emaciation, it was determined that a bird would be unlikely to survive greater than 50% weight loss, as it would be in stage three starvation (Duerr & Klasing, 2015), so a minimum weight of 2.05kg was determined as the lower cut-off weight for juveniles or adults, when other information to indicate age was unknown.

2.2.2 Spatial investigation of marine predation events in yellow-eyed penguins

Yellow-eyed penguins were examined for evidence of marine predator injury at breeding sites across their mainland range, in the two-year period from February 2018 to January 2020 inclusive. Photographic data were collected during usual population monitoring surveys and opportunistically when birds were handled during research projects, in association with several Wildlife Act Authority Permits (78325-FAU, 50925-FAU, 63792-FAU, 91377-FAU).

Field observations were only made when there were concurrent requirements for individuals to be handled (e.g. for the purposes of marking, blood/faecal collection, device attachment/retrieval), to check that the bird was not injured. During nest monitoring when penguins were incubating eggs, observations were collected whilst checking numbers of eggs, which involved elevating the bird's body with a transponder stick reader (Allflex RS420 Stick Reader, Allflex, Palmerston North, NZ) without removing it from the nest, and only viewing the visible aspects of the feet. Unmarked birds incubating eggs were uplifted for microchip placement, only if it was deemed safe to do so, depending on the nest location and whether potential predators (e.g. weka) were in the vicinity, in which case all four aspects of the feet could be viewed and photographed.

Yellow-eyed penguins were routinely assessed for the presence of active injuries, scars or no injuries to the limbs and body. During handling by conservation and research staff, their feet were photographed using a digital camera (Canon 1100D SLR, Canon, Sydney, Australia) from the dorsal, lateral, medial and plantar aspects, and any other additional injuries or scars were also photographed. Birds deemed to have active injuries requiring veterinary treatment were uplifted and taken to the local veterinary wildlife hospital. Notes were taken after the handling event and photographs were reviewed by the author retrospectively to determine the injury status and location of injury on the animal. The majority of data was collected by the author, however in some instances other researchers provided data (photographs and field notes) which were reviewed by the author to categorise injuries. Researchers were briefed to take photos of all aspects of the feet, regardless of whether or not they believed injuries or scars were present, to standardise observations.

Penguins at various life-stages were readily distinguished by morphological differences in plumage and eye colour. Adult and juvenile birds were photographed, with unfledged pulli excluded. In accordance with standard procedure in the yellow-eyed penguin monitoring program, all birds were checked for flipper bands and scanned for a microchip using a transponder stick reader by Conservation and research staff. Unmarked birds were microchipped with a passive integrated transponder (TROVAN, Microchips Australia, Victoria, Australia) in the subcutaneous tissue of the interscapular region, in accordance with the Department of Conservation best practice for transponder use in yellow-eyed penguins protocol (Young & McKinlay, 2012). Morphometric measurements were routinely collected as part of the usual monitoring program, and were used to indicate the most likely sex of the bird (see Section 1.2.5).

When unique identification information was present, this information was searched on the Yellow-eyed Penguin Database to confirm demographic details such as age and sex.

As assessed by photographs and field observations, an active injury was defined as a bleeding, discharging, swollen or weeping wound on the body, flipper or leg. Wounds covered by a scab or exudate, which had not yet re-epithelialised or contracted, were also regarded as active. Degloving injuries were included in cases where the bird was found alive.

Based on information attained from historical necropsy findings, scars were defined as black, grey or white in colour, linear to curvilinear, or V-Y shaped imprints in the skin. They also included regions of pinched in skin that had contracted over a previous injury site, as well as pinpoint or circular scabs. Any lesion on the plantar aspect of the foot appearing as a circular impression or callus consistent with pododermatitis was not included, nor was sloughing of the skin in this region; both commonly observed during moulting while the bird remains on land for an extended time. Regions of feather loss across normally feathered areas of the body without evidence of damage to the skin, were not included. Damage to the webbing without other injuries were also not regarded as marine predator scars. Pigment changes to the skin, which appeared as discolouration without signs of previous injury, potentially related to melanin deposition in an abnormal area, were not regarded as scars. Blistering of the skin on the dorsal aspect of the foot was also disregarded, as the cause of this was unclear.

Penguins were regarded as having no evidence of marine predator attack if they had undamaged feet with no indications of scarring, as described above. Several birds had both active injuries and scars suggestive of multiple predator attacks at different times, or perhaps the same attack with different healing times for separate injuries. These cases were classified according to their most recent injury and hence were included in the active category.

Breeding sites were categorised by region. These were Banks Peninsula, North Otago, Otago, Catlins and Southland. Multiple breeding locations were visited in each region, some of which had suitable individuals for observations whereas others did not (Table 2.1). Sites were visited at various times of the year. The majority of birds in the Otago and Catlins regions were sampled from February – June 2018, North Otago from April – May 2018, Southland in October 2018 and Banks Peninsula in October 2018 and January 2020. Based on yellow-eyed penguin breeding localities, it was more appropriate to consider the Catlins as a separate group rather than incorporating it into the council regions of Otago or Southland, as this council division occurs in the middle of the Catlins yellow-eyed penguin breeding sites.

Table 2.1: Breeding sites visited and sampled across the northern range of yellow-eyed penguins.

	Locations where observations made	Locations visited but no suitable birds located or observed
Banks Peninsula	Ōtanerito Bay Shell Bay	Red Bay Goughs Bay Flea Bay
North Otago	Bushy Beach Tavora Reserve (Bobby's Head)	
Otago	Otaphi Reserve (Alfred and Cicily Beaches) Victory Beach Pipikaretu Beach Papanui Beach Boulder Beach Fuchsia Gully	
Catlins	Long Point Nugget Point Roaring Bay Penguin Bay Hinahina Cove Te Rere Mahaka Point	
Southland	Rollers Beach (Stewart Island) Golden Beach (Stewart Island) The Neck (Stewart Island) Tommy Island Goat Island Crayfish Island	Refuge Island

For birds with active injuries or scars, information was grouped into multiple categories according to injury location on the animal. This included anatomical region injured, specifically body, flipper, foot/leg wounds (hereafter just referred to as leg wounds). Additionally, injury position in a vertical plane was assessed, specifically tibiotarsal (TT), tarsometatarsal (TMT), or digits. Injury classification across a horizontal plane included aspect of the foot (dorsal, medial, plantar or lateral), and for further distal injuries specifically classing into digit location (digit 1, 2, 3, 4). Categories were not mutually exclusive, as often there were multiple injuries present on different anatomical locations on the body, and injuries commonly crossed multiple categories in either a horizontal or vertical plane.

Chi-squared tests were performed to examine differences between geographical region and the presence of active injuries, scars or no injury, using an online chi-squared calculator (Preacher, 2001). Additional statistical tests examining the injuries more closely were performed with IBM SPSS version 27. Cochrane's Q tests were performed to investigate differences in injury prevalence between different anatomical locations. *Post-hoc* testing with Bonferroni's correction was performed in instances where a significant difference was shown between the groups. Broader relationships were also examined to determine possible correlations with age, sex and region with anatomical region on body, as well as injury location in the vertical plane, horizontal aspect and digit injury location using chi-squared tests, or likelihood ratio where the data did not meet

the criteria for chi-squared tests. Odds ratios were calculated for all data which corresponded to a 2x2 table. A one-sample binomial test was performed to examine differences between unilateral and bilateral foot injuries.

Significant results were interpreted in conjunction with the description in Ganesh and Cave (2018). Specifically, that p-value results can be used as more of a continuous scale in accordance with the strength of evidence, such that $p < 0.001$ indicates very strong evidence, $p < 0.01$ provides strong evidence, $p < 0.005$ equates to moderate evidence, $p < 0.1$ suggests weak evidence and $p > 0.1$ provides insufficient evidence.

2.2.3 Investigation of potential marine predators

In January 2018, an adult yellow-eyed penguin (microchip number 982000063627639) was uplifted from Penguin Bay in the Catlins and presented to the Wildlife Hospital Dunedin, knuckling when walking, with three chronic, necrotic lacerations proximal to the intertarsal joint, and a 3cm long puncture wound containing maggots. A tooth was extracted from the puncture wound. No photographs of the wound were taken at the time, and the animal was successfully released after treatment. The tooth was stored at room temperature in a sterile container until the DNA extraction process. The tooth was measured, photographed and examined by various fish and shark scientists in New Zealand and internationally, either in-person or by photographic depiction, prior to preparation of the tooth for molecular analysis.

Morphological assessment

Attempts were made to identify the predator morphologically, firstly by the author, through examining a collection of fish skulls from species found in New Zealand coastal waters at Te Papa Tongarewa Museum and comparing these with the tooth located in the penguin leg wound. Maxillary and mandibular jaw segments were viewed for 54 species, as detailed in Table 2.2.

Table 2.2: List of New Zealand coastal fish species skulls examined in the Te Papa Tongarewa Museum.

Common Name	Scientific Name	Common Name	Scientific Name
Gemfish	*Rexea solandri*	**Skipjack tuna**	*Katsuwonus pelamis*
New Zealand blue cod	*Parapercis colias*	**Yellow-fin tuna**	*Thunnus albacares*
Barracouta	*Thyrsites atun*	**Wahoo**	*Acanthocybium solandri*
Oilfish	*Ruvettus pretiosus*	**Black cod**	*Notothenia angustata*
Grouper	*Polyprion oxygeneios*	**Leatherjacket**	*Meuschenia scaber*
John dory	*Zeus faber*	**Elephantfish**	*Callorhinchus milii*
Red scorpionfish	*Scorpaena cardinalis*	**Blue warehou**	*Seriolella brama*
Scarpee	*Helicolenus percoides*	**Spotty**	*Notolabrus celidotus*
Kahawai	*Arripis trutta*	**Southern pigfish**	*Congiopodus leucopaecilus*
Red snapper	*Centroberyx affinis*	**Dark toadfish**	*Neophrynichthys latus*
Butterfish	*Odax pullus*	**Trevally**	*Pseudocaranx dentex*
Blue mackerel	*Scomber australasicus*	**Trumpeter**	*Latris lineata*
Yellowtail kingfish	*Seriola lalandi*	**Pilot fish**	*Naucrates ductor*
Orange roughy	*Hoplostethus atlanticus*	**Marblefish**	*Aplodactylus arctidens*
Bluenose	*Hyperoglyphe antarctica*	**Blue maomao**	*Scorpis violaceus*
Hoki	*Macruronus novaezelandiae*	**Yellow-eyed mullet**	*Aldrichetta forsteri*
Frostfish	*Lepidopus caudatus*	**Scarlet wrasse**	*Pseudolabrys miles*
Australasian snapper	*Chrysophrys auratus*	**Witch**	*Arnoglossus scapha*
Estuarine stargazer	*Leptoscopus macropygus*	**New Zealand sole**	*Peltorhamphus novaezeelandiae*
Giant stargazer	*Kathetostoma giganteum*	**Brill**	*Colistium guntheri*
Spotted stargazer	*Genyagnus monopterygius*	**Black flounder**	*Rhombosolea retiarii*
Ling	*Genypterus blacodes*	**Greenback flounder**	*Rhombosolea tapirina*
Bastard red cod	*Pseudophycis breviuscula*	**Sand flounder**	*Rhombosolea plebeia*
Red cod	*Pseudophycis bachus*	**Greenback jack mackerel**	*Trachurus declivis*
Blue moki	*Latridopsis ciliaris*	**Yellowtail jack mackerel**	*Trachurus novaezelandiae*
Pacific red gurnard	*Chelidonichthys kumu*	**Slender jack mackerel**	*Trachurus murphyi*
Giant boarfish	*Paristiopterus labiosus*	**Tarakihi**	*Nemadactylus macropterus*
Skipjack tuna	*Katsuwonus pelamis*	**Skipjack tuna**	*Katsuwonus pelamis*

Fish and shark experts in Australia and New Zealand were also consulted in person or via email, for their professional input to determine the most likely origin species.

Molecular assessment

Molecular analysis of the tooth extracted from the penguin wound was undertaken to attempt to definitively confirm the identity of the host. A range of DNA extraction methods were trialled with various fish teeth, and a Chelex method was found to be the best method for amplifying DNA from these samples. This involved suspending a fragment of tooth in 20µL of PBS, and adding 40µL of 6% w/v Chelex 100 (BioRad, CA, USA) and 1µL 20mg/ml proteinase K (Ambion, CA, USA) to the solution. The solution was then incubated at 56°C for 1 hour, before an additional 8 minute incubation at 100°C. Positive controls included barracouta and school shark teeth extracted with the Chelex method, and skeletal muscle from barracouta, gemfish (Rexea solandri) and frostfish (Lepidopus caudatus) extracted using a DNeasy Kit (Qiagen, CA, USA). Teeth were prepared by breaking them into smaller pieces using sterile bone cutters. From the sample tooth, a section of tooth with dried blood adhered (presumed to be tooth pulp) was chosen for the extraction.

PCR was initially performed using the first two forward and two reverse primers in the COI-3 combination listed in Ivanova et al. (2007) (Table 2.3). Primers were resuspended to 100µM, then diluted with water to make a final concentration of 10µM. A stock solution was made using 0.2µL of each 10µM primer (VF2_t1, FishF2_t1, FishR2_t1, FR1d_t1), 4µL Solis BioDyne FIREPol®, 2µL of template and water added to make a final volume of 20µL. PCR was undertaken using a FISH thermocycler program, which involved a denaturing step of 95°C for 15 minutes, followed by 40 cycles of 95°C for 30 seconds, 52°C for 40 seconds, 72°C for 1 minute, and a final extension step of 72°C for 10 minutes.

PCR products were visualised on a 1.3% w/v agarose gel (Bioline, London, UK) at 100V for 30 minutes using 0.5x TBE as the running buffer. Gels were visualized using a UVP MultiDoc-It 120 Imaging System Transilluminator. Samples that produced a PCR amplicon were sent for bi-directional Sanger Sequencing at Massey Genome Service (Massey University, Palmerston North, New Zealand). Resulting sequences were compared to available data in National Center for Biotechnology Information (NCBI), https://www.ncbi.nlm.nih.gov/ utilizing the BLAST algorithm (Altschul et al., 1990).

Two additional PCR attempts were performed using the same methods above with a different set of primers, described in Boussarie et al. (2018), involving a novel reverse primer specific for sharks using the COI gene (Shark-COI-MINIR) and the forward primer FishF1, producing an estimated 127bp amplicon. Products were observed on a 1% w/v agrose gel in 1xTAE. After multiple bands were produced, the band most similar in size to the predicted amplicon size (127bp) was cut out and sent for bi-directional Sanger Sequencing at Massey Genome Service (Massey University, Palmerston North, New Zealand).

A final PCR was run using a set of primers outlined in Truelove et al. (2019), targeting the 12S ribosomal RNA gene for fish (170bp), using the conditions described above. Relevant bands were cut out and sent for bi-directional Sanger Sequencing at Massey Genome Service (Massey University, Palmerston North, New Zealand).

Table 2.3: Fish primers used in molecular analyses of the tooth extracted from a yellow-eyed penguin leg wound.

Name	Sequence	Publication
VF2_t1	5'-TCAACCAACCACAAAGACATTGGCAC-3'	Ivanova et al. (2007)
FishF2_t1	5'-TCGACTAATCATAAAGATATCGGCAC-3'	Ivanova et al. (2007)
FishR2_t1	5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'	Ivanova et al. (2007)
FR1d_t1	5'-CACCTCAGGGTGTCCGAARAAYCARAA-3'	Ivanova et al. (2007)
Shark-COI-MINIR	5'-AAGATTACAAAAGCGTGGGC-3'	Boussarie et al. (2018)
FishF1	5'-TCAACCAACCACAAAGACATTGGCAC-3'	Boussarie et al. (2018)
shark_12S_F	5'-GTCGGTAAAACTCGTGCCAGC-3'	Truelove et al. (2019)
Shark_12S_R	5'CATAGTGGGGTATCTAATCCCAGTTTG-3'	Truelove et al. (2019)

Measurements of potential marine predator dentition

A pilot study was undertaken to examine the dentition of barracouta and several other species within the order Scombriformes, specifically gemfish and frostfish which are commonly located in New Zealand coastal waters. Four deceased barracouta, one gemfish and one frostfish were sourced from local fish suppliers. A series of measurements were collected from these specimens including standard length, fork length, total length, body depth, head length, gape length, tooth length and tooth spacing.

Complete dentition consisted of six elongated teeth on the rostral maxilla (three sets of two teeth, located either side of midline, from rostral to caudal) for all three species, in addition to strongly seated rows of teeth in the maxilla and mandible. The gemfish had elongated teeth on the rostral mandible, resembling two long canine teeth. The elongated palatine teeth were very mobile, and frequently these teeth were missing, therefore it was not possible to complete all measurements on these teeth.

In addition, two New Zealand sea lion skulls were examined to determine features of dentition. Measurements taken included length and width of all four canine teeth, and intercanine distances of the maxillary and mandibular canines, measured from both the base and tip of the tooth, termed proximal and distal intercanine distances respectively. Skull length was measured from the most rostral section of the maxilla to the occipital condyle, to standardise the measurements.

Bite mark impressions

Modelling clay (Jovi© Air Hardening Clay) was rolled into a flat rectangular section. Maxillary and mandibular sections of the New Zealand sea lion and barracouta skulls were used to create bite mark impressions, by providing pressure with the teeth held perpendicular to the clay. Additionally, teeth rake marks were also made by dragging the jaws of barracouta through the modelling clay; both with the jaw held open in full extension, and with the jaw in a resting position, ensuring to make contact with the palatine teeth in the maxilla. As some specimens were missing teeth, the barracouta and New Zealand sea lion specimens with the most teeth intact were chosen for this activity.

2.2.4 Pathological description of wounds and links to possible predators

Yellow-eyed penguin wounds were retrospectively assessed using descriptions and photos collected during necropsy examination at Massey University from the individuals outlined in Section 2.2.1. Wounds were classified into 10 categories according to arrangement and shape of puncture marks, approximate depth, and location on the body, as outlined in Table 2.11. Representative photographs were included and sketches were made to further illustrate defining features of each category.

In most cases, photographs were necessary to accurately assign wounds to these categories, unless necropsy information was very detailed and not only described the dimensions of the wounds, but also the spatial relationship between wounds when multiple wounds were present. Length and width of wounds were measured from photographs where scale bars were included. Accurate depth measurements could not be discerned from the photographs, however wounds were categorised as either superficial or deep as determined by the tissue structures disrupted by the overlying wounds.

Only individuals with active wounds were included in this study, as scars were unlikely to be representative of the nature of the original wounds. Individuals with evidence of underlying tissue infection from a previous wound, but with no active injury, were excluded. As the categories were not mutually exclusive, some of the individuals displayed characteristics of multiple categories, so up to two categories were selected for each individual where applicable.

2.3 Results

2.3.1 Temporal investigation of marine predation events in yellow-eyed penguins

Retrospective review of veterinary hospitalisation records

There has been an increasing frequency of yellow-eyed penguin admitted into veterinary clinics for marine predator injury over time (Figure 2.1). The highest number of admissions occurred in 2015, with a total of 63 yellow-eyed penguins treated at veterinary practices; equating to 12.5% of the estimated 504 mainland breeding individuals that year (Birdlife International, 2021). A high incidence of injuries was also noted in 2018 and 2019, with 52 and 54 individuals admitted to veterinary hospitals, representing 11.5% and 11.8% of the population respectively (BirdLife International, 2020; Yellow-eyed Penguin Trust, 2023). The average number of admissions of yellow-eyed penguins with marine predator wounds to veterinary hospitals in the 1990s was 4.3, in the 2000s was 5.9 and in the 2010s was 30.2. Wildlife hospitals have seen the majority of injured yellow-eyed penguins since 2016. Data was not collected from general veterinary practices for 2019, however anecdotally most injured penguins in North Otago and more southerly regions were taken to the Wildlife Hospital Dunedin for assessment, so it is likely we have captured most injured penguins after this time in our dataset.

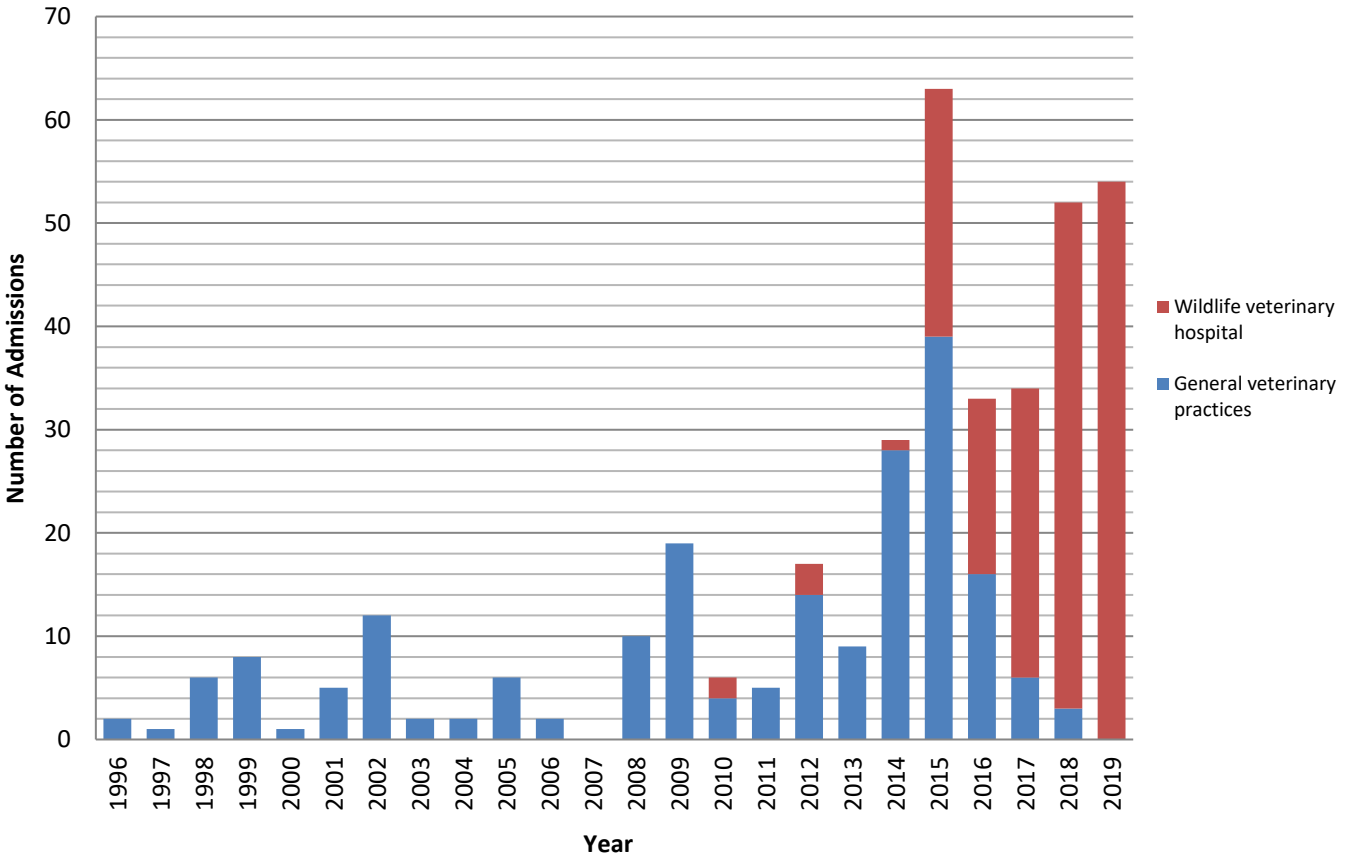


Figure 2.1: Admission of yellow-eyed penguins with marine predator injuries into veterinary clinics from 1996-2019.

Retrospective review of necropsy records

A similar increasing trend has been observed in the number of deceased yellow-eyed penguins submitted to Wildbase Pathology (Figure 2.2). The highest number of injured penguins submitted to this service was seen in 2015, with 34 individuals suspected to have been injured by a marine predator. High numbers of submissions were also observed in 2018 (16 individuals) and 2019 (25 individuals), mirroring veterinary hospital data. The number of yellow-eyed penguins submitted to Wildbase Pathology with marine predator injuries averaged 1.6/year in the 2000s and 13.2/year in the 2010s.

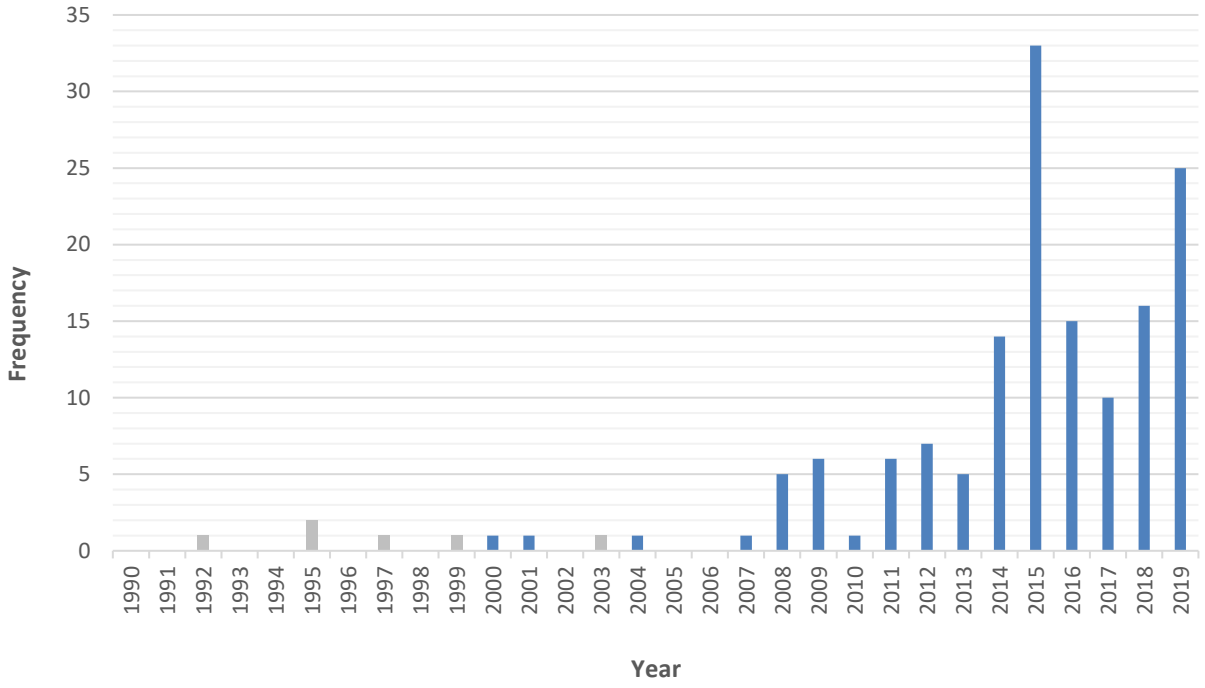


Figure 2.2: Number of yellow-eyed penguins submitted to the Wildbase Pathology necropsy service determined to have been attacked by a marine predator, from 1990-2019.
Grey region shows possible uncertainty in results due to samples that were frozen as museum specimens and were submitted several years later. These were reported as the year that the animal died.

2.3.2 Spatial investigation of marine predation events in yellow-eyed penguins

A visual depiction of the typical active injuries, scars and normal uninjured feet of yellow-eyed penguins encountered in this study is illustrated in Figure 2.3.

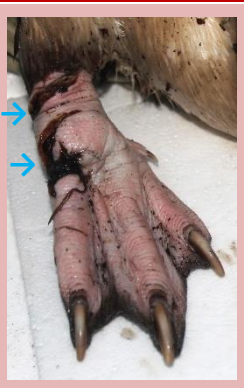
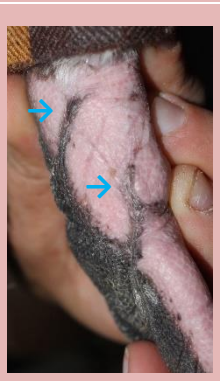
Injury Classification	Dorsal Aspect	Plantar Aspect	Lateral Aspect
Active			
Scar			
None			

Figure 2.3: Photographic display of typical foot injuries and scars, as well as uninjured penguin feet, seen in wild yellow-eyed penguins examined during fieldwork, as noted from various aspects of the foot. *Arrows demonstrate wounds and scars.*

A total of 128 birds were examined; 117 adults and 11 juveniles. Yellow-eyed penguins showed evidence of marine predator injuries across their entire northern range (Figure 2.4). A total of 63/128 (49%) showed evidence of current or previous marine predation

attack. Active injuries were only seen in Otago and the Catlins during field surveys. There was no evidence for an association between all five geographical regions surveyed and the presence of active injuries, scars or no injuries (Yates $\chi^2 = 11.764$, df = 8, p = 0.162). Closer examination of a smaller subset of the Otago and Catlins penguins provided moderate evidence for a difference between injury types ($\chi^2 = 7.183$, df = 2, p = 0.028).

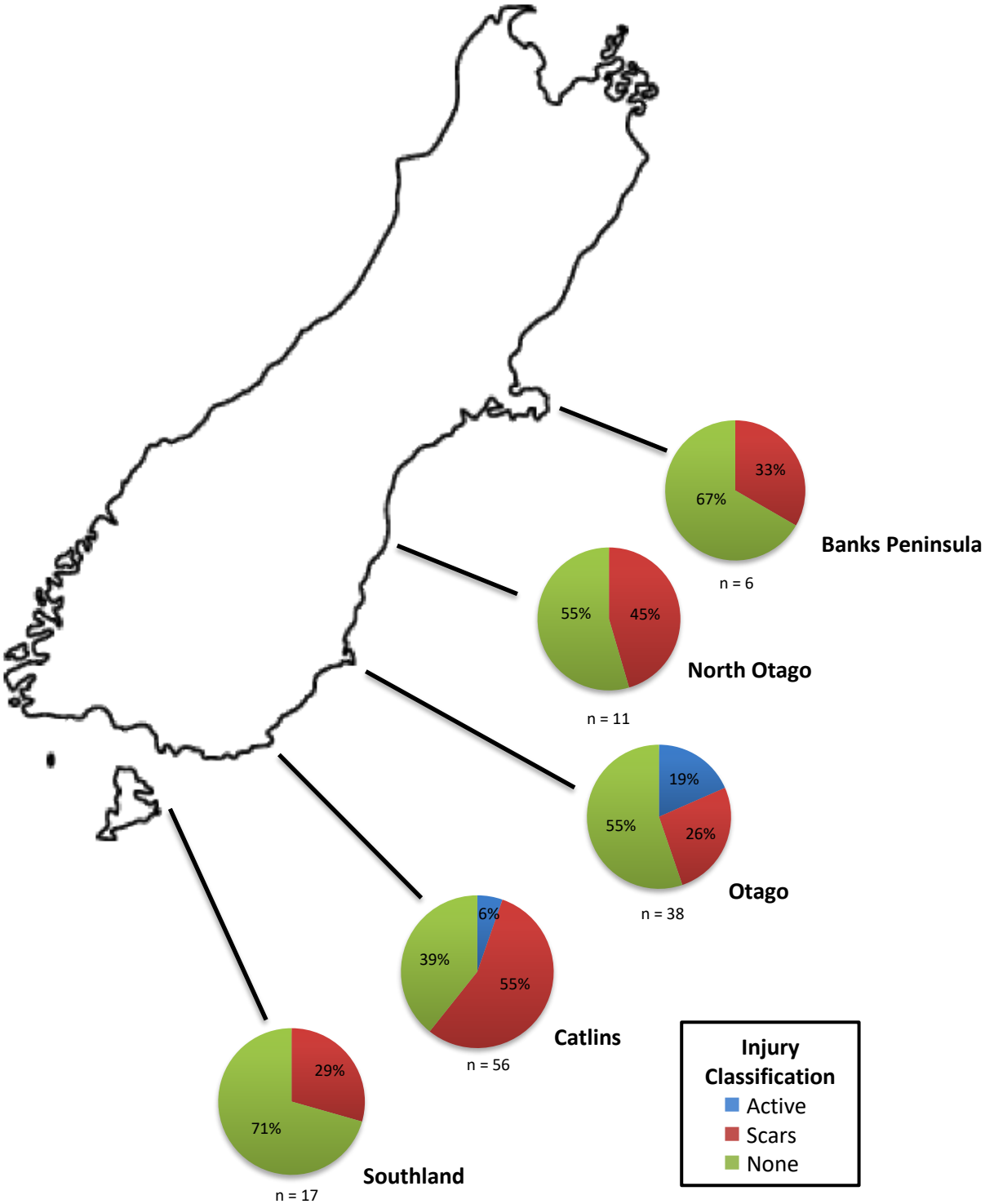


Figure 2.4: Map of the New Zealand South Island showing prevalence of active injuries, scars or no injuries consistent with marine predator attacks in wild yellow-eyed penguins. *Total n = 128.*

Anatomical region injured

All birds with active injuries or scars suggestive of marine predator attack had at least one leg injury, and some additionally had body and flipper injuries (Figure 2.5).

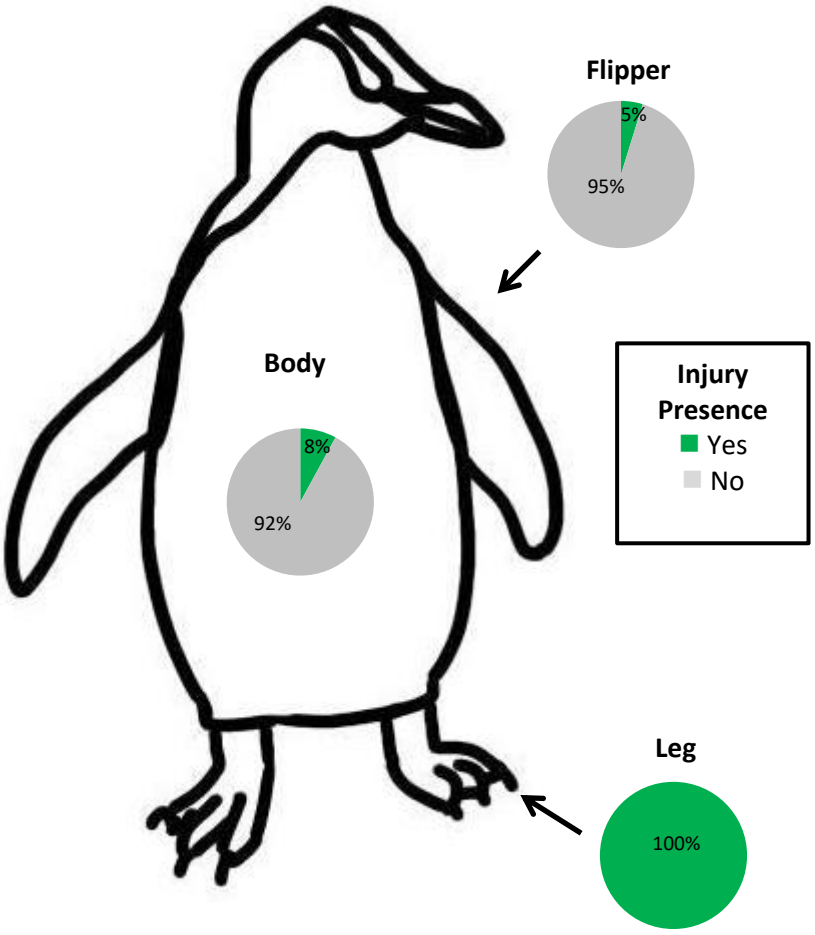


Figure 2.5: Anatomical region affected by marine predator injuries in wild yellow-eyed penguins examined in the field survey.
n = 63. Template by Ji Ye Ahn.

There was very strong evidence to suggest there was a significant difference between the prevalence of injuries at the three anatomical sites on the body (χ^2 = 114.23, df = 2, p <0.001). Pairwise comparisons suggest that leg wounds were significantly more prevalent than flipper or body wounds (Table 2.4).

Table 2.4: Pairwise comparisons between injury prevalence across sites on the body of yellow-eyed penguins.

Comparative Groups	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
Flipper & Body	-0.032	0.101	-0.314	0.754	1.000
Flipper & Leg	0.952	0.101	9.409	<0.001	**<0.001**
Body & Leg	0.921	0.101	9.095	<0.001	**<0.001**

There was no significant difference between the unilateral and bilateral leg injuries in marine predator attacks (p = 0.801).

Vertical distribution of injuries on the distal leg

Penguins displaying injuries to the distal leg had digit injuries 92% of the time (57/62) (Figure 2.5).

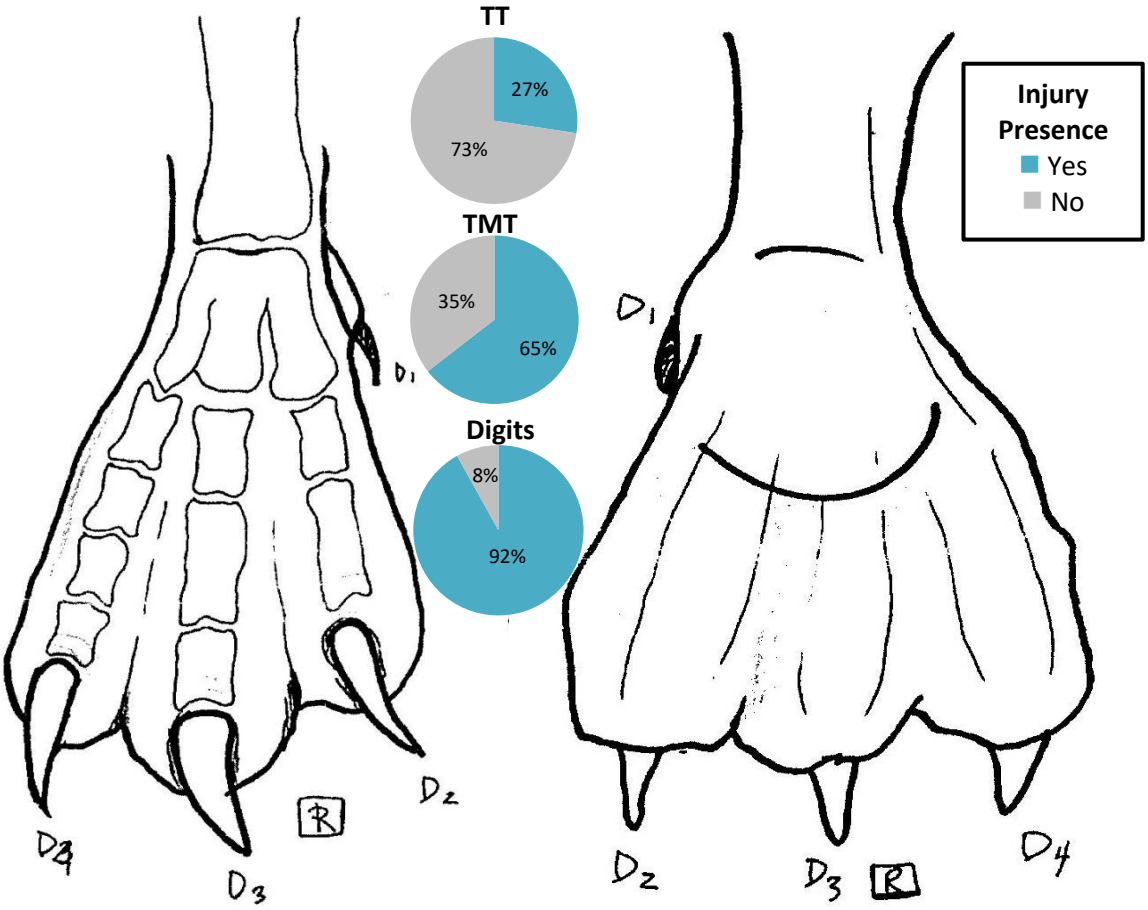


Figure 2.6: Horizontal location of marine predation injuries across the distal leg of yellow-eyed penguins examined in the field survey.
n = 62. Template by Ji Ye Ahn.

There was very strong evidence to suggest there was a difference in injury prevalence with vertical location across the leg ($\chi^2 = 50.375$, df = 2, $p < 0.001$). Pairwise comparisons revealed a significant difference whereby injuries were more common at the distal end of the foot than the proximal end (Table 2.5).

Table 2.5: Pairwise comparison between injury prevalence and vertical location on the distal leg.

Comparative Groups	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
Tibiotarsus and tarsometatarsus	0.371	0.091	4.066	<0.001	**<0.001**
Tibiotarsus and digits	-0.645	0.091	-7.071	<0.001	**<0.001**
Tarsometatarsus and digits	-0.274	0.091	-3.005	0.003	**0.008**

Horizontal distribution of injuries across various aspects of the leg

More than 80% (52/63) of affected birds had injuries to the dorsal aspect of the foot, and nearly two thirds of birds displayed injuries on the lateral side of the foot (Figure 2.7). There was very strong evidence for a significant difference between the prevalence of injury across different directional planes of the leg ($\chi^2 = 76.259$, df = 3, $p < 0.001$).

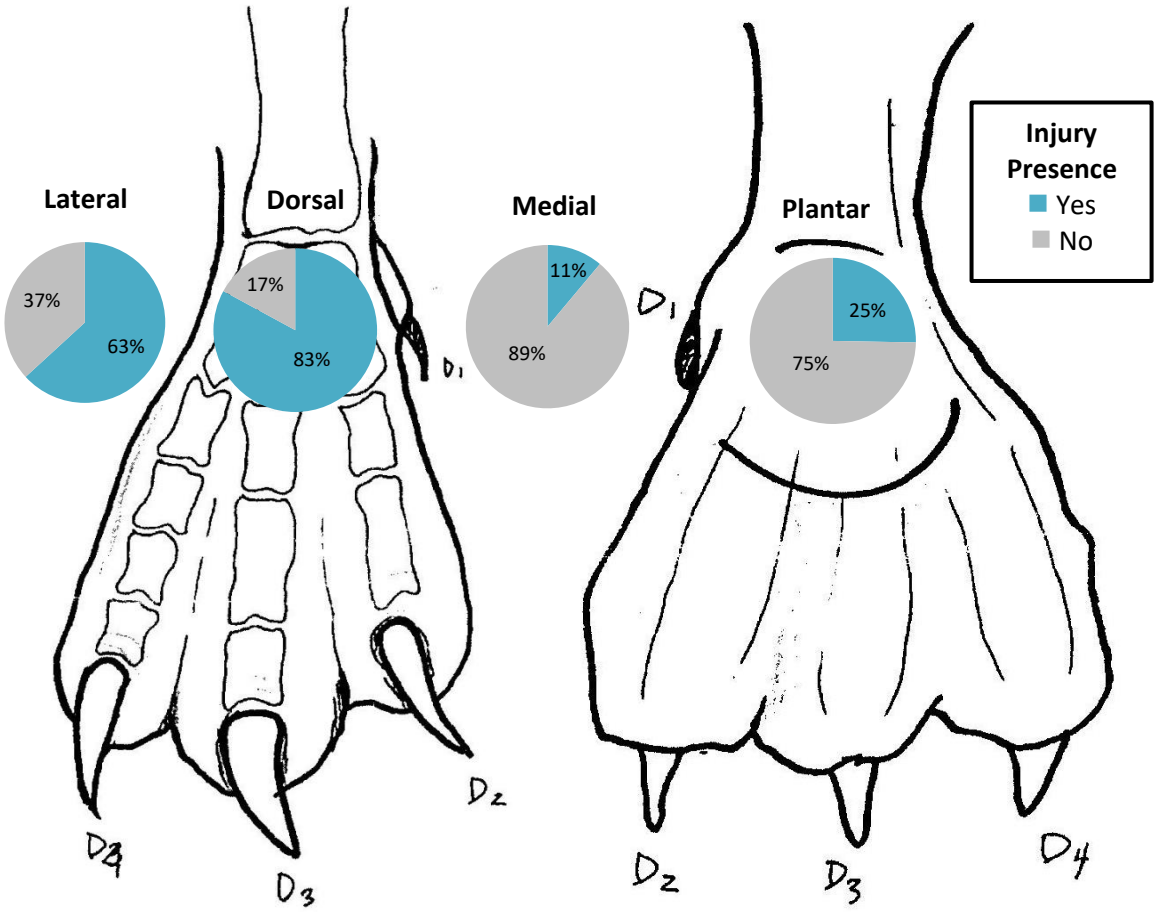


Figure 2.7: Injury presence on different aspects of the foot of yellow-eyed penguins examined in the wild.
n = 63. Template by Ji Ye Ahn.

Pairwise comparison between injury location in horizontal planes (dorsal, plantar, lateral, medial) demonstrated very strong evidence for a higher prevalence of injury on the dorsal and lateral surfaces of the foot, compared to the plantar and medial surfaces (Table 2.6).

Table 2.6: Pairwise comparisons of yellow-eyed penguin injury location across different aspects of the foot.

Comparative Groups	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
Medial & Plantar	0.143	0.093	1.540	0.124	0.742
Medial & Lateral	0.524	0.093	5.646	<0.001	**<0.001**
Medial & Dorsal	0.714	0.093	7.699	<0.001	**<0.001**
Plantar & Lateral	-0.381	0.093	-4.106	<0.001	**<0.001**
Plantar & Dorsal	0.571	0.093	6.159	<0.001	**<0.001**
Lateral & Dorsal	0.190	0.093	2.053	0.040	0.240

Horizontal distribution of injuries over digits

Approximately two thirds of yellow-eyed penguins with foot injuries had an injury involving digit 3 or 4 (Figure 2.8).

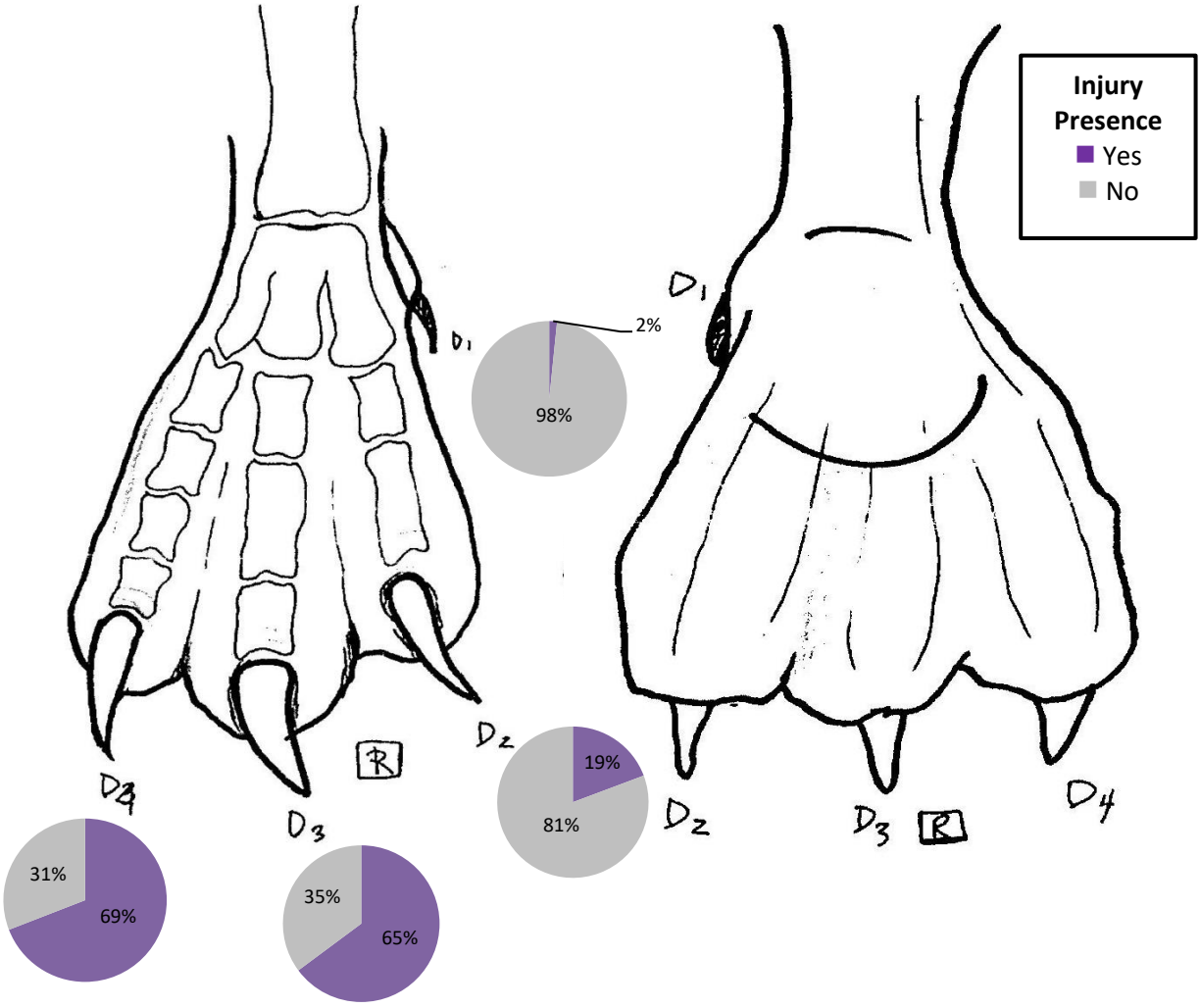


Figure 2.8: Location of marine predator injuries across the digits of yellow-eyed penguins examined in field survey.
n = 57. Template by Ji Ye Ahn.

There was very strong evidence to suggest a difference in injury prevalence across different digits of the foot (χ^2 = 79.79, df = 3, p < 0.001). Pairwise comparisons showed there were significantly more injuries involving digit 3 and 4, than digits 1 and 2 (Table 2.7).

Table 2.7: Pairwise comparisons between injury prevalence across different digits.

Comparative Groups	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
D4 & D3	0.048	0.092	0.528	0.598	1.000
D4 & D2	0.500	0.092	5.452	<0.001	**<0.001**
D4 & D1	0.677	0.092	7.386	<0.001	**<0.001**
D3 & D2	0452	0.092	4.924	<0.001	**<0.001**
D3 & D1	0.629	0.092	6.859	<0.001	**<0.001**
D2 & D1	0.177	0.092	1.934	0.053	0.318

There was no statistically significant relationship ($p > 0.05$) between anatomical location of injury (flipper, body or leg/foot), with age, sex and geographical region. Similarly there was no relationship was observed between horizontal aspect (dorsal, lateral, plantar, medial) or vertical distribution (TT, TMT, digit 1, 2, 3, 4) with age, sex or geographical region.

2.3.3 Investigation of potential marine predators

The tooth extracted from an injured yellow-eyed penguin leg wound measured 8mm long x 2.5mm wide x 2mm breadth, and was slightly convex on one side, and relatively flat on the other side (Figure 2.9). On the flat side, the tooth was broken transversely, half-way along its length. On the convex side it was broken off near the base. There was a small amount of dried, red material present on the flattened side of the tooth across the fracture site, which was at the time presumed to be either tooth pulp or penguin blood. Along the edge of the tooth, spanning approximately half of its circumference distally, there was a thin, smooth, flattened ridge, which lacked serration.

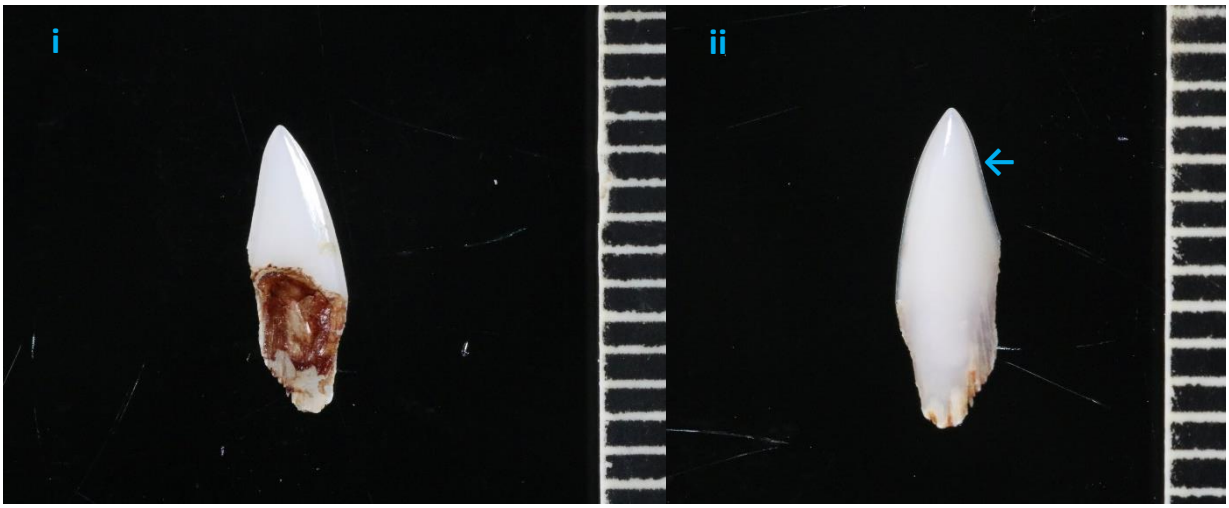


Figure 2.9: Images of the unknown predator tooth extracted from a yellow-eyed penguin foot wound, from the flat side (i) and the slightly convex side (ii).
Arrow shows smooth ridge around distal edge of tooth.

Morphological assessment

Of the 54 New Zealand fish skull specimens examined at Te Papa Tongarewa museum, there were no skulls containing teeth that resembled the tooth located in the penguin leg wound.

Subsequent consultation with expert marine scientists suggested the tooth morphologically resembled teeth from the Lamnidae genus. Its shape is believed to be most consistent with either a shortfin mako shark (*Isurus oxyrinchus*) or porbeagle shark (*Lamna nasus*) (F. Leach, personal communication, 2018; C. Duffy, personal communication, 2019; W. White, personal communication, 2019). As the tooth is missing its base, it is unknown whether it originally had base cusplets, the main distinguishing feature between these two species.

Molecular assessment

Molecular testing was inconclusive for the predator identification. DNA was amplified in all PCR attempts. Sequencing from all sets of primers suggested contamination with the injured host penguin's DNA, as yellow-eyed penguin DNA was amplified instead. A second PCR attempt using Shark-COI-MINIR and Fish F1 as primers (Boussarie et al., 2018) revealed a sequence that matched poorly with other known sequences, with the closest possible match being of a morepork (*Ninox novaeseelandiae*), which is nonsensical.

Measurements of potential predators

Results from the pilot study investigating the tooth measurements of barracouta, gemfish, frostfish and New Zealand sea lions are presented in Table 2.8 and Table 2.9. Images of dentition of these species is shown in Table 2.10.

Table 2.8: Fish teeth measurements and standard measurements for standardisation of specimens.

Palatine tooth spacing was provided when there was >1 tooth present in the same row, otherwise it is listed as NA. When measurements were not taken but teeth were present, this was listed as U (unknown). When more than one tooth was present, either a range was provided (e.g. mandibular or maxillary arcades), or if two teeth were present (e.g. palatine teeth), both measurements were given.

Measurements (mm)	*Barracouta A*	*Barracouta B*	*Barracouta C*	*Barracouta D*	*Gemfish*	*Frostfish*
Standard Length	542	680	668	577	580	1290
Fork Length	552	690	682	582	635	1315
Total Length	630	770	753	650	680	1340
Body Depth	74	93	92.5	75	110	105
Head Length	127	168	163	135	160	198
Horizontal Gape Length	47-50	65-71	60-62	50-55	75	70
Anterior Palatine Tooth Length	5	3	2, 4	5	10	7, U
Anterior Palatine Tooth Spacing	4	4	3	NA	3	0.8
Middle Palatine Tooth Length	U	U	8	8	11	11
Middle Palatine Tooth Spacing	NA	7	NA	NA	6	NA
Posterior Palatine Tooth Length	10	9	4, 10	10	12	8
Posterior Palatine Tooth Spacing	14 (tip), 7 (base)	7 (base)	14 (tip), 7 (base)	NA	8	NA
Mandibular Canine Tooth Length	NA	NA	NA	NA	7	NA
Mandibular Canine Tooth Spacing	NA	NA	NA	NA	10	NA
Maxillary Teeth Length	<1-2	<1-2	<1-3	1-2	1-4	0.5-3
Maxillary Teeth Spacing	2-3	1-2	0.5-3	1-2	0.5-3	0.3-2
Mandibular Teeth Length	<1-3	<1-3	1-4	0.5-3.5	0.5-6	0.5-2
Mandibular Teeth Spacing	3-4	1-4	0.5-4	1-4	1-6	0.5-1

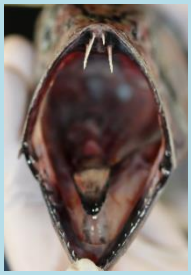
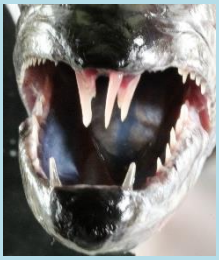
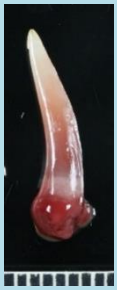
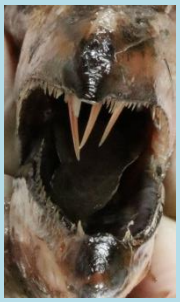
The fork length of barracouta and gemfish suggest these specimens are around the age of sexual maturity (Withell & Wankowski, 1989; Australian Fisheries Management Authority, 2015; Devine et al., 2022). Although the age of sexual maturity of frostfish is unknown, the fish included in our sample represents a specimen that is almost at maximum length for females and beyond maximum length for males, so it was likely that this was an adult fish also (Horn, 2013). Other representative body measurements were also collected for standardising of the results.

New Zealand sea lion skulls examined had a noticeably wider intercanine distance between the maxillary canines, compared to the mandibular canines, both for the proximal and distal intercanine measurements.

Table 2.9: New Zealand sea lion canine teeth measurements.

Measurements (mm)	New Zealand Sea Lion 1 (NZSL 1)	New Zealand Sea Lion 2 (NZSL 2)
Skull Length	285	270
Canine Upper L Length	22.3	22
Canine Upper L Width	16	13.5
Canine Upper R Length	20.5	22.5
Canine Upper R Width	13	13.7
Canine Lower L Length	27.5	27
Canine Lower L Width	15	13
Canine Lower R Length	26	26
Canine Lower R Width	11	12.3
Upper Proximal Intercanine Distance	32.5	33
Upper Distal Intercanine Distance	59	52
Lower Proximal Intercanine Distance	19.5	13.6
Lower Distal Intercanine Distance	48	43.5

Table 2.10: Photographic depiction of dentition of barracouta, gemfish, frostfish and New Zealand sea lion.

Species	Upper Jaw	Lower Jaw	Palatine/Canine Teeth
Barracouta			
Gemfish			
Frostfish			
New Zealand Sea Lion	Maxillary arcade  Mandibular arcade 	Maxillary arcade  Mandibular arcade 	

Bite mark impressions

Barracouta

Figure 2.10 shows the perpendicular bite mark arrangement of barracouta teeth, as depicted by their impressions into modelling clay. Note the deep puncture wounds from the palatine teeth in the maxillary impression.

Figure 2.10: Perpendicular bite impressions from barracouta jaws, showing maxillary (i) and mandibular (ii) arcades.
Rostral end at the top of the image.

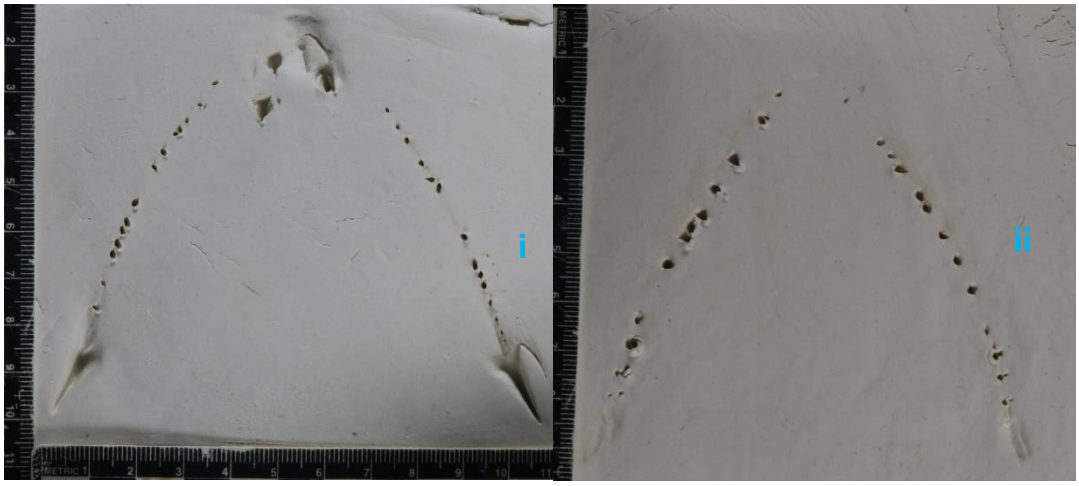
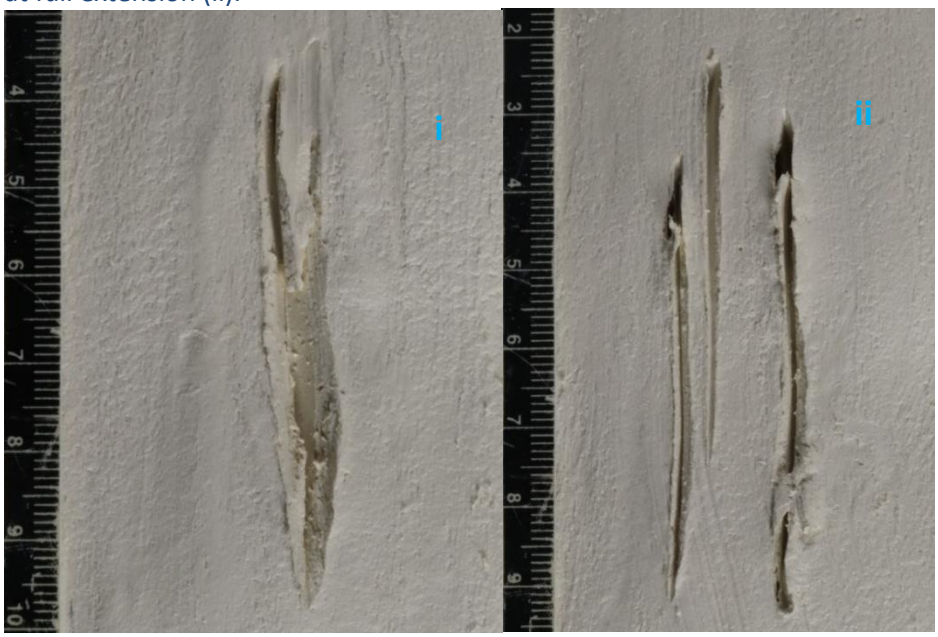


Figure 2.11 shows teeth rake marks from the palatine teeth of barracouta created in modelling clay with the jaw in a resting position (i) and with the mouth fully open in extension (ii). Impressions made from the jaw in a resting position appeared as Y-shaped gouges. Linear marks made during full extension were approximately parallel to one another, 4 and 10mm apart.

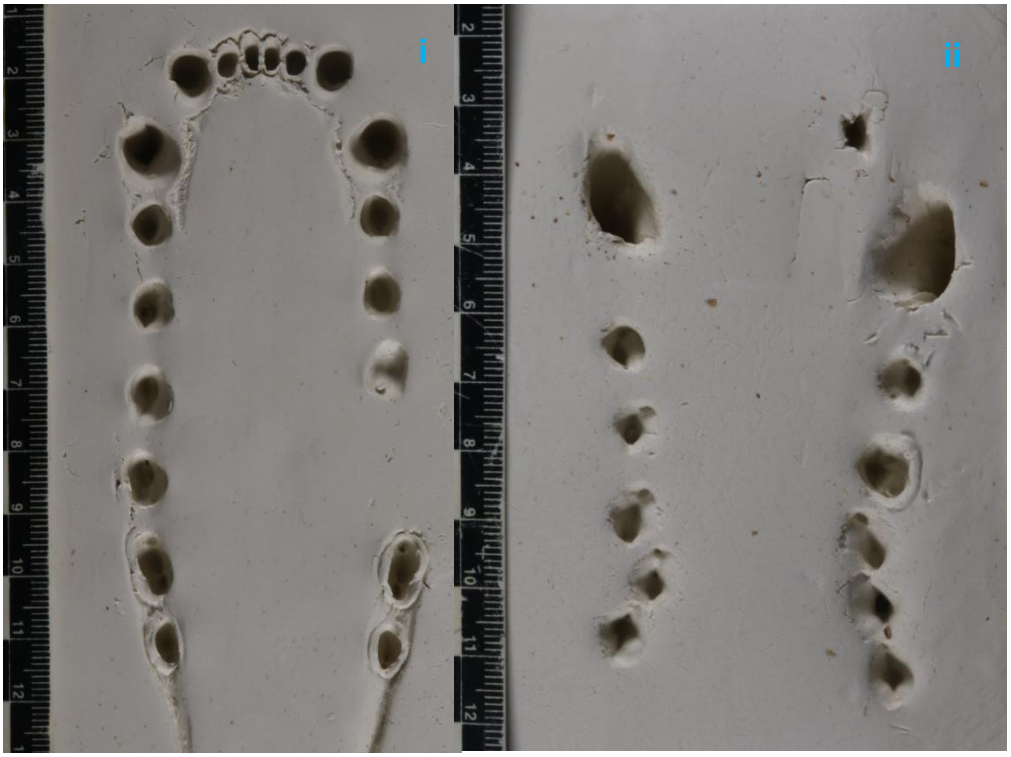
Figure 2.11: Teeth raking from barracouta palatine teeth, with jaw at a resting position (i) and at full extension (ii).



New Zealand Sea Lion

The bite impressions of NZSL 1 are depicted below in Figure 2.12, displaying the maxillary and the mandibular arcades, with perpendicular contact into modelling clay. Several teeth are missing including the left maxillary premolar 4, left and right mandibular incisor 1 and right mandibular incisor 2.

Figure 2.12: Perpendicular bite impressions from New Zealand sea lion maxillary (i) and mandibular (ii) arcades.
Rostral end at the top of the image.

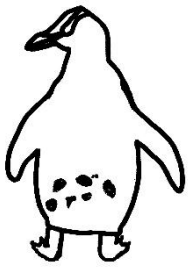
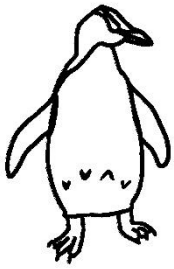
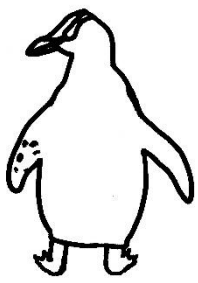
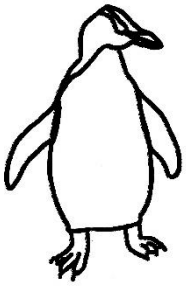


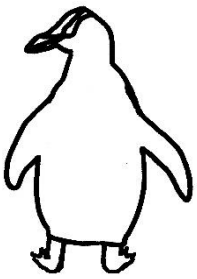
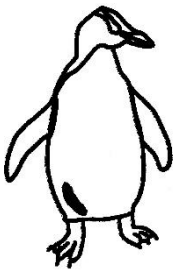
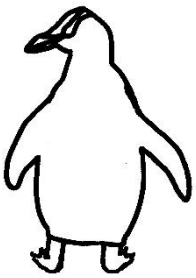
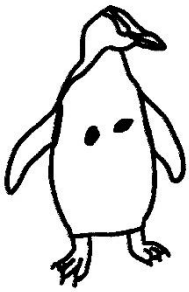
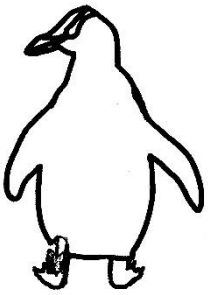
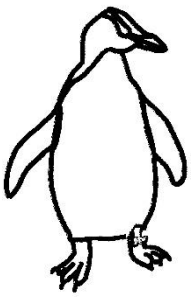
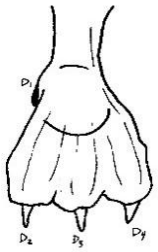
2.3.4 Pathological description of wounds and links to possible predators

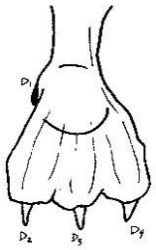
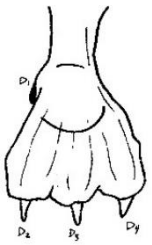
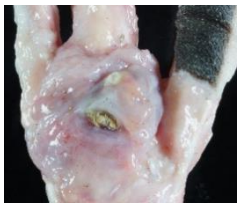
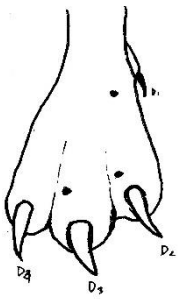
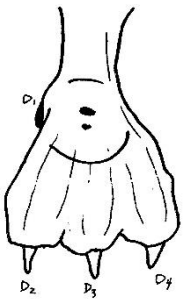
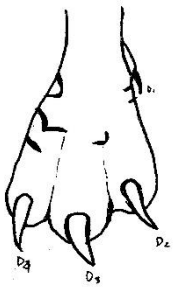
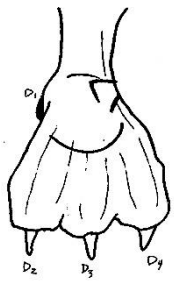
There was a broad range of injury presentations exhibited in necropsied yellow-eyed penguins. There was sufficient information from 48% (73/153) of necropsies to classify individuals into categories based on wound characteristics. These categories were not exclusive, so a total of 83 classifications (N) were assigned to these penguins, as 10 individuals were included in two categories. Table 2.11 depicts the typical injury types exhibited and the prevalence of each category in these birds at necropsy.

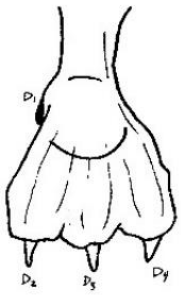
Type A and B wounds included birds with injuries on one side, and some with lesions on the contralateral side. The feathers surrounding these wounds were often cut partway along the rachus. Type C injuries sometimes involved a disarticulation or amputation of a limb. Type D injuries often led to exposure of underlying structures, such as muscle or bone. Type E injuries sometimes involved a fracture or dislocation of a joint.

Table 2.11: Categorisation of various bite wound characteristics made by marine predators, as observed at necropsy.
Template by Ji Ye Ahn.

Injury Type	Description	Photographs	Diagram of Dorsal Surface	Diagram of Ventral/ Plantar Surface	Proportion Observed (%) n/N
A	Deep puncture wounds in a large arc. Wound spacing (of major punctures in the same plane) ≥3cm.	 			2% 2/83
B	Puncture wounds arranged in a small arc formation. Spacing between the punctures <3cm.				4% 3/83

C	Single or several deep, continuous, linear-curvilinear lacerations, ≥3cm in length over the body, flipper or upper leg.				17% 14/83
D	Single or several deep puncture wounds on the body, flippers or upper leg, >0.5-<3cm in length.				17% 14/83
E	Degloving injury.				4% 3/83
F	Single linear or curvilinear laceration of the flipper or distal foot.				6 5/83

G	Multiple lacerations parallel to one another on the flipper or distal foot.				10% 8/83
H	Combination of lacerations which are parallel, perpendicular or diagonal to one another on the flipper or distal foot.				14% 12/83
I	Pinpoint or small oval-shaped puncture wounds, ≤5mm diameter.	 			2% 2/83
J	V, Y or triangular shaped wounds or flaps of tissue on feet.				7% 6/83

K	Loss of large areas of skin over feet, flipper or body, ≥15mm wide at widest point.				17% 14/83

Three categories accounted for more than half of the wound types seen; specifically Type C, D and K. Of individuals who were assigned multiple categories, two penguins each showed both Types H and K injuries, and a further two had Type D and G injuries. Foot injuries were seen in a couple of individuals who also displayed large, deep wounds to the body, including one bird that had both Type A and Type J, and another showing Type D and Type F injuries.

2.4 Discussion

2.4.1 Temporal investigation of marine predation events in yellow-eyed penguins

The increasing frequency of marine predation injuries to yellow-eyed penguins seen in this study is consistent with other reported findings from wildlife rehabilitators. In a non-peer reviewed conference presentation, Lalas et al. (2017) reported the average number of yellow-eyed penguins admitted to a wildlife rehabilitation centre for marine predator injuries increased over the last few decades. With the caveat that Lalas et al.'s study uses very different methods, the results are comparable. On average there were 1.9 yellow-eyed penguins admitted to this rehabilitation facility for foot injuries each year in the 1990s, 3.6/year in the 2000s and 5/year in 2010s (Lalas et al., 2017), suggesting an increase of admissions of more than 2.5 times between the 1990s and 2010s (Lalas et al., 2017). Their study suggests that yellow-eyed penguins are admitted for injuries up to 7 times more frequently in the 2010s, compared to the 1990s. Lalas et al. (2017) also showed that penguin foot wounds have been noted by rehabilitators for as long as rehabilitation efforts began in the 1980s, indicating that this is not a novel threat to yellow-eyed penguins, despite an increasing frequency.

Our findings likely represent an underestimate of the total number of yellow-eyed penguins injured by marine predators annually. This is because many injured penguins may remain undiscovered; as they may have died at sea because of severe injuries, their bodies may have been consumed by marine predators, and some that do return to shore may not be located, as many landing beaches are remote and not constantly monitored. Our data was dependent on the degree of search effort and interventions undertaken (e.g. uplifting injured birds and taking them to one of the aforementioned veterinary clinics for assessment). Due to the brevity of some of the veterinary records, some injured yellow-eyed penguins may not have been counted if the penguin species was not detailed. Rehabilitation information was not included in the total number of cases requiring treatment in our dataset, to avoid duplication due to the lack of recorded information about individual penguin identification from many of the general veterinary practice records. Most penguins receiving treatment from rehabilitators had some veterinary oversight and therefore should be captured in our records.

General veterinary practitioners without substantial training in wildlife medicine were predominantly treating yellow-eyed penguins at local veterinary clinics in the 1990s, 2000s and first half of 2010s. Birds with severe injuries were flown to specialised wildlife veterinary hospitals in the North Island for more intensive treatment during this time. Input from wildlife veterinarians has significantly increased since the mid-2010s, with Massey University veterinarians travelling to Dunedin in 2015 to assess and treat penguins, the establishment of a temporary penguin hospital in Dunedin in the summer of 2016 and 2017, and the opening of the Wildlife Hospital Dunedin in 2018. Since then, the majority of penguins that come into captivity for marine predator wound treatment receive veterinary assessment and treatment prior to rehabilitation.

Necropsy data mirrored a similar trend to that shown by the veterinary hospital data, providing further evidence for an increase in the frequency of attacks over time. This

builds on the findings made by Hunter et al. (2015). On average, more than eight times as many yellow-eyed penguins examined by Wildbase Pathology showed evidence of marine predator attack in the 2010s, compared to the 2000s. Whilst the wildlife pathology service at Massey University has been operational since 1991, a human medical physician based in the South Island was also completing necropsy examinations on yellow-eyed penguins locally during this time. Between November 1996 and July 2003, 114 necropsies were completed on adult or juvenile birds found deceased between the Waitaki River Mouth and the Catlins. In this seven-year period, only three cases were believed to have died due to marine mammal attack, and seven cases due to other suspected marine predators, such as sharks, averaging 1.2 marine predation cases/year (Hocken, 2005). This information may suggest a low frequency of attacks historically, however without a non-veterinary and non-pathological background, it is difficult to be confident in the findings.

Some cases may appear in both the veterinary treatment and necropsy data, for example when an individual received treatment in a veterinary clinic but subsequently died and the bird was necropsied; hence these two datasets are not mutually exclusive and the number of cases each year in Figure 2.1 and Figure 2.2 should not be summated. Veterinary pathologist interpretation, clinical history and characteristics of wounds were used to make a subjective assessment of whether the wound was likely caused by a marine predator. There is some acknowledged difficulty in distinguishing pinniped and dog bite wounds, but the most accurate assessment was made with the best available information at the time to limit incorrect classifications.

It is difficult to determine from our dataset how changes in monitoring or interventions over time have affected these results, compared to larger ecosystem changes. There is substantial evidence that ocean ecosystems are changing around the world. Climate change is causing increases in sea surface temperature, increased ocean acidity and changes in ocean currents, with the most pronounced changes observed in polar regions (Hoegh-Guldberg & Bruno, 2010). More than 30,000 terrestrial and marine species that have been shifting in geographical range as a result of the effects of climate change, with marine species making movements towards the poles six times faster than terrestrial species (Lenoir et al., 2020). Resultantly there are changes in food webs, as the distribution and abundance of prey and predators change (Hoegh-Guldberg & Bruno, 2010), which may lead to increased predation of some species. Similar to findings in our study, the incidence of white shark predation on sea otters in California has more than tripled between 1990 and 2013, despite similar carcass search effort throughout (Tinker et al., 2016), although reasons for this increase are unclear. There is a lack of reliable data to accurately estimate abundance of shark and teleost fish, such as barracouta, in New Zealand waters, with many estimates related to catch per unit effort (CPUE), hence it is difficult to know if there is a true increase in predators in local waters with the information available (Francis & Finucci, 2019; Devine et al., 2022).

An alternative hypothesis is that yellow-eyed penguins are attacked more frequently by marine predators when the penguins' preferred food availability is poor. This may be due to a change of foraging patterns by penguins, or increased time spent at sea, although evidence to support this is lacking as penguins have been shown to follow

consistent paths using seafloor landmarks to direct them (Mattern et al., 2007) and no data has been published demonstrating penguins have been undertaking longer fishing trips. The northern yellow-eyed penguin population is strongly affected by the Southern Oscillation Index, whereby they thrive during periods of El Niño when food resources are more abundant, due to lower sea surface temperatures bringing more nutrient-rich waters from the south. Conversely, in periods of La Niña, food is scarce and adult mortality is higher, and fledging success reduced (Peacock et al., 2000). In our study, although years with high marine predator attack frequency (2015, 2018, 2019) did not correlate to strong La Niña events (Stats NZ, 2020), there was a severe starvation event seen in the northern population of yellow-eyed penguins in the summer of 2018/2019, whereby very few chicks fledged naturally and were uplifted for hand-raising (Webster et al., 2019), as adults were unable to provide adequate quantities of appropriately sized food, due to a prey switch which was observed that year (Mattern et al., 2019). Alternatively marine predators such as sharks might also undertake prey-switch events and predate more on penguins when the abundance of fish is low.

2.4.2 Spatial investigation of marine predation events in yellow-eyed penguins

The field survey to investigate marine predation in the northern population of yellow-eyed penguins showed nearly half had been attacked by a marine predator at some point in their lifetime. Marine predation was evident in all five regions in their northern range. Due to time and resource limitations, no penguins were sampled in the southern (subantarctic) population during this study, although anecdotal reports suggest yellow-eyed penguins in this region are rarely seen with active wounds or scars, resembling those seen in the northern population (C. Muller personal communication 2020; R. French personal communication, 2020; M. Young personal communication, 2023).

The number of active injuries reported in our field survey was low, with only 10 birds with active injuries found, despite 52 yellow-eyed penguins being admitted into veterinary hospitals with injuries during 2018 when the majority of sampling was undertaken. This is likely related to the timing of sampling. Fieldwork for this project began in late February 2018, after many injured penguins had already been admitted into veterinary wildlife hospitals that year. Active injuries were only identified in birds from Otago and the Catlins during field surveys, however this is likely also reflective of timing, which overlapped with the end of the peak attack period in these regions, compared to the other regions which were sampled at different times of the year. In the Catlins, Otago and North Otago, tracking devices were being placed on yellow-eyed penguins concurrently, in conjunction with other research, so these areas were revisited on subsequent nights whilst attempting to recapture the same individual. By comparison, sampling in the Southland region and Banks Peninsula was mainly undertaken during nesting surveys at incubation, in which instance most sites were only visited once, sampling a smaller selection of birds.

There was no strong evidence for a difference in the proportion of active injuries, scars or uninjured penguins across different regions of the northern range of yellow-eyed

penguins. Small sample sizes within some regions may have limited the identification of subtle differences between populations. Despite this, there were 225 pairs noted on the mainland in 2018, so our sampling efforts represented more than one quarter of all adult penguins on the mainland. Our numbers at each site more often reflected the respective population sizes of the regions, with some regions having small or declining populations, such as several sites on the Banks Peninsula where no nests were identified in 2018, despite being used as nesting beaches in previous years.

Leg injuries were significantly more common than body or flipper injuries, based on our field surveys. Penguins are more likely to survive leg injuries, than injuries to the body; which may involve deeper wounds, increased blood loss and damage to coelomic organs; or flipper injuries, which may have a greater effect on the mobility of the bird underwater. The findings in Randall et al. (1988) reflect this, whereby leg wounds were more common in penguins found alive, whereas ventral and head wounds were more common in dead penguins in some locations. Additionally, whilst active wounds were easier to detect anywhere on the body in this study, due to the presence of blood, discharge or temporary loss of feathers around the wound site, scars were more difficult to see, except on unfeathered regions of the legs. Digit injuries were significantly more prevalent than tarsometatarsal or tibiotarsal injuries, which is logical, as the distal part of the foot is more accessible for a predator to get purchase on.

Most injuries affecting the distal legs showed a common pattern of distribution. Injuries were significantly more likely on the dorsal and lateral surfaces of the leg. Digit injuries also reflected this, being much more likely over digits 3 and 4. Concurrent wounds were rarely present on the opposite side of the foot. Randall et al. (1988) also found African penguins injured by sharks which were found alive were more likely to have dorsal or lateral wounds, although this included wounds on all regions of the body, not leg wounds specifically, unlike in our study.

Injury distribution on the foot is dependent on the location of the penguin and attacker in relation to each other, the angle from which the attack proceeds and the region of the mouth contacted. When the penguin is swimming horizontally and in an upright position, the dorsum of the foot is directed towards the seafloor. A predator approaching the penguin in a parallel orientation (upright, horizontal) from any direction (in front, behind or to the side), would be contacting the penguin with its lower jaw, for wounds to occur most frequently on the dorsal aspect of the foot. A predator approaching a penguin on a perpendicular angle (making an approach from above or below) would contact the bird with the upper jaw when approaching the penguin front on, and the lower jaw when approaching from behind. The penguin could theoretically be approached from behind around the side of the foot, with predator's upper jaw contacting the dorsal aspect of the foot, and the lower jaw contacting the lateral aspect of the foot, or alternatively only single jaw contact may be made.

2.4.3 Investigation of potential marine predators

Our study was unable to definitively identify the marine predator species attacking yellow-eyed penguins. Despite the discovery of a tooth in a leg wound, molecular testing was inconclusive, and morphological assessment could not discern between two species; the porbeagle shark and the shortfin mako shark. With the base missing, the most distinguishable feature between the two species, the presence of small base cusplets on porbeagle shark teeth which are not present in mako shark teeth, was absent (Moyer et al., 2015). Each species also has a slightly different inside and outside pitch angle (Frazzetta, 1988), which could not be calculated due to the missing base of the tooth.

The primers used for the various PCR attempts were not specific for shark DNA, and instead isolated DNA from other taxa, including the host penguin's DNA. It is likely the blood present on the tooth was contamination with blood from the host penguin, rather than tooth pulp. Sharks exhibit three main types of teeth, but none of these contain a central pulp, either in their developing stages or as a mature tooth (Jambura et al., 2018; Jambura et al., 2019; Jambura et al., 2020). Whilst bony fish teeth are known to have pulp within a pulp cavity (Berkovitz & Shellis, 2017), this tooth did not resemble a fish tooth morphologically.

Ideally different sections of tooth would have been collected and tested separately, similar to the process described by Presečki et al. (2000), but adapted for shark tooth anatomy; specifically testing of the enameloid and dentine (either osteodentine or orthodentine depending on the species) (Schnetz et al., 2016). Use of a tissue grinder to grind the tooth into a fine powder may also have been beneficial (Presečki et al., 2000). The tooth could have been prepared by cleaning the surface first, such as with bleach, as this has been used to minimise potential contamination from environmental DNA when examining ancient DNA (Kemp & Smith, 2005). It is possible that the tooth DNA was severely degraded by the time DNA extraction was performed; nearly 18 months later. DNA and RNA may have been better preserved by storing the sample at -80°C rather than ambient temperature, or using a preservation agent (Smith & Morin, 2005; Hernandez et al., 2009), particularly important for tooth samples which notoriously provide both low quantity and low quality DNA and RNA (Lee et al., 2013).

The geographical distribution of both shortfin mako sharks and porbeagle sharks extensively overlap with yellow-eyed penguins, fitting the theory that one or both species may injure yellow-eyed penguins. Shortfin mako sharks have a slightly more northern distribution, most commonly found at latitudes of 20-45°S, with some individuals migrating to southern Pacific waters in winter and spring, returning to New Zealand in summer (Francis et al., 2019). They inhabit waters between 14-27°C, although can be found in waters as cold as 9°C. The majority of shortfin mako sharks in New Zealand are juveniles, measuring <100cm in length (Large et al., 2022), with most adults located in waters elsewhere in the world (Francis et al., 2019). Porbeagle sharks are usually found at 39-50°S in summer, 31-41°S in winter, and in between these latitudes at other times of the year. They usually inhabit waters between 7-19°C. Most porbeagle

sharks are resident in New Zealand waters, and are found around much of the coastline of New Zealand (Francis et al., 2015).

Shortfin mako and porbeagle shark distributions also overlap vertically in the water column with yellow-eyed penguin distribution. Porbeagle sharks are usually found in waters between 0-370m deep, (McMillan et al., 2011), although they are capable of deeper dives to greater than 1000m in depth. They do, however spend most of the day in waters below 200m moving vertically throughout the water column to feed, coming up to shallower waters along the continental shelf at night (Francis et al., 2015). Shortfin mako sharks are usually found in waters 0-500m deep, often pelagically over the continental shelf or in the open ocean (McMillan et al., 2011), and have been observed in water as shallow as 20m off Stewart Island (C. Duffy, personal communication, 2023).

Shortfin mako sharks have been reported to consume seabirds (Procellariiformes) as a minor proportion of their diet elsewhere in the world (Lopez et al., 2009), however there are no reports of porbeagle sharks consuming marine birds. The diets of both species of shark predominantly consist of fish and cephalopods, and neither species have been reported to consume penguins to date, based on Southern Hemisphere diet studies (Cliff et al., 1990; Cherel & Duhamel, 2004; Lopez et al., 2009; Horn et al., 2013; Groeneveld et al., 2014; Klarian et al., 2018; Belleggia et al., 2021). In New Zealand waters, approximately 20-40kg juvenile shortfin mako sharks have been observed attempting to eat fluttering shearwaters (*Puffinus gavia*) rafting at the surface (C. Duffy, personal communication, 2023), and therefore it would be unsurprising if they also attacked penguins.

The pilot study detailing tooth measurements of several teleost fish species and New Zealand sea lions provides a rough guide for bite wound depths and interdental distances. With such small sample sizes, it is not intended to provide a reference range for interdental or intercanine distances for various species, as it does not provide an accurate representation of age, sex and individual variation of these species. However, these measurements may still provide useful information when assessing wounds, to ascertain whether certain injury dimensions may be feasible for various species. Bite impressions of barracouta and New Zealand sea lions also provide a guide as to the types of injuries that these predators might inflict on prey species, and this approach has been useful to reveal differences in terrestrial predation injuries on penguins in Africa (Vanstreels et al., 2019a).

2.4.4 Pathological description of wounds and links to possible predators

There are likely multiple species of marine predators attacking yellow-eyed penguins, based on the variable nature of injuries exhibited. Type A wounds were similar to Type I wounds in Randall et al. (1988), except our criteria for minimum wound spacing was 3cm instead of 5cm. These wounds were often attributed to white shark attack in South Africa. Smaller spaced bite wounds in an arc shape (Type B injuries), closely resembled Type III wounds in Randall et al. (1988), suggested to be caused by white sharks, hammerhead sharks, broadnose sevengill sharks (*Notorynchus cepedianus*), and Carcharhinid sharks in South Africa. All of these species also inhabit New Zealand waters and are potential culprits. The feathers adjacent to Type A and B wounds were usually cut part-way along their length, also a feature of shark wounds (Randall et al., 1988).

Type C wounds may be caused by a large marine predator due to many of these wounds being very deep. They may be caused by the slashing action of a shark with serrated teeth, creating long, deep, mostly linear wounds (Randall et al., 1988). Propeller wounds have been implicated in deep curvilinear lacerations in little penguins, with characteristics that closely resemble Type C wounds in this study, although there is not definitive proof that propellers were actually the cause of these injuries (Cannell et al., 2016), and in some marine species such as otters, similar wounds that were once thought to be of propeller origin have now been determined to be caused by sharks (Randall et al., 1988). Propeller injuries from confirmed cases in humans often involve multiple, deep, parallel lacerations through various skin and muscle layers, and sometimes limb fractures or amputations have been observed. Occasionally only a single deep wound will be present from propellor strike (Ihama et al., 2009b). Similar lesions have been seen in marine mammals with suspected propeller injury, although no witnessed events occurred to link the pathological findings in these cases (Beck et al., 1982; Byard et al., 2012; Byard et al., 2013).

Type D wounds are also likely caused by a large marine predator. Randall et al. (1988) showed that large sharks were capable of single or a couple of deep bite wounds on the body of penguins. It is possible that New Zealand sea lions could inflict similar wounds, however this is less likely as New Zealand sea lions have been reported to consume pieces of the carcass in most attacks (Lalas et al., 2007).

Degloving injuries (Type E) have been observed in pinniped attacks on various species of penguins both in New Zealand and overseas (Marks et al., 1997; Du Toit et al., 2004; Lalas et al., 2007). Degloving wounds have occasionally been reported in human shark attack injuries, when victims attempt to withdraw bitten limbs away from the predator, whilst being held in its gape (Woolgar et al., 2001; Khalil, 2021). Dogs occasionally cause degloving injuries to humans (Byard & Langlois, 2020), but their ability to deglove the skin of penguins is unknown. Vanstreels et al (2019a) reported that African penguins which had been suspected to have been killed by dogs, based on characteristic footprints nearby, rarely had full thickness skin punctures, instead severe soft tissue damage. This included subcutaneous and muscular haemorrhage or bruising, and sometimes crushed and haemorrhagic lungs and kidneys, likely due to shaking or stomping on the animal once caught.

The array of foot injuries seen in yellow-eyed penguins is also unlikely to be caused by a single predator species. Some foot wounds were noted concurrently with Type A and D injuries, indicative of a larger predator such as a shark. One penguin had both Type A wounds on the body and Type J wounds on the feet, suggesting that sharks may be able to create V or Y-shaped wounds or triangular flaps in the skin over the feet. Several penguins had Type D injuries along with Type F or Type G, suggesting both deep puncture wounds and superficial foot lacerations are possible from the same predator. Foot and flipper wounds were a common finding in African penguins believed to have been attacked by sharks, some of which had concurrent injuries to the main body (Randall et al., 1988). Type K injuries may be caused by a predator removing a section of skin at the time of attack, or secondary loss of skin to ischaemic necrosis or infection. These injuries were commonly associated with tendon ruptures when they occurred over the intertarsal joint.

Sharks have a range of tooth shapes, and they use these in different ways to perform various actions, including grasping, cutting, tearing or crushing. This invariably affects the different types of injuries afflicted on their prey. The most abundant shark species which are potential penguin predators inhabiting yellow-eyed penguin foraging locations include the broadnose sevengill, white, shortfin mako, porbeagle and blue sharks (*Prionace glauca*) (C. Duffy personal communication, 2023). Sevengill sharks produce distinctive bite marks, due to their thin, smooth maxillary teeth with various cusps and cusplets depending on the tooth, and large comb-like mandibular teeth. A combination of circular holes, often seen in pairs; and long cuts with prominent fluting are created by the jaw of this species (Randall et al., 1988). White sharks have serrated teeth that are narrow at the tip and widen towards the base, which are more effective at slicing and cutting prey (Whitenack, 2008). They have a wide bite area (20-60cm), large teeth and have no overlap of adjacent teeth (Long & Jones, 1996). Shortfin mako shark jaws produce superficial lacerations to the flippers, when dragged through a penguin model, or multiple round irregular holes when sunk in perpendicularly (Randall et al., 1988). Unlike other species of shark, they have more than one functional row of teeth present at a time (Long & Jones, 1996). Porbeagle sharks have similar shaped teeth to shortfin mako sharks, which presumably function in a similar way, but have small base cusplets at the lateral margins, believed to be additional grasping surfaces for prey (Frazzetta, 1988). Blue sharks have overlapping teeth which are finely serrated along the maxilla, and are narrow and smooth along the mandible, creating clean cut and smooth bite wounds and a bite width of less than 30cm (Long & Jones, 1996).

Fish also have a wide variety of prey manipulation strategies, which varying accordingly with the anatomical structures in the mouth. The bite mechanics of barracouta have not been extensively studied. However, the bite mechanics of other fish with elongated palatine teeth have been investigated, including the great barracuda (*Sphyraena barracuda*), which use these teeth to impale or grasp prey prior to cutting it into smaller pieces with the rest of their teeth. It is unknown whether barracouta teeth function similarly, as they do not have as greatly developed maxillary and mandibular arcades of teeth (Grubich et al., 2008). Although our study provides evidence that some penguin foot wounds may be caused by Lamnidae sharks, we are unable to exclude the possibility that barracouta or other teleost fish species are creating some of these wounds also.

Feet are a common site injury site in penguins. They are a less streamlined location on the body, with no feather covering, and relatively thin skin on the dorsal, lateral and medial surfaces, making these areas more vulnerable to injury. Whilst other studies have reported foot injuries in penguins (Randall et al., 1988; Luna-Jorquera & Culik, 1995; McFarlane, 1996; Cannell et al., 2016), none describe these in detail, making comparison difficult. This thesis therefore serves as a starting point from which other observations can be built to determine commonalities and link evidence as more becomes available. It would be unsurprising if there are multiple aetiologies for foot injuries in penguins, both in New Zealand and worldwide.

There were several challenges in assessing wounds retrospectively at necropsy, as the ability to assign wounds to the appropriate category was dependent on the detail of wound descriptions, and representation of wounds by photographs where available. Wounds assessed on different individuals were often at various stages of healing. Although scars weren't assessed, there was still a substantial difference between fresh injuries and wounds that were predominantly healed. Surgical intervention such as debridement and suturing of wounds made it difficult to accurately assess the traits of original wounds, and hence many of these cases were excluded entirely from the dataset. Type C, D and K injuries were most prevalent, suggesting that many wounds were deep, long or had quite substantial tissue damage. It was easier to definitively categorise injuries where only one wound was present (e.g. Type C and F), as detailed information regarding this wound in proximity to other wounds was not required, so it is possible that some of these categories are over-represented proportionally to others.

Wound depth may help to distinguish between potential predators. As most of the wounds from yellow-eyed penguins were assessed retrospectively from necropsy reports and photos, exact depth was rarely known due to the lack of information recorded and inability to accurately ascertain this information from photographs. Most teleost fish teeth in New Zealand, as examined in the Te Papa Museum, are small (measuring only a few millimetres in length), with the exception of a few species with elongated palatine teeth, such as barracouta, gemfish and frostfish, rarely measuring more than 10-12mm in length. Teleost fish can be excluded as the culprits for wounds much deeper than this, which are likely due to a larger marine predator. Depth could only be used to rule out potential predators based on tooth length, as the teeth from larger predators could make shallow wounds depending on how the tooth contacts the animal, if the tooth encounters hard structures, such as bone, and if there is compression of surrounding tissue by the force of the bite.

An extension of the pilot study measuring intercanine distances and tooth length, width and breadth of New Zealand Sea Lions, as well as arc diameter, interdental spacing and tooth measurements of common New Zealand shark species could be useful in the future to more accurately discern between injury types based on pathological characteristics. Further work investigating bite wound impressions of common New Zealand shark species could also be useful.

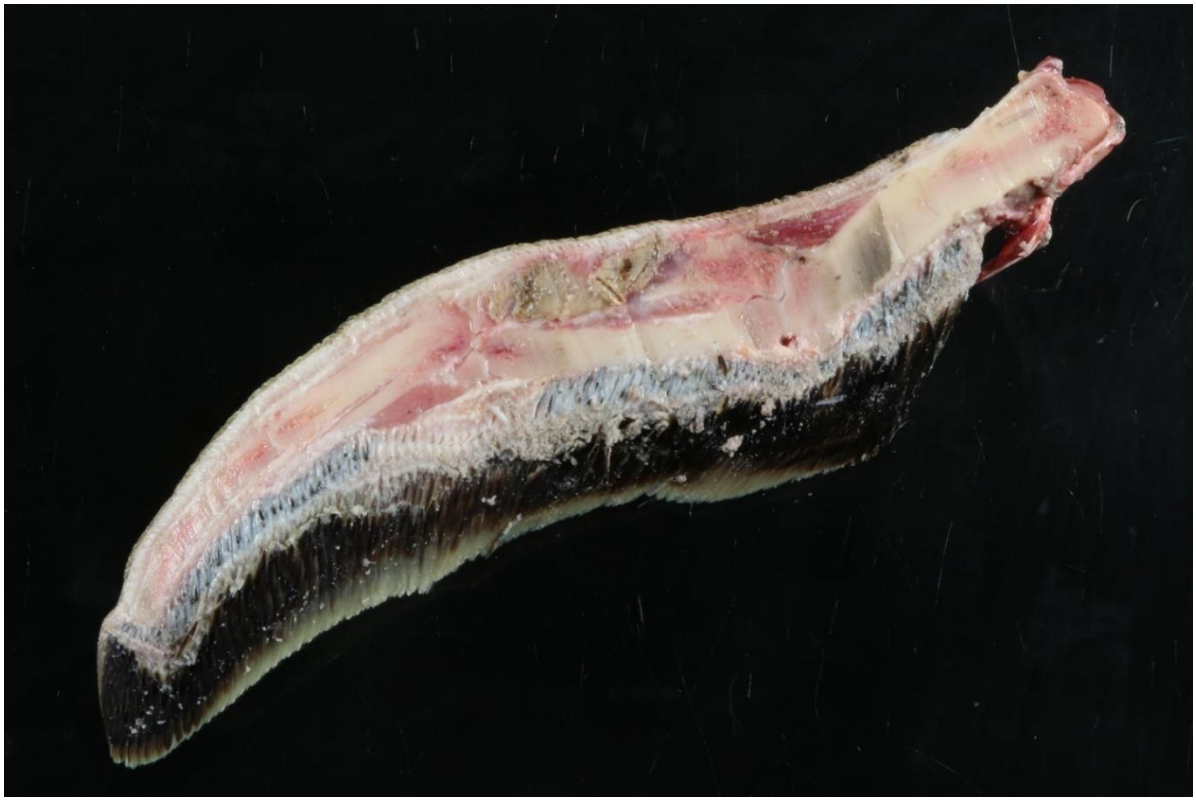
2.5 Conclusion

Findings from this study show that a large proportion of yellow-eyed penguins are impacted by marine predators across their northern range, with a reasonable proportion of the population affected in any given year, and the majority enduring a marine predator attack at some point in their lifetime. The wide array of injuries seen suggests that multiple species of predators may be involved. In order to understand the ecological dynamics between yellow-eyed penguins and their marine predators, and possible changes to the marine environment in recent years, direct or indirect evidence confirming predators is required. Whilst direct observation of predation events may be possible from land for attacks in the nearshore zone, such as the witnessed predatory attacks of New Zealand sea lions, other observations of predation at sea may be more challenging. Rear-facing Go Pro© cameras attached to the dorsum of penguins may provide an indirect way of viewing events in the marine environment, and are already being used (in a forward-facing direction) for penguin foraging and diet studies.

Alternatively molecular testing could be performed on wound swabs to detect remnant DNA from the predator. This could also be helpful to definitively correlate predator species with specific wounding injuries. This work has successfully been undertaken to detect shark species predating on fish during commercial fishery operations, identifying that most fish were attacked by Carcharhinus sharks (Fotedar et al., 2019). These fish were sampled shortly after the attacks, once they were caught and brought onto a boat. By contrast, yellow-eyed penguins are unlikely to be uplifted until hours, days or weeks after an attack, therefore wound swabs may not be as informative if seawater has already washed away most of the predator's genetic material from the wound. Multiple displacement amplification may be useful in this instance to replicate a very small number of copies of DNA, and has been successfully used in to identify the presence marine species in seawater long after the departure of the animal (Truelove et al., 2019), and to screen for pathogens in seawater (Wilson et al., 2017; Gong et al., 2018). Digital droplet PCR is another technique by means of detecting environmental DNA (eDNA) in marine species, previously used to detect the presence of killer whales in the oceans several hours after they have passed through an area (Baker et al., 2018), which could be useful in such investigations as well. Should these techniques be employed to investigate such interactions further, it should be recognised that there is a significant potential for confounding using these methods due to incidental contaminant DNA in the environment, however they may still be useful as a screening tool.

CHAPTER THREE

Clinical, pathological and treatment review of hospitalised cases



3.1 Introduction

The previous chapter presented information on the temporal, geospatial and morphological distribution of marine predator induced injuries on penguins. Veterinary treatment of marine predator wounds in penguins has been largely unstudied around the world. The high number of injured penguins presented to New Zealand wildlife hospitals for medical treatment in the previous decade provides a unique opportunity to understand the clinical and pathological features of predation wounds, examine treatment regimens and correlate this information with prognostic and ecological outcomes.

Additionally, with the large dataset of yellow-eyed penguins necropsies performed by veterinary pathologists, common necropsy features of marine predator attacks can be examined. These include local complications at the site of injury, as well as the occurrence of secondary hospital complications.

This chapter examines the risks and benefits of treating yellow-eyed penguins in a captive hospital or rehabilitation setting, considering overall outcomes, and highlights the risks of captive care by identifying common secondary hospital complications as determined at necropsy. It also outlines the current state of antibiotic resistance in contaminated marine predator wounds, so that this can be monitored on an ongoing basis.

3.2 Materials and Methods

Individual patient records from three wildlife hospitals treating the majority of injured yellow-eyed penguins in New Zealand were included; namely the Wildlife Hospital Dunedin, Wildbase Hospital and The Nest Te Kōhanga at Wellington Zoo. Records from general veterinary practices were insufficiently detailed for inclusion in this section of the study.

Data was captured for all yellow-eyed penguin presented to these hospitals, from 2010-2019, with injuries suggestive of marine predator attack. Inclusion criteria were defined by clinical history or physical examination findings detailing the presence of active wounds. Penguins were also included if they had radiographic evidence of lysis of bone in a limb, without a concurrent active wound, to capture suspected chronic cases where the original skin wound had healed.

Hospitalised yellow-eyed penguins often had multiple identification numbers. On hospital admission, all birds received a unique hospital identification number linked to their patient record. Many birds also were identified with a flipper band or microchip, either in the field as part of the ecological monitoring program, or just prior to release. Birds that were unmarked at admission, and subsequently died or were euthanised in care often did not have a unique identification tag, but their clinical and necropsy records could be linked to their hospital identification number.

Records were retrospectively reviewed to ascertain information. Life history, including age, sex and breeding information, was obtained from admission forms, hospital records or the 'Yellow-eyed Penguin Database'. Admission details noted for each patient included the date the injury was first observed, date uplifted, location uplifted, presence of a flipper band, description of injuries and release date. Post-release outcomes were determined by reviewing all ecological data available for each bird (Yellow-eyed Penguin Database, and Google Spreadsheets of field data). Clinical records detailing physical examination findings and radiographic information were examined, and where possible radiographs were independently reviewed by the author for consistency. Corresponding haematology, biochemistry and culture and sensitivity information was also collected.

3.2.1 Grading of injuries

Grading scales were developed to categorise the nature and severity of injuries. These were a modified pododermatitis scale, osteomyelitis grading scale and chronicity assessment. The proportion of individuals with suspected nerve or tendon damage were also examined independently of these grading scales.

A pododermatitis scale was modified as an overall injury grading tool based on the depth of injury, anatomical structures involved, and pathological features. The scale was developed based on an existing seven-grade pododermatitis scale (Burgmann, 2022) altered to reflect features of marine predation injury. Higher grades were associated with increased injury severity. Categories were Grade 1: partial thickness skin wounds; Grade 2: full thickness skin wounds; Grade 3: necrotic plugs or infection; Grade 4: cellulitis, muscle damage, temporary nerve palsy; Grade 5: tenosynovitis, joint exposure, partial tendon/ligament rupture; Grade 6: full tendon/ligament rupture, joint ankylosis, permanent nerve damage, joint dislocation; Grade 7: osteomyelitis. When multiple wounds of varying severity were present, the injury with the highest grade on the scale was reported.

Injury grade was determined by reviewing all available case information. This included wound descriptions from history and clinical reports, radiographs and necropsy findings and photographs where available. It was assumed that temporary nerve damage was present in birds with an absence of superficial or deep pain responses, or knuckling of a foot that demonstrated clinical resolution or significant improvement within several weeks. Permanent nerve damage was suspected in cases presenting identically at initial assessment, but which failed to improve over time, and when subsequently euthanised and necropsied, had no evidence of tendon damage to the feet. Ruptured nerves or significant neuritis were not easily discernible on gross necropsy examination, so this was a diagnosis of exclusion. Tendon ruptures were determined by descriptions of lacerated tendons visible during physical examination or necropsy. Rupture of a major extensor tendon of the foot usually correlated with knuckling during walking attempts. Partial tendon rupture was determined by direct visualisation, as reported in clinical records or at necropsy.

An osteomyelitis grading system was developed based on a modified Cierny-Mader system (CM osteomyelitis scale), commonly used in human medicine to assess osteomyelitis. The usual grading system involves a numerical scale (1-4) indicating the location and depth of osteomyelitis within bone, and an alphabetical scale (A-C) reflecting the systemic condition of the patient, including other diseases and local factors impeding healing (Calhoun et al., 2009). Due to limited information including intrinsic difficulties assessing the systemic condition of avian patients, only a numerical scale was used in this assessment. The scale was modified to include a fifth category, to better reflect the types of lesions seen, specifically Grade 1: medullary involvement only; Grade 2: cortical involvement only; Grade 3: medullary and cortical involvement not involving the entire diameter of the bone; Grade 4: entire thickness of bone resulting in an infected non-union; Grade 5: osteomyelitis involving a joint. Only confirmed cases of osteomyelitis were included, as determined by radiographic, gross necropsy or histological findings. In some instances, initial radiographs did not show evidence of osteomyelitis but subsequent radiographs several weeks later showed the development of this condition, so in these cases only the latter radiographs were used to assess the extent of the osteomyelitis.

Chronicity was categorised as acute or chronic. Acute wounds were classified as less than two weeks in duration. Wounds were assigned to this category in accordance with the patient history and clinical description (i.e. bleeding when uplifted, wounds in the early stages of wound healing). Alternatively, cases with no radiographic evidence of osteomyelitis despite bone exposure were also regarded as acute, based on the assumption that most cases of osteomyelitis have a radiographic delay of several weeks' duration (Calhoun et al., 2009; Jaramillo, 2011; Pineda et al., 2011). Chronicity was also informed by ecological data and previous history. In one instance, an uninjured bird was sighted at a local nesting site by researchers and seen again five days later with injuries to the feet. Chronic injuries were classified as wounds that were at least two weeks old. Injuries were determined to be chronic if there was radiographic evidence of osteomyelitis or a known reoccurrence of a previous injury.

3.2.2 Haematology and biochemistry

Haematological and biochemical information was examined retrospectively to investigate the systemic health status of patients. Blood results from all yellow-eyed penguins attending veterinary wildlife hospitals for predation injuries were reviewed, including haematology and biochemistry. In some instances, blood samples were collected from the same individual multiple times during care, however only results from the first test collected were included.

Estimated total and differential white cell counts were manually performed from blood smears by veterinarians in-house, or at a commercial veterinary laboratory within New Zealand (New Zealand Veterinary Pathology, Gribbles Veterinary Pathology or IDEXX). Packed cell volume (PCV) was measured manually after centrifugation of whole blood in microhematocrit tubes. Biochemistry profiles were run on whole blood collected in lithium heparin tubes using VetScan® Avian/Reptilian Profile Plus reagent rotors on

Vetscan® II machines, measuring thirteen analytes. These were aspartate transaminase (AST), creatine kinase (CK), bile acids (BA), uric acid (UA), glucose (Glu), total protein (TP), calcium (Ca), phosphorus (P), calcium: phosphorus ratio (Ca:P ratio), sodium (Na), potassium (K), albumin (Alb) and globulin (Glob). Results for albumin and globulin were discounted from the study, due to the unreliability of the bromcresol green dye-binding method used to measure albumin in penguins (Cray et al., 2011) and other birds (Greenacre et al., 2008), and as globulin is calculated from the measured total protein and albumin levels.

Total protein measurements was either measured by refractometer or the biuret method on the Vetscan® profile (Abaxis, 2007). In 11 cases, a full biochemistry was not performed, and total protein was only obtained by refractometer method. To maintain consistency of results, the refractometer measurement was chosen in all cases over the biuret method when there were two different readings available for the same sample.

Biochemistry results were occasionally affected by sample quality or parameter values extending beyond the testing range of the equipment. Samples with mild (1+) or moderate (2+) haemolysis were included in the study. In samples affected by gross haemolysis (3+), an error message was reported for certain analytes including AST and potassium, and hence these analytes were excluded from the dataset, although the rest of the results were still reported (Abaxis, 2013). When analyte values were outside the measurable range, these were reported as the lowest or highest result that the testing equipment could measure. Minimum values for bile acids were 35µmol/L. Maximum measurable values of other parameters included uric acid at 2975µmol/L, glucose at 38.9mmol/L, AST at 2000U/L and CK at 14000 U/L.

Mean and standard deviation was calculated for each haematological and biochemical analyte, and minimum and maximum values were noted. As there are no reference ranges for yellow-eyed penguins, reference ranges for African penguins were used for haematology (Gallo et al., 2019) and biochemistry (Parsons et al., 2015), as they are a closely related penguin species. Clinical pathology data were analysed with survival and chronicity information using general linear models to determine correlations in SPSS version 27 (IBM). Significant results were determined in accordance with views described in Ganesh and Cave (2018).

3.2.3 Culture and sensitivity

Culture and sensitivity results, where available, were reviewed retrospectively for all eligible patients. Samples included wound and joint swabs, as well as tissue and bone biopsies, as decided by the attending clinician. All samples were tested at commercial laboratories within New Zealand (New Zealand Veterinary Pathology, Gribbles Veterinary Pathology or IDEXX). Aerobic cultures were requested in all 18 cases, anaerobic cultures in 15/18 individuals and fungal cultures in 7/18 cases. Due to the low number of fungal cultures, only aerobic and anaerobic culture results were included in the study. Antibiotic sensitivity panels were not routinely performed on anaerobic bacteria, although some results were available for facultative anaerobe species.

Repeat culture and sensitivity testing was undertaken for three patients during their hospitalisation. Only initial culture and sensitivity samples were reported, as they were more likely to contain bacteria inoculated by the predator. They were included regardless of the duration that the patient had been in hospital and despite some birds having prior antibiotic therapy. Culture samples collected from multiple sites on the same patient concurrently and submitted for separate culture, were amalgamated into a single result. Samples collected from other unrelated sites (e.g. endotracheal tube sample) were not included. Culture and sensitivity samples taken at necropsy were also excluded, as they were more likely to be contaminated by post-mortem overgrowth of commensal bacteria.

Antimicrobial sensitivity profiles were reported across a range of antibiotic classes, varying between laboratory protocols and clinician requests. Bacteria were categorised as sensitive, intermediate and resistant. Bacteria were defined as non-susceptible if they displayed full resistance or intermediate/moderate sensitivity, consistent with recommendations by Magiorakos et al. (2011). When a bacterial organism was found to be resistant to one antibiotic in a class, it was inferred that all antibiotics within that class were resistant due to cross-resistance (Weinstein et al., 2018). In the instance where multiple antibiotics within the same class showed different sensitivity profiles, the group was assumed to have a sensitivity level of the antibiotic within that class displaying the most resistance.

Isolates were assessed for multi-drug resistance, defined as non-susceptibility (resistance, moderate sensitivity, intermediate sensitivity) to three or more antibiotic classes to which there is no intrinsic resistance (Schwarz et al., 2010). Intrinsic resistance was considered for bacterial genera, as outlined by Lubbers et al. (2023), and any reported sensitivity results for bacteria with intrinsic resistance for certain antimicrobials were removed. As not all bacteria were identified to species level, intrinsic resistance was considered present in the entire genus regardless of whether it is usually only present in certain species within that genus. Specifically and of relevance to the results, *Enterococcus* is intrinsically resistant to cephalosporins, lincosamides, folate pathway inhibitors and aminoglycosides; *Proteus* is intrinsically resistant to cephalosporins and tetracyclines; *Pseudomonas* to beta-lactams, folate pathway inhibitors and tetracyclines; and *Staphylococcus* to cephalosporins (Lewis II et al., 2020). Information about intrinsic resistance of *Azoarcus, Kocuria* and *Pantoea* have not been published, and so it is assumed there is no intrinsic resistance in these species. Statistical analyses were not performed on culture and sensitivity results due to the low sample size.

3.2.4 Treatment review

Treatment protocols were reviewed for each patient and summarised into four main descriptors. These were the number of antibiotics used, duration of antibiotic treatment, wound management technique and length of time in care. Other medications provided, such as anti-inflammatories, opioid analgesia, fluid therapy,

prophylactic antifungal use and thiamine supplementation were relatively standardised across all three hospitals and so were not independently analysed.

Number of antibiotic classes provided

The total number of antibiotics used in each presentation was examined. Different antibiotics within the same overarching class were regarded as a single antibiotic. This information was reflective of the overall antimicrobial medication provided to an individual during its time in hospital and did not consider whether antibiotics were used in unison or separately. In some cases, antibiotics were prescribed for other conditions (e.g. doxycycline for avian malaria treatment), however this administration may have contributed to the success of wound treatment or the prevention of infection recrudescence, so was included in the total number. Topical antibiotic or antibacterial agents were not included in this tally.

Antibiotic duration

Antibiotic duration was calculated for each patient. It was then categorised into 7 groups, specifically: 1 = ≤7 days, 2 = 8-14 days, 3 = 15-21 days, 4 = 22-28 days, 5 = 29-35 days, 6 = 36-42 days, 7 = >42 days. After the commencement of a single dose of antibiotic the animal was assumed to have had one day of antibiotic therapy, with this number increasing with every 24-hour period, such that no partial days were counted. When antibiotic treatment was provided intermittently, the lengths of the individual antibiotic courses were combined.

Wound management technique

The management of wounds was grouped into three categories: management as an open wound, closed wound, or staged closure. These approaches also involved wound debriding and bandaging, but such information was not discerned from the data.

Open wound management involved healing by secondary intention with no suture placement. Closed wound management employed primary wound closure to fully close wounds in a single surgery during the initial assessment under general anaesthetic. Staged wound management involved a combination of closed and open approaches for the same injury. This included injuries that were only partially closed during surgery, or wounds that were either initially managed as open wounds before being subsequently closed, or wounds that were originally sutured closed to allow for initial healing, before being reopened and debrided.

Inclusion criteria included the presence of one or more wounds, and sufficient information available describing wound management. Patients were excluded if they were euthanised or died before wound could be treated. Occasionally separate wounds on the same patient were managed using different approaches. In these scenarios, the management method of the most severe wound was selected, based on available information.

Length of time in care

Length of time in care was calculated as the total number of days that the individual was held in captivity for each admission. This commenced the day the bird was uplifted and

included both the time in hospital and in rehabilitation. No half days were included, and the bird was assumed to be in hospital one day from the moment it was uplifted, with each new 24-hour period counting as another day.

3.2.5 Outcomes of treatment

Hospital survival and post-release outcomes were determined for yellow-eyed penguins treated at veterinary wildlife hospitals for marine predator injuries. Birds were divided into two categories based on hospital outcome; dead or survived. Birds that died naturally or were euthanised were grouped together as 'dead', whereas birds that were released back to the wild were considered to have 'survived'.

Post-release outcomes were also examined for the subset of patients that survived to release. As yellow-eyed penguins are not permitted to remain in permanent captivity in New Zealand, all surviving birds were released. Outcomes were determined as 'not resighted', 'resighted non-breeding' or 'resighted breeding'. A bird was classified to be 'resighted breeding' if it was found tending to chicks or eggs, regardless of breeding outcome. Post-release outcomes were only assigned to individuals more than one month after release. Two individuals were excluded from the post-release analyses as they did not have unique identification tags on release.

Ecological data from yellow-eyed penguin monitoring surveys or independent research studies was retrospectively reviewed to assess each outcome status. Data was accessed via the Yellow-eyed Penguin Database and through several Google Document Excel spreadsheets containing ecological information that had yet to be inputted into the database. Additionally veterinary clinical records, rehabilitator notes and Massey University's Post-Mortem Database were searched to determine outcomes. Outcome information was obtained until the 31st January 2020.

A post-release outcome was determined for all individuals after each presenting marine predator attack, meaning that for birds with multiple hospital admissions, an outcome was assigned to each new admission. This meant that a single individual may have been resighted after one attack and been rebreeding after another. Adult and juvenile hospital outcome and post-release outcome were examined separately.

Records were collated to investigate injury recrudescence. This included birds presenting with lameness or swelling in the same location on the body as a previous marine predator injury. Sometimes birds were uplifted with new wounds, but also showed active pathology from their previous injuries, so these cases were included as recrudescent based on a subjective assessment of the information. Patient admission for a recrudescent injury was not regarded as a new attack unless the bird had also sustained new injuries from another attack concurrently.

The total number of admissions for separate marine predation attacks for each individual was determined by hospital and rehabilitation data. Radiographs were reviewed alongside the clinical history to determine whether all injuries present were a

due to the current attack, or if a previous attack suspected. For example, radiographs revealing a chronic osteomyelitis injury to the foot in a different region to active wounds suggested the bird was likely injured in a previous attack.

3.2.6 Prognostic factors

Statistical analyses were performed comparing clinical presentations and treatments with outcomes, to determine useful prognostic indicators and examine the response to treatment. Outcomes of interest included hospital outcome (dead, released), post-release outcome (released, resighted non-breeding, resighted breeding), and recrudescence of the original injury. Risk factors for penguins with repeat predator attacks were not assessed due to a low sample size.

Chi squared (χ^2) tests were performed in SPSS version 27 (IBM) to determine associations between presentation and treatment factors with hospital survival, post-release survival and injury recrudescence Presentation factors assessed included age class (adult, juvenile), anatomical region injured (flipper, leg, body) and grading of injury (modified pododermatitis scale, modified Cierny-Mader osteomyelitis scale, and chronicity assessment). In addition, the presence of osteomyelitis, tendon/ligament damage and nerve damage were examined separately for effect on outcome. Various treatment regimens examined included wound management approach (open, closed, staged closure), number of antibiotics classes (1, 2, >2) and duration of antibiotic therapy (≤7, 8-14, 15-21, 22-28, 29-35, 36-42 and >42 days). Likelihood ratios (λ) were used when the sample was not suitable for chi-squared analysis.

Post-hoc analysis with Bonferroni correction was performed analysing hospital survival data for all significant presenting and treatment factors that did not correspond to a 2x2 table, including modified pododermatitis scale, wound management approach, number of antibiotics and duration of antibiotics. Similarly, a *post-hoc* analysis of post-release outcomes was performed for the modified pododermatitis scale only.

Variables affecting hospital survival were analysed using a backward stepwise (Wald) binomial regression. These factors included site of injury, chronicity and the presence of complications such as osteomyelitis, tendon/ligament damage and nerve damage. The Cierny-Mader osteomyelitis scale and modified pododermatitis scale were not successfully included in the analysis. Non-parametric modelling was performed to examine correlations between length of time in care with hospital survival (Mann-Whitney U-test), while parametric analysis was used to assess time in care with post-release outcome (one-way ANOVA).

3.2.7 Necropsy review

The same criteria, as described in Section 2.2.1 of this report, was used to select necropsies of yellow-eyed penguins attacked by marine predators. Information from the necropsy reports was used to identify secondary complications from the initial wounding event, as well as secondary complications of captive care, where applicable.

Wild submissions were distinguished from captive submissions. For the purposes of this report, 'captive' submissions referred to any yellow-eyed penguin that presented to a wildlife veterinary hospital, general veterinary practices or rehabilitation centre. Due to the movement of penguins between wild and captive populations for treatment, certain criteria were established to assign birds into these categories. Birds found dead in the wild or which were euthanised or died on admission at veterinary hospitals, without diagnostic investigation or attempted treatment, were classified as wild submissions. Birds that were brought into captivity, and for which stabilisation or ancillary testing had been performed (e.g. bloods, radiographs) were regarded as captive submissions, regardless of time in care. Similarly, any individual found dead in the wild after recent hospitalisation (i.e. within a month of release from captivity) was considered part of the captive cohort to capture possible complications from captivity.

History, clinical information, gross pathological assessment, histopathological information, photographs and ancillary testing (e.g. culture and sensitivity, PCR testing of specific pathogens) were used to inform the severity of injury and presence of other diseases in patients. Data was reviewed to determine complications from the initial injury, and secondary complications from captivity, where relevant. Complications from the original injury included tenosynovitis, tendon/ligament damage (including partial and full rupture), nerve damage (suspected and confirmed), fractures and joint dislocations. Tenosynovitis was determined grossly by erythema, swelling or caseous material within tendon sheaths, or histologically by the presence of inflammatory cells with or without infectious organisms within these structures. Legs were dissected to examine tendon and nerve structures when clinical history indicated the bird was dragging its leg pre-mortem, or a loss of sensation had been noted, as previously outlined in Section 3.2.1.

The captive subset of birds was further examined for pathological findings which may have been attributed to the captive environment. Complications deemed related to captivity included mycotic respiratory disease, bacterial respiratory disease, tracheal stenosis, death under general anaesthesia, gastrointestinal ulceration or perforation, bacterial gastroenteritis, avian malaria (presumptive diagnosis from gross and histopathological findings or confirmed diagnosis by PCR), gout, pododermatitis, hepatic lipidosis, heat stroke and salt toxicity.

3.3 Results

A total of 178 wildlife veterinary hospital admissions of yellow-eyed penguins injured by marine predators occurred between 2010-2019. This comprised of 163 different individuals, specifically 81% adults and 19% juveniles. Of these, 57% were male, 35% female and 8% unknown sex.

A consistent seasonal and temporal trend was highlighted by the veterinary hospital admission data. Over one third of cases (34%) were admitted in February, and 85% cases between January and April (Figure 3.1).

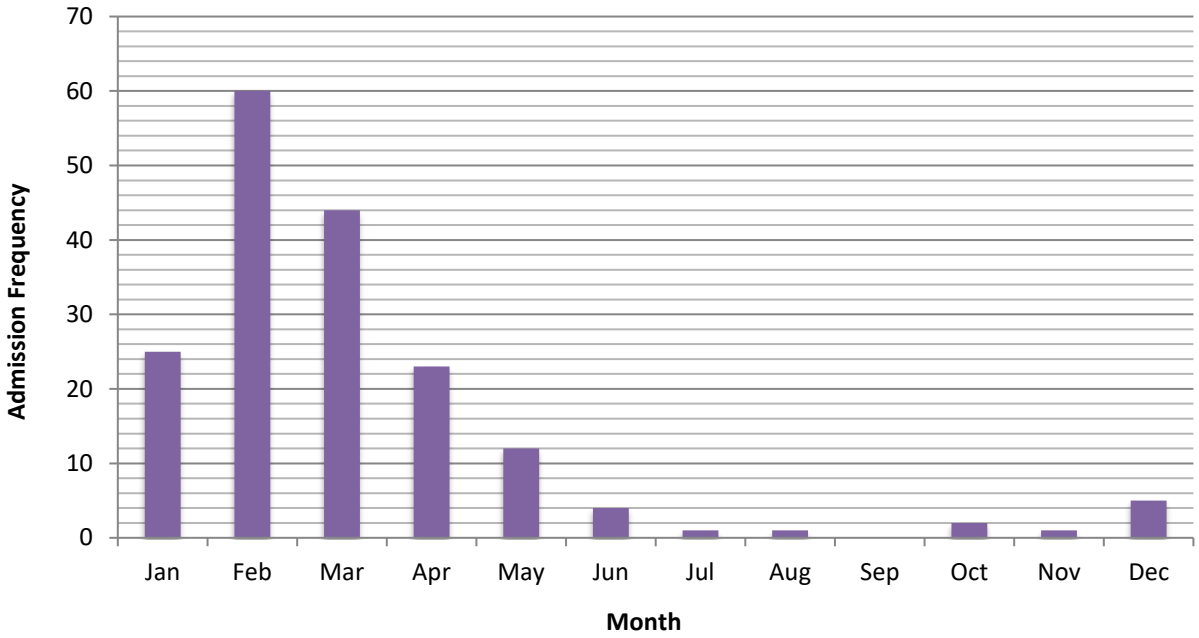


Figure 3.1: Monthly admissions of yellow-eyed penguins with marine predator injuries into veterinary wildlife hospitals in Aotearoa New Zealand between 2010-2019.

As previously reported in Chapter 2, the majority (57%) of admissions to veterinary wildlife hospitals occurred in 2018 and 2019, coinciding with the opening of a local wildlife veterinary hospital in Dunedin.

The majority of birds were admitted from the Otago (49%) and Catlins (41%) regions, reflective of the locations with the largest population sizes (Figure 3.2).

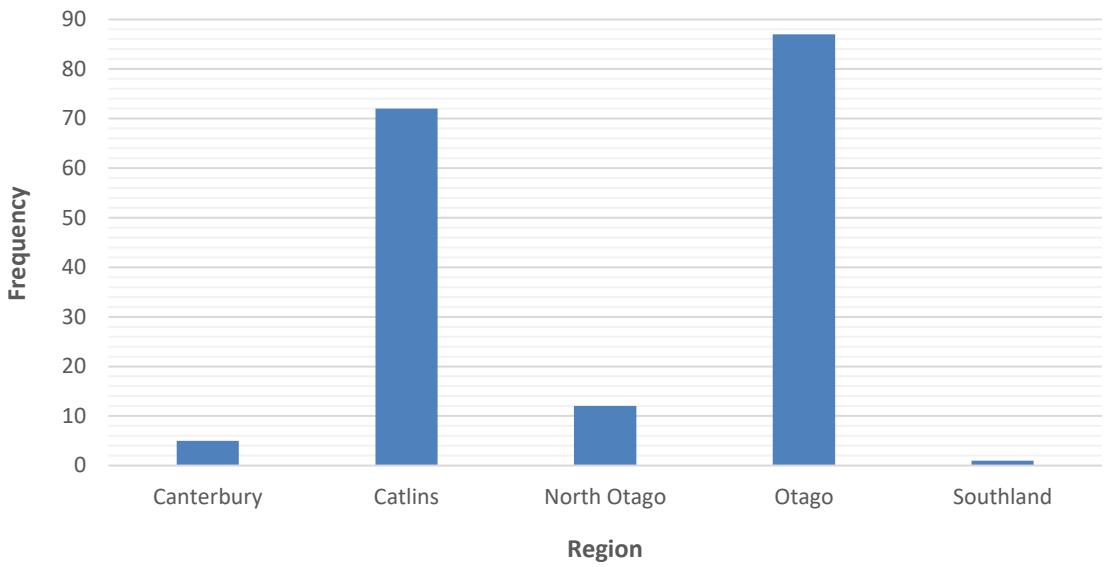


Figure 3.2: Region of origin for yellow-eyed penguins admitted to wildlife hospitals for marine predator injuries.

Nearly 85% (151/178) of wounded penguins hospitalised had injuries on the hindlimb (Figure 3.3). A higher proportion of body and flipper injuries were seen in the hospitalised patients, compared to the field surveys of injured birds (Figure 2.5).

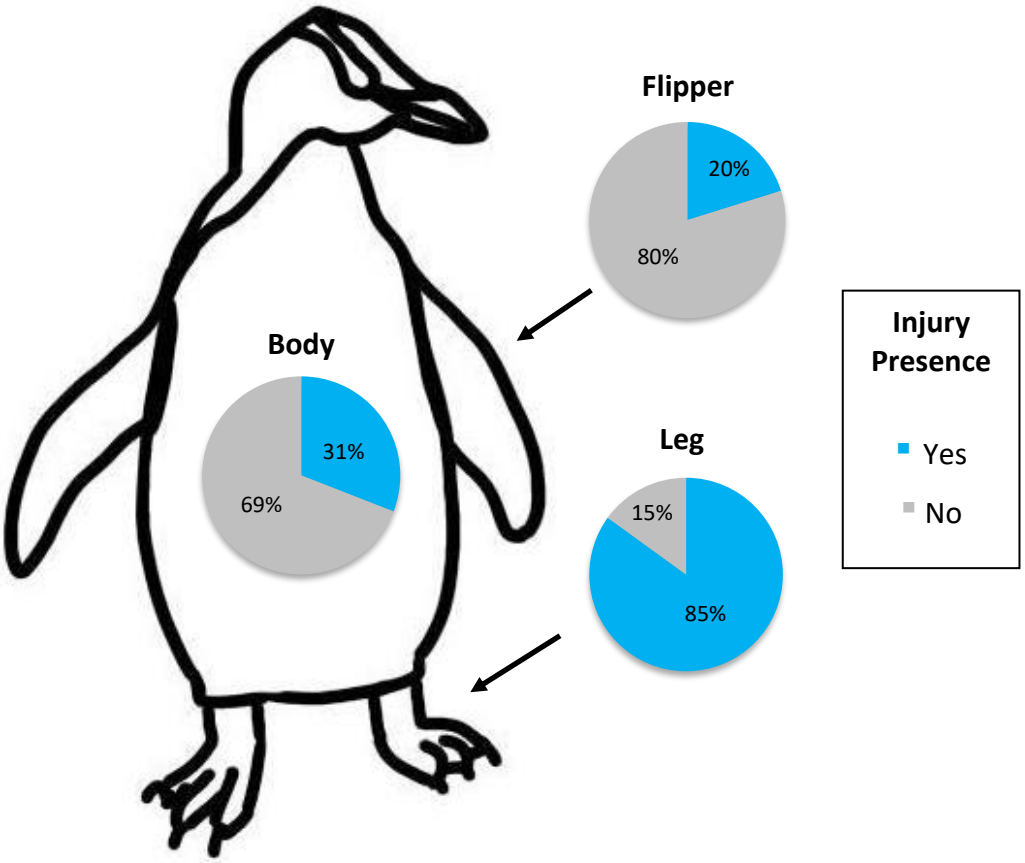


Figure 3.3: Marine predator injury anatomical location on yellow-eyed penguins treated at veterinary wildlife hospitals.
n = 178. Template by Ji Ye Ahn.

3.3.1 Grading of injuries

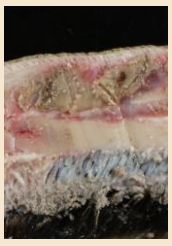
Multiple grading scales were applied to yellow-eyed penguin marine predator injuries. All hospital admissions were assessed by the modified pododermatitis scale and chronicity, whereas only penguins with osteomyelitis were eligible for assessment using the modified Cierny-Mader osteomyelitis scale.

Modified pododermatitis scale

Injuries grades are shown in Table 3.1. Grade 1-2 were regarded as mild, Grade 3-5 as moderate and Grade 6-7 as severe injuries. Moderate-severe injuries constituted 84% (149/178) of hospital admissions.

When examined independently, 15% (26/172) of birds had some degree of tendon damage and 9% (16/171) had temporary or permanent nerve damage.

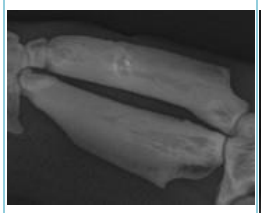
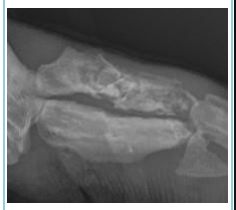
Table 3.1: Assessment of marine predation injuries to yellow-eyed penguins using the modified pododermatitis scale.

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6	Grade 7
Partial thickness wounds	Full thickness wounds	Necrotic plugs or infection	Cellulitis, muscle damage, temporary nerve palsy	Tenosynovitis, joint exposure, partial tendon/ ligament rupture	Full tendon/ ligament rupture, joint ankylosis, permanent nerve damage, joint dislocation	Osteomyelitis
						
1/178	28/178	3/178	48/178	13/178	16/178	69/178
0.6%	15.7%	1.7%	27%	7%	9%	39%

Modified Cierny-Mader osteomyelitis scale

Osteomyelitis was a common feature of marine predation injuries, occurring in 71/178 (40%) cases. Osteomyelitis was located at a joint (Grade 5) in more than three quarters of affected individuals (Table 3.2). Osteomyelitis was most prevalent in the distal joints (e.g. phalangeal or intertarsal joints), reflective of wound location on the distal leg. Concurrent marked soft tissue swelling or adjacent periosteal reaction was frequently evident radiographically. Lesions were rarely confined to the cortex (Grade 2) or medulla (Grade 1) only.

Table 3.2: Assessment of yellow-eyed penguin marine predator wounds using the modified Cierny-Mader osteomyelitis scale.

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Medulla only	Cortex only	Cortex and medulla, not entire diameter	Cortex and medulla, non-union fracture	At joint
				
1/71	7/71	5/71	4/71	54/71
1%	10%	7%	6%	77%

Chronicity

Acute injuries comprised 58% (103/178) of presentations with the remaining 42% (75/178) being characterised as chronic.

3.3.2 Haematology and biochemistry

Blood results were available for 84 patients, with full haematology and biochemistry panels accessible for 23 and 34 patients respectively. Haematological and biochemical parameters of yellow-eyed penguins wounded by marine predators showed some variation from expected values (Table 3.3). A wide range of PCVs were observed, extending beyond the minimum and maximum expected range for penguins. On average, yellow-eyed penguins with marine predator injuries were mildly anaemic on admission. Several animals were polycythaemic, however the total protein for all affected animals was within the normal range. In terms of leukocyte profiles, although normal penguins usually have a predominantly lymphocytic blood profile, injured hospitalised penguins were more heterophilic. Monocyte, basophil and eosinophil levels were similar to normal reference range values.

Table 3.3: Haematological parameters of injured yellow-eyed penguins at initial blood sample collection.
(*) Comparative reference ranges with African Penguins (Spheniscus demersus) (Gallo et al., 2019). Values lower than the reference ranges are marked blue and values higher than the reference ranges are marked red.

Parameter	Mean	Standard Deviation	Minimum and maximum range	n	Comparative Normal Penguin Range (*) mean (min-max range)
PCV	**37.7**	12.5	**12**-**64**	75	47.64 (39-56)
Estimated WCC	27.5	16.9	**7**-87	26	25.12 (7.7-122.2)
Heterophils (x10^9/L)	21.3	15.9	3.4-**79.2**	26	11.31 (2.69-43.99)
Heterophils (%)	74.2	15.2	43-**94**	26	46.91 (13-88)
Lymphocytes (x10^9/L)	5.0	4.5	**0**-16.6	26	10.96 (1.17-72.10)
Lymphocytes (%)	21.1	15.6	**0**-54	26	39.56 (9.00-82.00)
Monocytes (x10^9/L)	0.8	0.9	0-2.9	26	0.98 (0.00-7.39)
Monocytes (%)	3.2	3.1	0-10	26	2.58 (0.00-16.00)
Basophils (x10^9/L)	0.3	0.7	0-**3.1**	26	0.03 (0.00-0.82)
Basophils (%)	0.7	1.7	0-**8**	26	0.17 (0.00-4.00)
Eosinophils (x10^9/L)	0.2	0.3	0-1.2	26	1.84 (0.00-9.94)
Eosinophils (%)	0.8	1.4	0-5	26	10.78 (0.00-41.75)

Biochemistry results are reported in Table 3.4. Values for AST and CK varied greatly from the reference range, indicating significant muscle trauma. Uric acid levels also differed considerably, with several birds recording values beyond the maximum measurable range of the test. Phosphorus levels also ranged widely, with the highest values seen in birds with concurrently high uric acid, suggestive of end-stage renal failure. A wide range of total protein values were also seen. Results for bile acids were omitted from this table, as they were all less than or equal to 35µmol/L.

Table 3.4: Biochemistry results at initial presentation.
(**) Comparative reference ranges with African Penguins (Spheniscus demersus) (Parsons et al., 2015). Values lower than expected reference ranges marked blue and values higher than expected reference ranges marked red.

Parameter	Mean	Standard Deviation	Minimum and maximum range	n	Comparative Normal Penguin Range (**) mean (min-max range)
AST (U/L)	407.5	360.6	121-**2000**	36	218 (90-627)
CK (U/L)	1065.0	2240.5	80-**14000**	37	419 (77-1554)
UA (µmol/L)	481.4	529.5	**68**-**2975**	38	394 (99-1363)
Glucose (mmol/L)	14.1	4.6	8.2-**38.9**	38	11.8 (8.2-19.5)
Calcium (mmol/L)	2.6	0.2	2.13-2.99	38	2.77 (1.96-5.91)
Phosphorus (mmol/L)	1.3	0.7	**0.31**-**4.24**	38	1.53 (0.6-3.8)
Ca:P ratio	2.7	1.4	0.57-7.71	38	
Total Protein (g/L)	55.5	11.4	**21**-74	49	59 (34-83)
Potassium (mmol/L)	4.6	1.5	2.6-11.8	36	5.09 (1.6-19.22)
Sodium (mmol/L)	148.7	4.4	142-168	38	154 (140-168)

Complete individual haematology and biochemistry results from initial samples of wounded yellow-eyed penguins are available in Appendix 1 and Appendix 2.

Haematological and biochemical analytes were not strongly associated with hospital survival or with chronicity. There was only weak evidence for a trend that eosinophilia was associated with survival ($F = 3.838$, $df = 1$, $p = 0.063$), and insufficient evidence for any of the other haematological parameters ($p > 0.1$).

Of the biochemical parameters tested, there was moderate evidence to suggest hyperuricaemia was associated with mortality ($F = 5.031$, $df = 1$, $p = 0.032$) and weak evidence that hyperkalaemia was associated with mortality ($F = 3.124$, $df = 1$, $p = 0.087$). All other parameters showed insufficient evidence for any association ($p > 0.1$). There was weak evidence for hypernatraemia to be associated with more chronic cases ($F = 3.806$, $df = 1$, $p = 0.060$) but insufficient evidence for other parameters ($p > 0.1$).

3.3.3 Culture and sensitivity

Bacterial culture testing was undertaken for 18 yellow-eyed penguins with marine predator wounds. Of these, 14 samples had antibiotic sensitivity testing undertaken, growing at least one aerobic organism. A total of 14 bacterial genera were cultured from the predation wounds of yellow-eyed penguins (Figure 3.4). The most commonly isolated genera included *Staphylococcus* (33%; 6/18), *Clostridium* (33%; 6/18), *Escherichia* (33%; 6/18). Other frequent isolates were *Enterococcus* (22%; 4/18) and *Pseudomonas* (17%; 3/18).

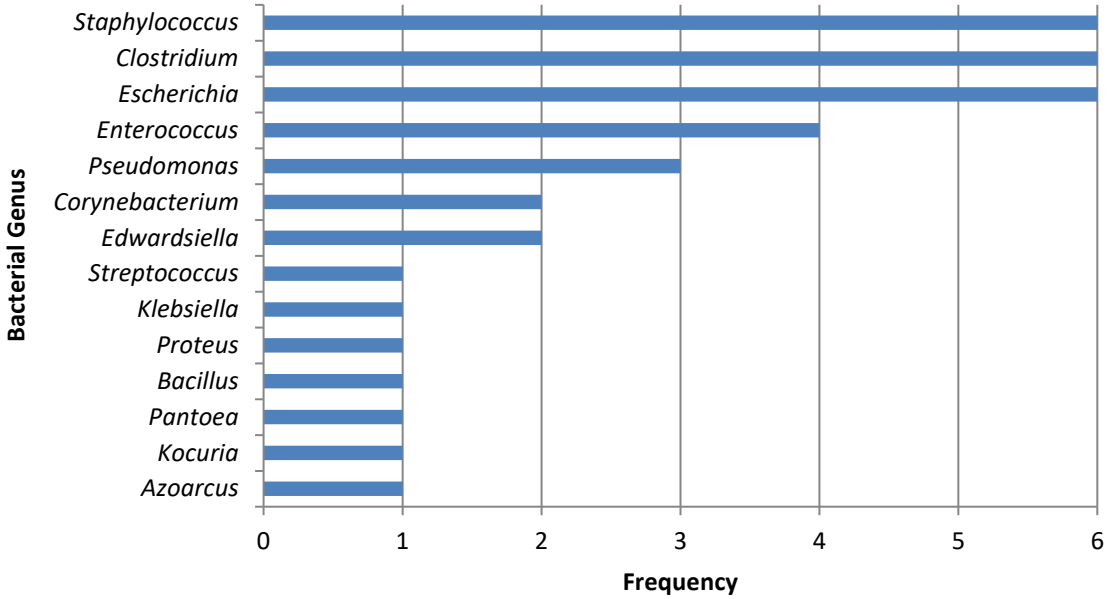


Figure 3.4: Frequency of bacterial organisms cultured from marine predator wounds of yellow-eyed penguins.

The majority of samples (61%) grew multiple organisms, with one wound containing five bacterial genera (Figure 3.5). In three samples, no bacterial growth was detected.

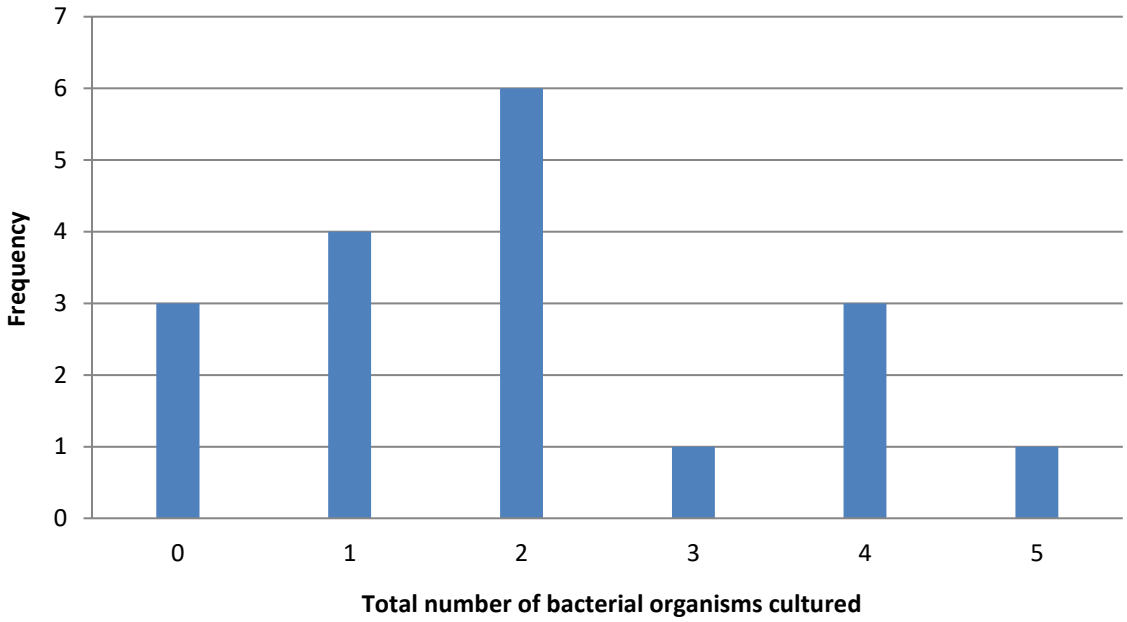


Figure 3.5: Number of bacterial isolates cultured from individual yellow-eyed penguins with marine predator wounds.

Antibiotic sensitivities were performed on a total of 28/36 (78%) of bacterial organisms grown. Antibiotic resistance was extremely common, with acquired resistance observed to at least one antibiotic class in 22/28 (79%) of isolates (Table 3.5). Multi-drug resistance was also highly prevalent. It was observed in 12/28 (43%) of isolates cultured. Full bacterial culture and sensitivity results for the initial samples tested are available in Appendix 3.

Table 3.5: Bacterial isolates and antibiotic sensitivity profiles from first culture samples, and presence of multi-drug resistance (MDR).
BL (β-lactams), C (cephalosporins), FQ (fluoroquinolones), L (lincosamides), FPI (folate pathway inhibitors), TET (tetracyclines), AG (aminoglycosides), NIT (nitroimidazole), PP (polypeptides). R = resistant, I = intermediate resistance, M = marginal sensitivity, S = sensitive, IR = intrinsic resistance.

Bacterial Genus	BL	C	FQ	L	FPI	TET	AG	NIT	PP	# Non-susceptible classes	# classes tested	MDR (Yes, No)
Azoarcus	R	R	R	R	R		R	R		7	7	Yes
Bacillus	I	I	S	S	S		S	R	S	3	8	Yes
Corynebacterium	S	S	S		S	S	S			0	6	No
Edwardsiella	S	R	M		S	S	M			3	6	Yes
Enterococcus	S	IR	R	IR	IR	S	IR			1	3	No
Enterococcus	S	IR	R	IR	IR	S	IR			1	3	No
Enterococcus	S	IR	M	IR	IR	S	IR			1	3	No
Enterococcus	S	IR	S	IR	IR	S	IR			0	3	No
Escherichia	I	S	S	R	I		S	R		4	7	Yes
Escherichia	R	R	R	R	R	S	S			5	7	Yes
Escherichia	R	S	R		R	M	S			4	6	Yes
Escherichia	S	M	S	R	S	S				2	6	No
Escherichia		S	M		S	S	S			1	5	No
Escherichia	M		S	R	R	M				4	5	Yes
Klebsiella	R	S	R		R	S	S			3	6	Yes
Kocuria	S	R	R	S	S		S	R		3	7	Yes
Pantoea	S	S	S	R	R		S	R		3	7	Yes
Proteus	S	IR	S	R	S	IR	S	R		2	6	No
Pseudomonas	IR	R	R	R	IR	IR	S			3	4	Yes
Pseudomonas	IR	S	S	R	IR	IR				1	3	No
Pseudomonas	IR		M	R	IR	IR				2	2	No
Staphylococcus	S	IR	S	S	R		S	R		2	6	No
Staphylococcus	S	IR	S	S	S	S				0	5	No
Staphylococcus	R	IR	R	R	R	R	R			6	6	Yes
Staphylococcus	S	IR	S		S	S	S			0	5	No
Staphylococcus	S	IR	S	S	S	S	S			0	6	No
Staphylococcus	S	IR	S	S	S	S				0	5	No
Streptococcus	S	S	S	R	S		S	R		2	7	No

Antibiotic resistance was also examined for various antimicrobial agents (Figure 3.6). The two most frequently used antimicrobial classes in the treatment of yellow-eyed penguins with marine predator wounds were β-lactams and cephalosporins, for which only 67% and 53% of isolates were sensitive to respectively. The least susceptible antimicrobial classes included the nitroimidazoles (e.g. metronidazole) and lincosamides (e.g. clindamycin), with 0% and 33% sensitivity respectively. Only one bacterial isolate

was tested for sensitivity in the polypeptide class of antibiotic (polymyxin B), to which the tested bacteria was sensitive.

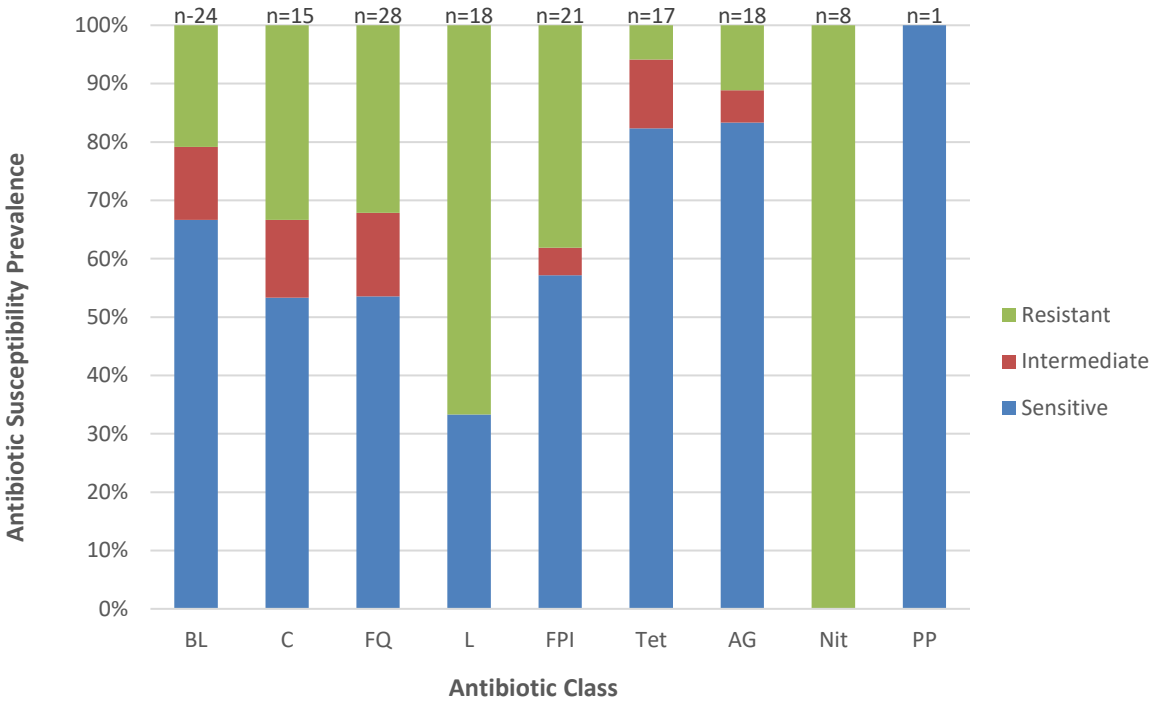


Figure 3.6: Resistance prevalence in various antibiotic drug classes from isolates cultured. *BL (β-lactams), C (cephalosporins), FQ (fluoroquinolones), L (lincosamides), FPI (folate pathway inhibitors), TET (tetracyclines), AG (aminoglycosides), NIT (nitroimidazole), PP (polypeptides).*

3.3.4 Treatment review

Wounded yellow-eyed penguins presenting to wildlife veterinary hospitals were treated with antibiotics, analgesia, preventative antifungals, fluid therapy and vitamin and mineral supplementation. Exact treatment regimens varied between institutions and patients, and changed over time.

Empirical treatment for yellow-eyed penguins in one facility included amoxicillin-clavulanic acid 125mg/kg (Noroclav, Norbrook, Auckland, NZ) PO BID combined with ceftazidime 50mg/kg (Ceftazidime, Mylan, Auckland, NZ) IV BID. In some cases, cephalosporin treatment was changed to oral cephalexin 40mg/kg (Cephalexin ABM, ABM Pharma, Auckland, NZ) PO BID. In the other facilities a variety of protocols were used including amoxicillin-clavulanic acid 125mg/kg (Curam, Novartis, Auckland, NZ) PO BID or amoxicillin clavulanic acid (Amoxiclav, Multichem, Auckland, NZ) IV BID as a single antibiotic, or in combination with enrofloxacin 15mg/kg (Enrotril, Troy Ilium, Auckland, NZ) PO BID and metronidazole 30mg/kg (Flagyl S Suspension, Sanofi, Auckland, NZ) PO BID or metronidazole 15-30mg/kg (Metronidazole Claris, AFT Pharmaceuticals, Auckland, NZ) slow IV BID. Antibiotic treatment was adjusted according to culture and sensitivity results where available.

Analgesia was provided in the form of tramadol 30mg/kg (Tramal, CSL, Auckland, NZ) PO BID or butorphanol 4mg/kg (Butorgesic, Troy Ilium, Auckland, NZ) IM BID for the initial stages of treatment. Meloxicam 0.5-1mg/kg (Boehringer Ingelheim, Auckland, NZ) PO BID was given for anti-inflammatory and analgesic effects. Itraconazole 5mg/kg (Sporanox, Janssen, Auckland, NZ) PO SID or itraconazole 10mg/kg (Itrazole, Mylan, Auckland, NZ) PO SID was provided as a routine preventative antifungal agent in all hospitalised yellow-eyed penguins for the duration of care, with dose and formulation variable across hospitals. Fluid therapy was provided to patients intravenously with balanced electrolyte solutions (Hartmann's, Baxter Healthcare Ply Ltd, NSW, Australia) or orally using either Hartmann's or Vytrate (Vytrate, Jurox, Auckland, NZ) diluted at a 1:12.5 ratio with water as per manufacturer instructions. One hospital routinely supplemented birds with iron sulphate 40mg/kg (Ferrograd, Mylan, Auckland, NZ) PO SID to support the regenerative response of anaemic patients who suffered blood loss. The same institution also prophylactically administered an antimalarial during an outbreak of avian malaria in captive birds, specifically a combination of atovaquone 15mg/kg and proguanil hydrochloride 6mg/kg (Malarone, GlaxoSmithKline, Auckland, NZ) PO once weekly.

Wound management usually involved a combination of cleaning with 0.05% aqueous chlorhexidine solution, lavaging with sterile saline and debridement of dead tissue under general anaesthesia. Wounds that were not initially closed were dressed with manuka honey (Active Manuka Honey, Aniwell, Auckland, NZ) or silver sulphadiazine topically (Flamazine, Smith & Nephew, Auckland, NZ), with bandage changes every 3-4 days. Six penguins were treated by amputation of affected digits to remove infected bone. In 5/6 cases, digit 4 was amputated at the tarsometatarsal-phalangeal joint by disarticulation of P1, and in the remaining case digit 3 was amputated by disarticulation of P3 from the P2-P3 joint. Outcomes were variable for these individuals. Two died in hospital of other causes. Of the four that were released, one was never resighted, two were resighted with recrudescent injuries and one was resighted breeding. Ruptured tendons were repaired in two animals, one of which was released and the other euthanised during treatment.

Birds were housed in individual hospital cages and hand-fed twice daily with salmon (*Arripis trutta*), pilchards (*Sardinops sagax*) or anchovies (*Engraulis australis*). A vitamin supplement was added to fish at 0.2g tablet/226g fish (Mazuri Vita-Zu seabird tablets, Mazuri, Missouri, USA). Penguins were given access to water for swimming once injuries had fully healed, after which the focus of rehabilitation was waterproofing and rebuilding fitness. In some facilities that only had access to freshwater pools, birds were provided with increasing concentrations of sodium chloride in oral fluids (initially a 1% solution increasing to a 3% solution over several days) to reactivate salt glands prior to release.

Number of antibiotics

Sufficient information was available detailing antibiotic regimes for all except one patient. The majority of cases 106/177 (60%) were treated with two antibiotic classes (Figure 3.7). Five distinct antibiotic classes were provided to 5/177 (3%) of patients during treatment. No antibiotic coverage was provided to 12/177 (7%) of patients.

The following antibiotic classes were prescribed to patients for the treatment of marine predator injury: β-lactams (amoxicillin clavulanic acid), cephalosporins (ceftazidime, cephalexin), fluoroquinolones (enrofloxacin, ciprofloxacin), nitroimidazoles (metronidazole), aminoglycosides (amikacin, toframycin), lincosamides (clindamycin) and tetracyclines (doxycycline).

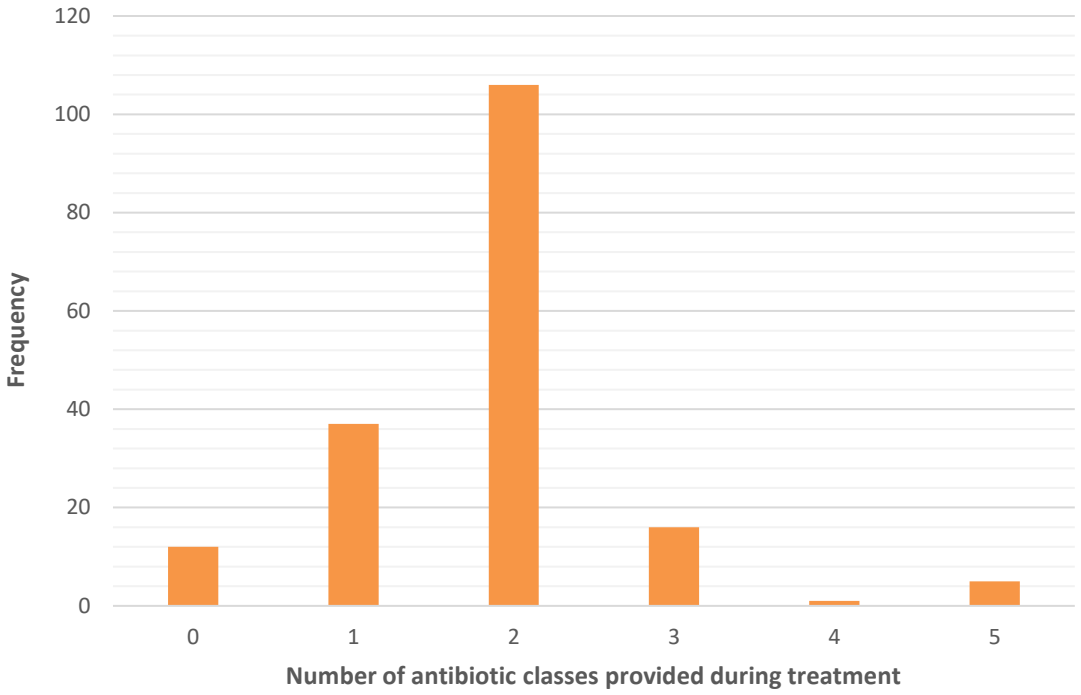


Figure 3.7: Frequency of cases treated with varying number of antibiotic classes.

Antibiotic duration

Nearly half (47%; 77/165) of patients prescribed with antibiotics were medicated for up to two weeks (Figure 3.8). Only 12% of cases (19/165) were treated with more than four weeks of antibiotics.

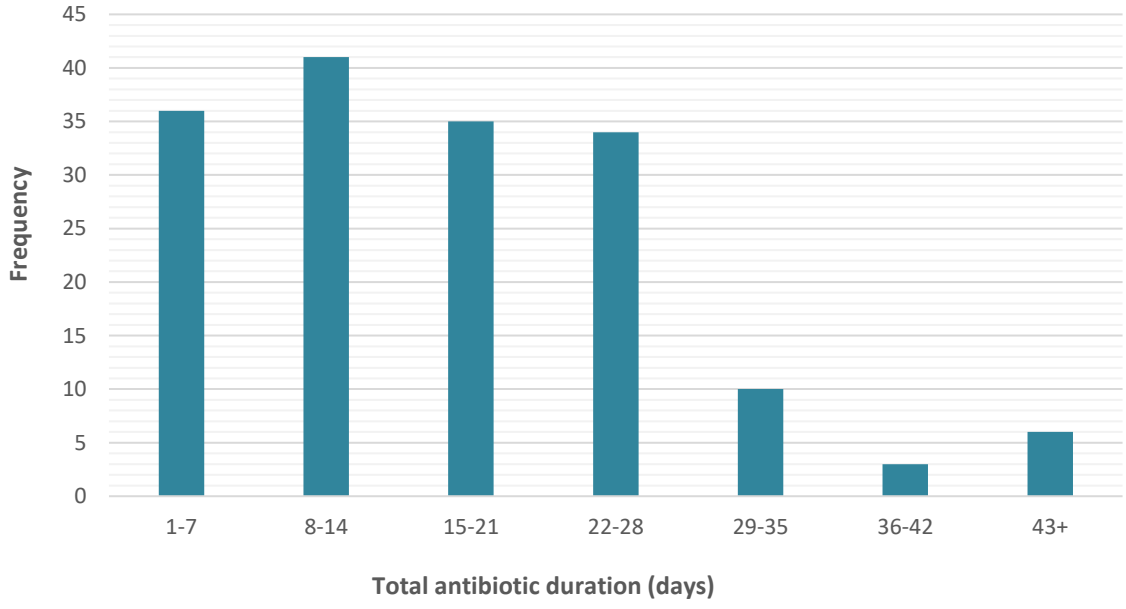


Figure 3.8: Duration of antibiotic course for each patient presenting for marine predator injury.

Wound management technique

Wound management approach varied considerably. More than two thirds (73%; 87/119) wounds were sutured closed either at the initial assessment under general anaesthesia, or later during treatment (Figure 3.9). Approximately one quarter (26%; 32/119) of wounds in yellow-eyed penguins were managed as open wounds. The most common method of wound management was staged closure, 45/119 (38%) of wounds managed by this approach.

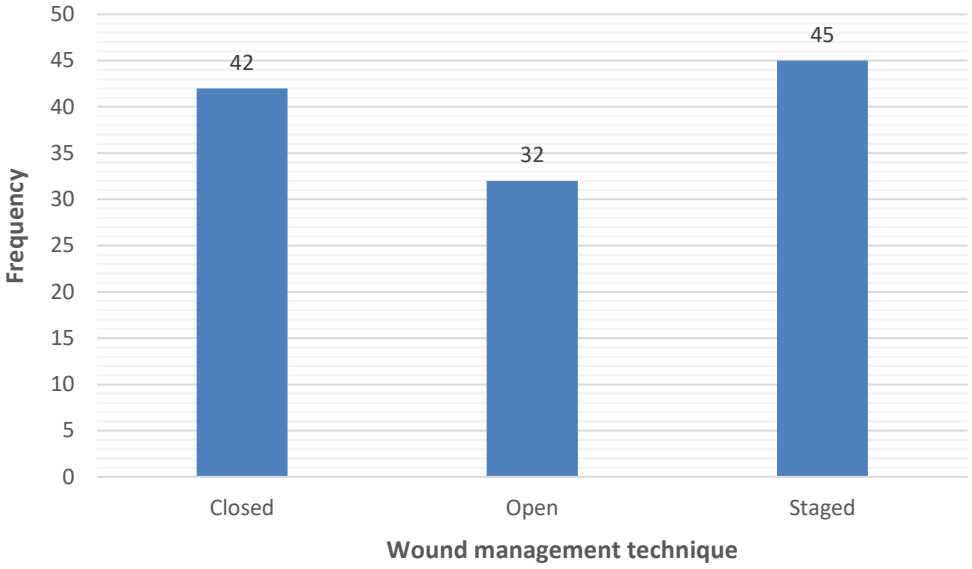


Figure 3.9: Wound management technique.

Length of time in care

The average length of time in care was 41 days, median time in care was 34 days, with a range from 1-335 days.

3.3.5 Outcomes of treatment

Approximately one third of penguins (34%; 60/176) were euthanised or died during treatment, whilst two thirds (66%; 116/176) survived and were released (Figure 3.10).

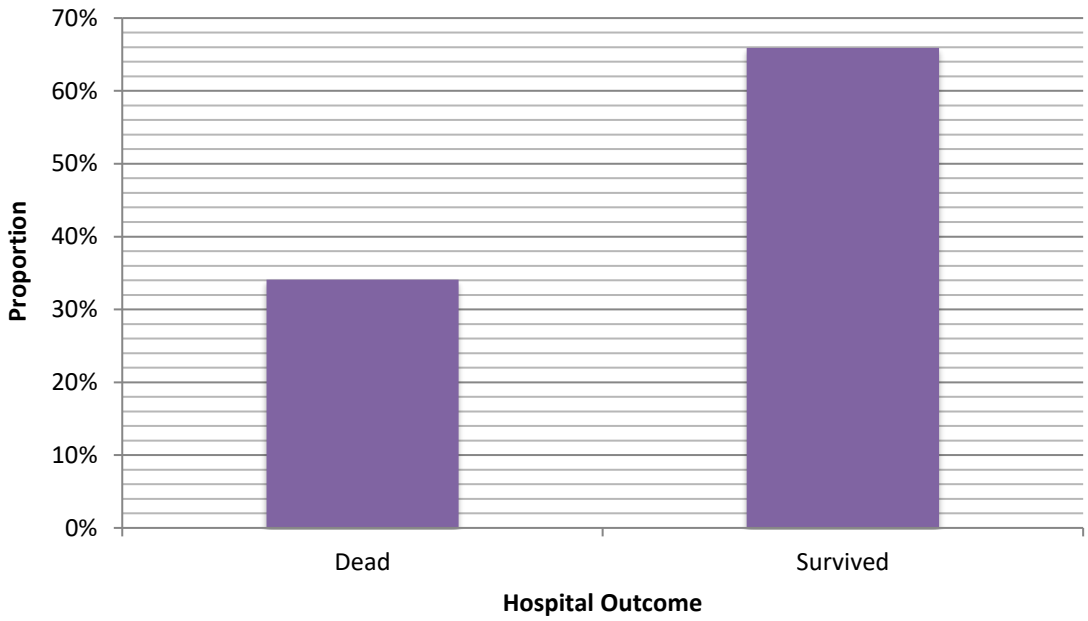


Figure 3.10: Outcome of yellow-eyed penguins hospitalised for marine predator wounds.

There was no evidence of a difference between adult and juvenile hospital survival, presented in Figure 3.11 and Table 3.6.

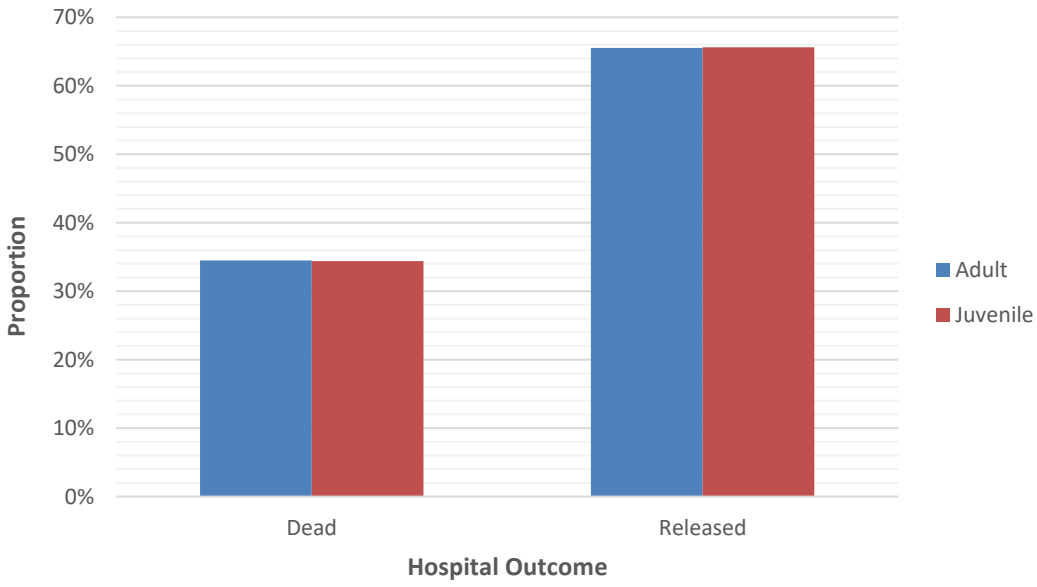


Figure 3.11: Proportional outcomes of adult and juvenile yellow-eyed penguins that died or were released from hospital.

Outcomes post-release revealed that nearly half of all birds (49.1%; 57/116) were not resighted after receiving veterinary care from wildlife hospitals for marine predator injuries. Just under one quarter of birds (22.4%; 26/116) were resighted, but not observed to breed, and just over one quarter of birds (28.4%; 33/116) were seen to be making breeding attempts post release (Figure 3.12). The post-release outcomes of adults and juveniles are presented separately in Figure 3.13, with no significant difference found between different age class and such outcome (Table 3.7).

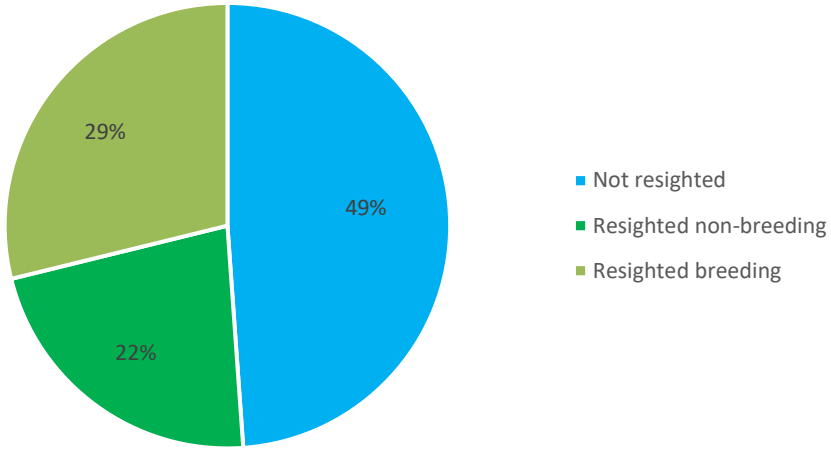


Figure 3.12: Post-release outcomes of yellow-eyed penguins following hospitalisation for marine predator injuries.

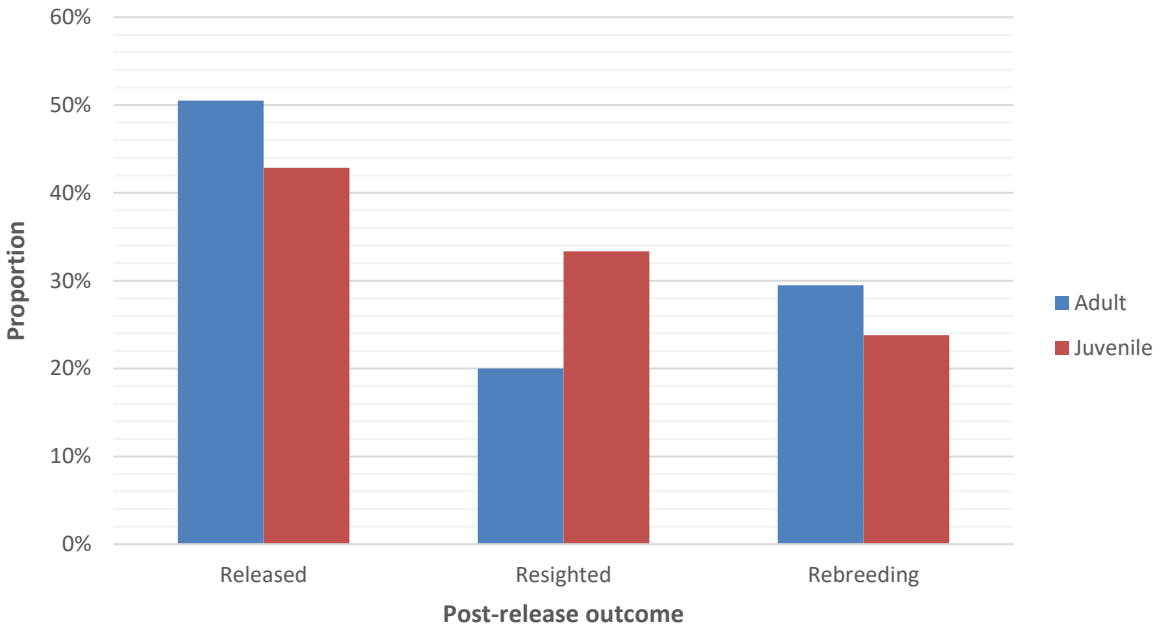


Figure 3.13: Proportional post-release outcomes of adult and juvenile yellow-eyed penguins hospitalised for veterinary treatment of marine predator wounds.

Injury recrudescence occurred in 5% (8/170) of penguins being re-hospitalised for complications associated with the initial injury (Figure 3.14).

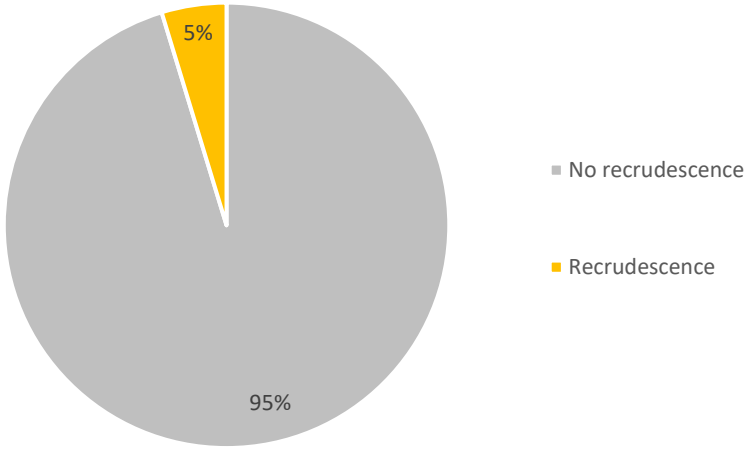


Figure 3.14: Proportion of yellow-eyed penguins admitted to veterinary hospitals for a recrudescent marine predator injury.

Repeat injury was common, with 15% (24/163) of penguins presenting to hospital with injuries from a second marine predator attack, and a single individual reported to be injured three times during the study period (Figure 3.15).

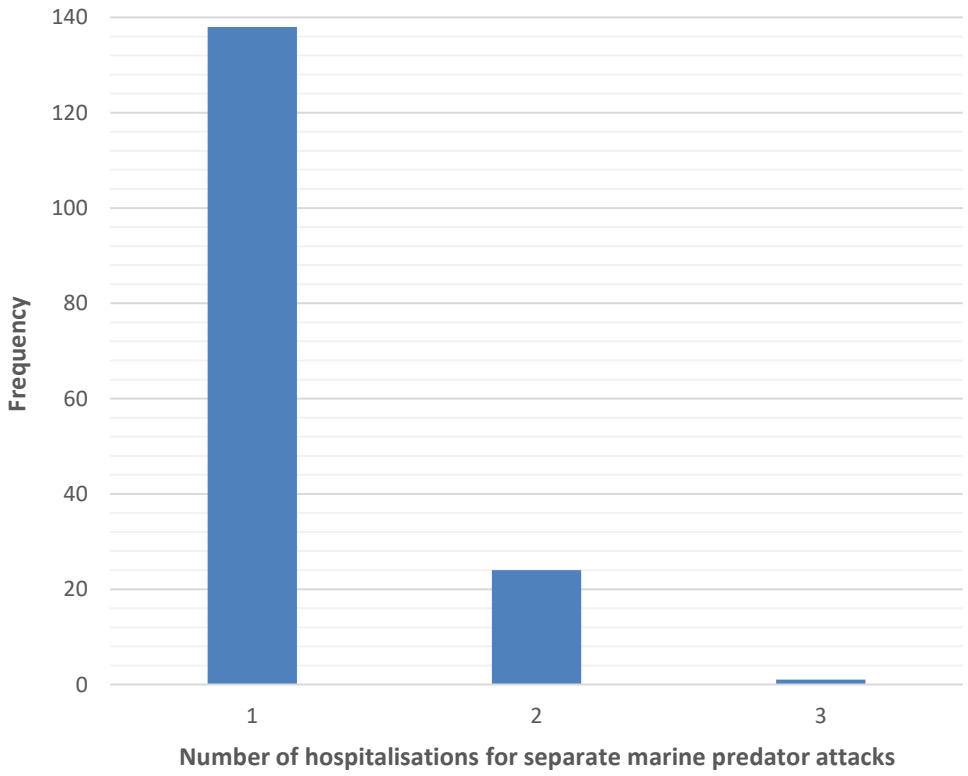


Figure 3.15: Number of separate marine predator incidents observed for individual yellow-eyed penguins hospitalised at veterinary wildlife hospitals.

3.3.6 Prognostic factors

Presenting factors and treatment factors were examined both independently and collectively to determine their effect on hospital survival and post-release outcomes. There was very strong evidence to suggest that lower injury grades using the modified pododermatitis scale was strongly associated with hospital survival ($\lambda = 32.594$, df = 6, p = <0.001) (Table 3.6), and moderate evidence of association with post-release outcomes ($\lambda = 28.070$, df = 6, p = 0.005) (Table 3.7).

Likewise, there was very strong evidence to indicate that survival in hospital was strongly associated with the absence of complications, such as osteomyelitis ($\chi^2 = 8.798$, df = 1, p = 0.003), tendon/ligament damage ($\chi^2 = 24.640$, df = 1, p = <0.001), or nerve damage ($\chi^2 = 9.158$, df = 1, p = 0.002). Longer duration of antibiotic treatment were also strongly indicative of hospital survival ($\lambda = 32.205$, df = 6, p = <0.001).

Table 3.6: Various presenting and treatment factors effect on hospital survival of yellow-eyed penguins hospitalised for marine predator injuries.
** Likelihood ratio performed rather than chi-square test due to unsuitability of data.*

Factor	**n**	**χ^2**	**Df**	**p-value**
Age class	177	<0.001	1	0.991
Site of injury (flipper)	177	1.993	1	0.158
Site of injury (leg)	177	0.330	1	0.566
Site of injury (body)	177	0.228	1	0.633
Modified pododermatitis scale	177	32.594*	6	**<0.001**
CM osteomyelitis scale	71	7.446*	4	0.114
Chronicity	177	1.766	1	0.184
Presence of osteomyelitis	168	8.798	1	**0.003**
Presence of tendon/ligament damage	175	24.640	1	**<0.001**
Presence of nerve damage	174	9.158	1	**0.002**
Initial wound management	119	7.484	2	**0.024**
Number of antibiotic classes	164	1.334	2	0.513
Antibiotic duration	164	32.205*	6	**<0.001**

Table 3.7: Various presenting and treatment factors effect on post-release outcomes of yellow-eyed penguins hospitalised for marine predator injuries.
** Likelihood ratio performed rather than chi-square test due to unsuitability of data.*

Factor	**n**	**χ^2**	**Df**	**p-value**
Age class	116	1.764	2	0.414
Site of injury (flipper)	116	4.201	2	0.122
Site of injury (leg)	116	7.098	2	**0.029**
Site of injury (body)	116	3.093	2	0.213
Modified pododermatitis scale	116	28.070*	12	**0.005**
CM osteomyelitis scale	37	2.702*	6	0.845
Chronicity	116	0.375	2	0.829
Presence of osteomyelitis	109	0.464	2	0.793
Presence of tendon/ligament damage	115	0.793*	2	0.673
Presence of nerve damage	114	2.509*	2	0.285
Initial wound management	87	1.176	4	0.882
Number of antibiotics	109	4.015*	4	0.404
Antibiotic duration	109	11.853	12	0.458

There was very strong evidence to suggest that chronicity predicted injury recrudescence ($\lambda = 14.766$, df = 1, p = <0.001). No other presenting or treatment factors predicted injury recrudescence.

A *post-hoc* analysis of chi-squared tests revealed that there was a significantly increased chance of an animal surviving to release with Grade 4 injuries (adjusted p = 0.001), and a significantly reduced chance with Grade 6 (adjusted p = <0.001) and Grade 7 injuries (adjusted p = 0.003). Additionally, there was moderate evidence to suggest birds with staged closure of injuries were more likely to survive to release, than any other wound management approach (adjusted p = 0.012). Hospital survival was lower for birds that received antibiotics for one week or shorter duration (adjusted p = <0.001), and higher for those receiving 22-28 days of antibiotics (adjusted p = 0.003). *Post-hoc* analysis of post-release outcome revealed only a significant difference for grade 3 injuries, whereby the number of birds resighted as non-breeders was significantly higher than the number not resighted, or resighted breeding (adjusted p = 0.001).

When presenting factors were considered concurrently using a binomial logistic regression, anatomical region location (flipper, leg, body) and chronicity were found to be non-significant factors determining hospital survival. The presence of severe injury complications including osteomyelitis, tendon/ligament damage or nerve damage were found to be significant predictors of hospital mortality ($\chi^2 = 37.876$, df = 3, p = < 0.001) with a -2 log likelihood 174.838, and the model predicting 75.2% of results correctly. Birds with osteomyelitis were approximately three times less likely to survive hospitalisation to release. Additionally, the presence of tendon and ligament damage reduced hospital survival approximately nine times. Birds with nerve damage were seven times more likely to die in hospital (Table 3.8).

Table 3.8: Reduced binomial logistical regression model of presenting factors affecting hospital survival of yellow-eyed penguins with marine predator injuries.

Presenting Factor	B	S.E.	Wald	df	Sig.	Odds Ratio	95% CI for Odds Ratio	
							Lower	Upper
Osteomyelitis	1.075	.378	8.089	1	**0.004**	2.929	1.397	6.143
Tendon/ligament damage	2.214	.535	17.114	1	**<.001**	9.150	3.206	26.116
Nerve damage	2.058	.750	7.534	1	**0.006**	7.830	1.801	34.039

The average length of time in care for all birds was 41.1 days. There was very strong evidence that birds which were released had a significantly longer time in care than those that died or were euthanised in hospital ($Z = 5870.5$, $p < 0.001$), with a mean of 22.7 days compared to 50.7 days (Figure 3.16).

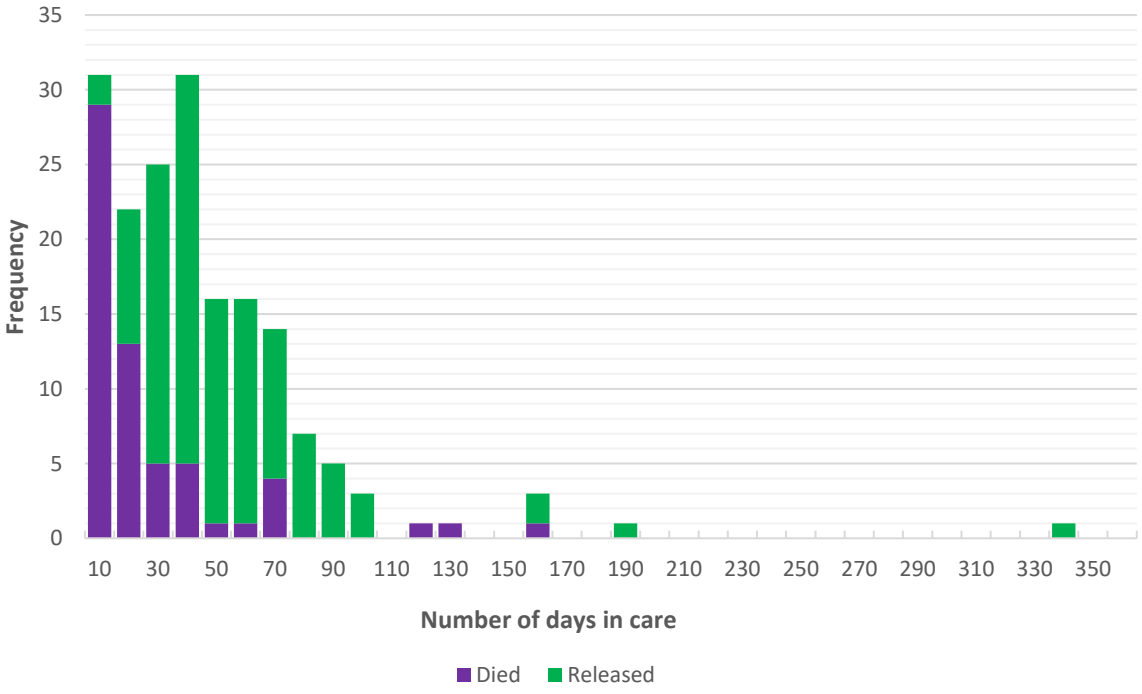


Figure 3.16: Length of time in care of yellow-eyed penguins hospitalised for marine predator wounds by hospital outcome.

There was no significant difference seen between the length of time in care with post-release outcomes, including resighting or breeding attempts ($F = 24.103$, $df = 1$, $p = 0.214$).

3.3.7 Necropsy review

A total of 453/930 (49%) yellow-eyed penguin submissions in the Massey University Pathology Database were identified as fledged birds, consisting of 126 juveniles and 326 adults. Of these, 34% (153/453) had been identified as being wounded by a marine predator. Hospitalised or rehabilitated penguins consisted of 63% (97/153) of these submissions, and 37% (56/153) of these were found deceased in the wild.

Eighty two percent (125/153) had a severe complication caused by the injury itself, or secondary to captivity. Specifically 67% (103/153) of complications were directly related to the original injury. The most prevalent complications in deceased birds included osteomyelitis, seen in 49% (75/153), as well as tenosynovitis (22%; 33/153) and tendon/ligament damage (18%; 27/153) (Figure 3.17).

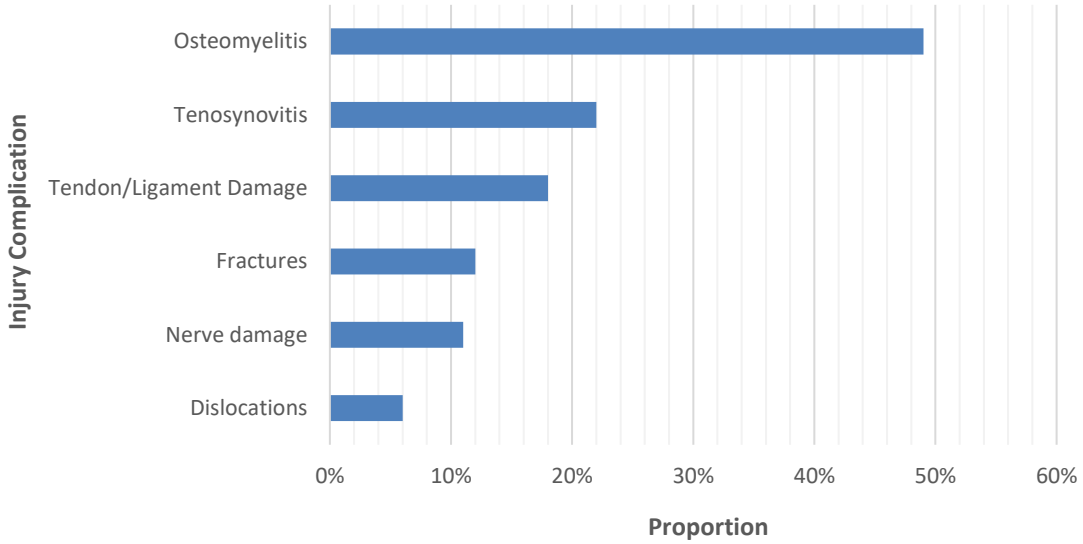


Figure 3.17: Prevalence of secondary injury complications in necropsied yellow-eyed penguins with evidence of a marine predation attack.

More than half of yellow-eyed penguins treated in captivity (51.5%; 50/97) developed a severe complication due to the captive environment. Often these complications were serious, and most were likely to have caused mortality. A total of 65 complications were reported in 50 patients, as some had concurrent ailments. The respiratory system was most commonly affected, accounting for 48% (31/65) of the hospital complications; gastrointestinal related diseases constituted 18% (12/65) and the urinary system in 8% (5/65) of hospital complications seen (Figure 3.18).

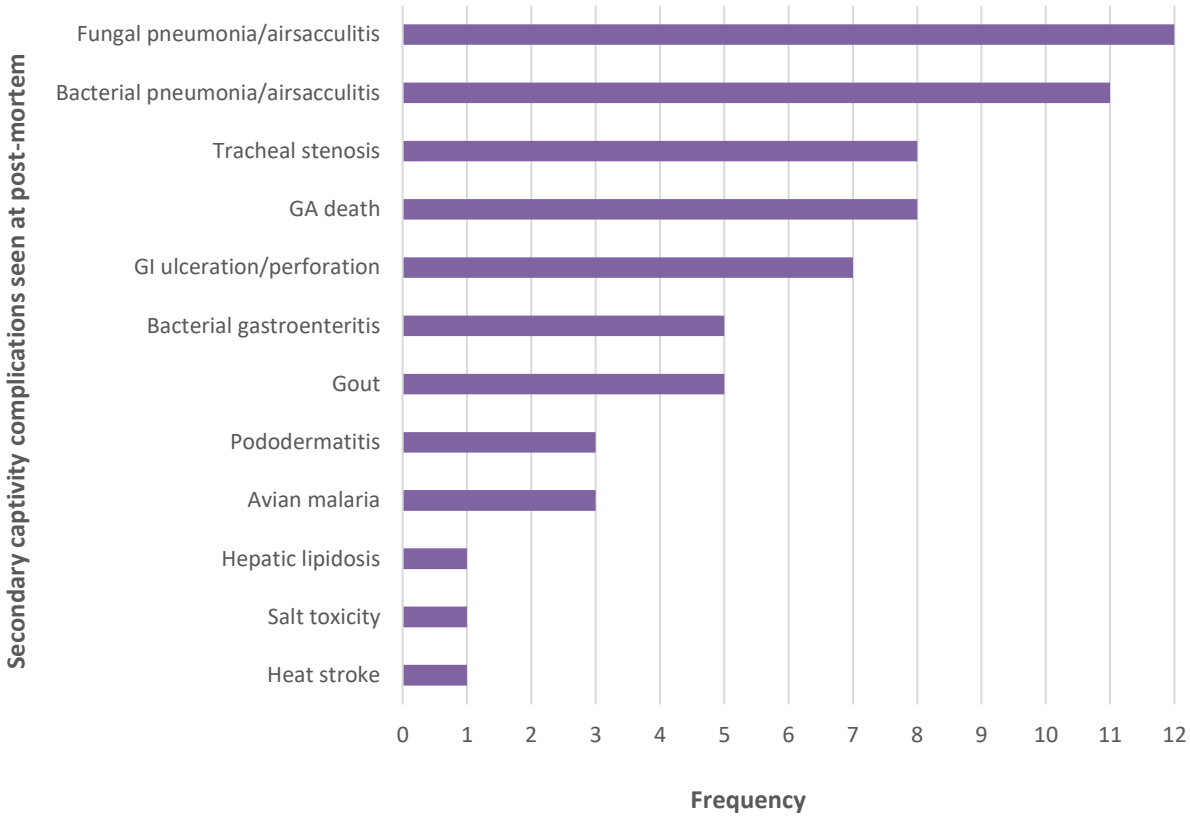


Figure 3.18: Most common complications related to captivity seen in hospitalised or rehabilitated yellow-eyed penguins with marine predator injuries.

Mycotic airway disease (pneumonia and air sacculitis) was observed in 12% (12/97) of captive patients presented for necropsy, and was the most common hospital complication seen. In two cases, tissues were cultured confirming *Aspergillus spp.*, although speciation was not performed. Seven individuals were located in veterinary wildlife hospitals and the remaining five were in either rehabilitation or general veterinary practices. The length of time in captivity was known for individuals from veterinary wildife hospitals, averaging 51 days in care (range 2-159 days). Six of these seven birds were on prophylactic antifungal therapy prior to death, including four on itraconazole 10mg/kg PO SID, and two on itraconazole 5mg/kg PO SID. Aspergillosis was seen across multiple veterinary hospitals, with several formulations of itraconazole used prophylactically, including liquid (Sporonox) and capsules (Itrazole) which were opened and granules medicated directly on the consumed fish. No statistical analyses were performed on this data due to small sample sizes.

3.4 Discussion

Uplifting wild animals for treatment in veterinary hospitals or rehabilitation centres is a major undertaking, not without risk for the individual animal and the entire population (Alden et al., 2021). Many species encounter considerable stress in an unfamiliar environment with regular handling, and nosocomial infections are possible. There are frequent conspecific and interspecific interactions directly and indirectly in veterinary hospitals, thus there is also the potential for disease transmission (Morley, 2002; Weese, 2012; Burgess & Morley, 2015).

With this in mind, an analysis of injury severity using grading scales, assessment of systemic disease status by haematological and biochemical testing and consideration of antimicrobial resistance in bacterial wound isolates, was warranted. Treatment regimens were similar for various wildlife hospitals, and have been broadly investigated. Review of pathological findings from penguins found deceased in the wild and in captive care investigates the proportion of deceased birds with serious injury complications and secondary disease processes that may have occurred from captivity. This allows an examination of disease severity attributed to the death of these cases, and identification of severe disease manifestations, which were likely to be at least partially attributed to the captive environment, so that preventative measures to reduce the risk of disease occurrence can be considered.

3.4.1 Grading of injuries

Grading of marine predator wounds into various categorical scales allowed injuries with complex descriptions to be grouped according to their features and severity, using a consistent approach. Mild injuries (Grade 1-2) were unlikely to require treatment in immunocompetent patients, as the bird's own immune system was likely to overcome minor superficial wound contamination. Moderate injuries (Grade 3-5) were likely to afflict the bird by affecting physical functioning within their normal environment or causing systemic illness. These cases would benefit from veterinary treatment and supportive care whilst overcoming the injury. Severe injuries (Grade 6-7) were significant physical manifestations, which would likely cause the death of a patient if untreated. Treatment required was very intensive and often involved long courses of antibiotics or significant surgical interventions.

A standardised grading scale may be a useful tool for in-field assessments of injured penguins to decide whether they should be uplifted for veterinary treatment. All birds with moderate to severe injuries (Grades 3-7) should be uplifted for immediate veterinary assessment. In cases of uncertainty, or for any injuries other than superficial cuts with minimal swelling or discharge, uplifting injured birds for veterinary assessment is recommended. Veterinary assessment and diagnostic imaging (e.g. radiographs) may be required for precise injury grading, to fully assess the extent of nerve, tendon or bone involvement.

More than 80% of marine predator wounded yellow-eyed penguins admitted to veterinary wildlife hospitals had moderate or severe injury. This indicates that a high proportion of individuals admitted to veterinary hospitals truly required veterinary intervention. Whilst this suggests that the majority of marine predator injuries are extensive, moderate and severe injuries may have been over-represented, as some individuals with mild, uncomplicated injuries may not have been uplifted. Conversely, some penguins with moderate or severe injuries may not have survived the initial attack or have been too encumbered to swim back to shore. Therefore, this grading scale provides an indication of the types of injuries presented to wildlife hospitals, but is not wholly representative of the true prevalence of injury characteristics occurring at sea.

The modified Cierny-Mader osteomyelitis scale was not useful for the assessment of marine predator wounds in penguins, compared to its application in human medicine. In the human Cierny-Mader scale (Grades 1-4), the higher categories represent more severe disease and a higher intensity of interventions required, such as longer courses of antibiotics and more aggressive surgical debridement (Calhoun et al., 2009). Most osteomyelitis caused by marine predator attacks involved infections at joints, with very few confined to the medulla or cortex only. This is likely because the bacterial invasion of bone originated from traumatic inoculation of bacteria during bites, and not from haematogenous spread. This led to the modification of the scale to include a fifth category. This scale has no practical implications for guiding treatment of these wounds and was not useful in predicting prognosis for hospital survival or post release outcome.

Assessing the chronicity of cases required several assumptions, and hence provides only an indication of the nature of the injuries seen. Radiographs have a low sensitivity and specificity for acute or less severe osteomyelitis cases (Lee et al., 2016), as radiographic changes are only seen where there is destruction of more than 50-75% of the bony matrix, and because there can be a two-week lag time for radiographic changes after bone infection (Calhoun et al., 2009). Hence there is the potential for misdiagnosis when radiographs are used as the only imaging modality. This may be offset by repeating radiographs two weeks after admission for patients with bone or joint exposure without radiographic evidence of osteomyelitis on initial radiographs, or employing other imaging modalities such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and nuclear medicine (Lee et al., 2016).

In human medicine, there are multiple osteomyelitis grading scales in use, with none of the scales used universally (Garwood & Kim, 2015). In marine predation wounds in yellow-eyed penguins, the modified pododermatitis scale was most valuable in gauging prognosis, and chronicity was useful to predict likelihood of injury recrudescence. The treatment of acute cases should focus on preventing secondary complications such as infection developing, whereas the treatment of chronic cases focusses on treating secondary complications from the original injury where present.

3.4.2 Haematology and biochemistry

Haematology and biochemistry results were largely unhelpful in predicting hospital survival and there did not appear to be strong correlations with injury chronicity. The haematological parameter most affected by predator attacks was PCV. Mean PCV for wounded yellow-eyed penguins was below the reference range for African penguins (Gallo et al., 2019), most likely due to whole red blood cell loss from haemorrhage, although in some cases anaemia may have also been caused by chronic disease. There was, however, quite a variable range of PCVs, with some birds presenting with absolute polycythaemia.

Plasma concentrations of uric acid was the most useful biochemical analyte to predict survival, with several animals having values beyond the upper measurable range. Severe gout was confirmed by necropsy examination of most cases with marked hyperuricaemia. Post-prandial elevation of uric acid associated with nitrogen metabolism can cause a transient physiological hyperuricaemia (Chitty & Monks, 2018), however it was less likely to have affected the results in this study as samples were usually collected under general anaesthesia, prior to which a 24-hour fasting period often preceded.

Despite our findings, haematology and biochemistry are still very useful in assessing the systemic condition of an individual patient, and providing specific patient care based on individual medical requirements, such as guiding the correction of dehydration. It may also highlight other disease concerns, such as the detection of mycotic respiratory disease or gout, and can be useful to guide antibiotic treatment duration. Therefore it is recommended that these diagnostic tests are still used to guide treatment, but are not predictive of hospital survival outcomes or injury chronicity.

3.4.3 Culture and sensitivity

Injuries from marine predator attacks were often heavily contaminated with numerous bacterial agents. Several animals had four or five distinct bacterial genera cultured from their wounds. The results may have been a conservative estimate of the number of infectious agents, as it does not account for agents that could not be identified to genus level. Similarly, as anaerobic and fungal cultures were not performed in all cases, entire spectrums of organisms may have been missed.

Half of bacterial isolates cultured were regarded as normal commensal skin flora of birds. Specifically these were *Staphylococcus, Escherichia, Pseudomonas, Corynebacterium, Streptococcus, Bacillus* and *Pantoea* (Lamb et al., 2014). Other organisms cultured have not been reported as regular skin bacterial flora, except *Clostridia* which has been cultured on the faces of scavenger birds such as vultures, believed to be from contamination of food items (Roggenbuck et al., 2014). The only study related to the normal flora of the integument of penguins details the normal ocular flora of captive Humboldt penguins, with *Corynebacterium* and *Staphylococcus* the most frequent isolates (Swinger et al., 2009).

A similar range of bacteria to those found in yellow-eyed penguins have been isolated in digit osteomyelitis in humans. Such organisms include *Staphylococcus, Streptococcus* and Enterobacteriaceae (Garwood & Kim, 2015), which constituted 45% of bacterial isolates in this study, and hence human treatment regimens may potentially be extrapolated to penguins. In humans, osteomyelitis is also usually caused by polymicrobial infections, and often a combination of aerobic and anaerobic organisms (Garwood & Kim, 2015).

The proportion of antibiotic resistance in bacterial isolates identified from the penguins' wounds was profound. To the best of our knowledge, many of these patients were receiving antibiotics for the first time, during their hospitalisation. However, case selection for culture and sensitivity testing was not random. In 41% (7/17) of cases, culture and sensitivity samples were collected more than two weeks after hospitalisation, and were potentially from individuals that were refractory to treatment, possibly over-representing levels of antimicrobial resistance. To ensure prudent antibiotic use, ideally culture and sensitivity testing would be performed on all individuals, before antimicrobial administration, to guide treatment. Bone or tissue biopsies are much more sensitive and specific than swabs collected from wounds (Conterno & Turchi, 2013; Heravi et al., 2019; Senneville et al., 2020), and are the preferred diagnostic samples for these contaminated wounds.

3.4.4 Treatment review

Successful treatment of wounded yellow-eyed penguins involves providing an optimal environment for wound healing, and resolution of infection in various tissues (e.g. soft tissue, tendon and bone). Thus, treatment is multifactorial, and the intensity and length of treatment is highly dependent on the extensiveness of infection and physical trauma.

One of the most severe and common complications associated with marine predator injuries in penguins is osteomyelitis, where the same principles apply for successful treatment (Lipsky et al., 2012). A widely accepted notion in the treatment of wounds is to debride and remove non-viable tissue, to assist healing (Anderson, 2006). Traditionally it was also believed that radical surgical excision of infected, but viable, bone in addition to adjunctive medical treatment was superior to medical treatment alone, by shortening the duration of antibiotic therapy required and reducing the risk of recrudescence or antibiotic resistance (Garwood & Kim, 2015). On the contrary, other studies indicate that resolution rates in humans with osteomyelitis are similar, regardless of whether bone debridement or amputation is undertaken when appropriate antibiotic therapy is delivered (Jeffcoate & Lipsky, 2004; Peters et al., 2020). To date, only a small number of yellow-eyed penguins have had digital amputations in the treatment of such injuries. Two of these individuals were found several years later with recrudescent infection at the amputation site, however due to such a small sample size, no firm conclusions can be made about this treatment approach. In the context of treating wildlife, digit amputations may shorten treatment duration, hence reduce time in captivity, reducing the likelihood of hospital complications.

Culture and sensitivity testing was used to guide treatment in less than 10% of cases. Of these, only half of the samples were collected within the first week of hospitalisation. The potential for the development of antibiotic resistance increases with increasing exposure to each dose of antibiotic, therefore with infections requiring a long course of antibiotics, the chance of developing resistance to an antibiotic is high (Lodise et al., 2007; Costelloe et al., 2010; Llewelyn et al., 2017). Choosing an appropriate antimicrobial as early as possible is therefore important.

Medication regimens adopted for yellow-eyed penguins resemble those used to treat human osteomyelitis. Recommended empirical treatment for mild osteomyelitis in humans includes β-lactam antibiotics, for their broad-spectrum activity and good penetration into bone (Garwood & Kim, 2015; Lipsky & Uçkay, 2021). However several systematic reviews have indicated that a range of antibiotics may be effective for the treatment of digital osteomyelitis, with no superiority proved for a particular agent (Peters et al., 2020). Based on our results, prior to the commencement of antibiotic treatment, samples should be collected from wounds for both aerobic and anaerobic culture. Antibiotic therapy should be adjusted accordingly once these results are available (Garwood & Kim, 2015; Lipsky & Uçkay, 2021).

All veterinary hospitals implemented a combined antibiotic therapy approach, often involving amoxicillin-clavulanic acid with the combination of a fourth- or first-generation cephalosporin (ceftazidime or cephalexin respectively), enrofloxacin or metronidazole. Triple antibiotic therapy was instigated in some instances. Due to the high level of importance of both fluoroquinolones and later generation cephalosporins (e.g. third and subsequent generations) in human medicine, they are now regarded as 'Critically Important' antibiotics, and their use is no longer recommended in veterinary medicine, unless culture and sensitivity results indicate resistance to all other antimicrobial classes (Office of Health Protection, 2018; New Zealand Veterinary Association, 2019; World Health Organisation, 2019b; AMR Vet Collective, 2022). Amoxicillin-clavulanic acid is regarded as 'Highly Important' (New Zealand Veterinary Association, 2019) and metronidazole as 'Medium Importance' with relation to human health, and would ideally also be used as second-line therapy (Office of Health Protection, 2018). However, there are also limitations in the pharmacological information available for the effectiveness of antimicrobials in avian medicine, specifically for the order Sphenisciformes (Hawkins et al., 2018), in addition to considerations regarding drug route and duration, which may limit available choices. Using a second-generation antibiotic (e.g. cefoxitin) may be an option if cephalosporin coverage is required, as it still provides broad spectrum coverage including for some anaerobic bacteria, although its use is off-label in animals, and it is still regarded as an antibiotic of Medium importance (Office of Health Protection, 2018). The presence of polyclonal infections in marine predator wounds and high levels of resistance may complicate antimicrobial options.

Recent changes have been made to the recommended route of antibiotics for human osteomyelitis. Traditionally intravenous antibiotics were recommended for 2-5 days prior to commencing parenteral antibiotics (Lipsky et al., 2012). There is now good evidence to suggest that intravenous antibiotics do not offer any significant advantages

to oral antibiotics, for the treatment of osteomyelitis in human patients (Conterno & Turchi, 2013; Li et al., 2019). However due to the limited availability of drugs in veterinary medicine, some may only come in an intravenous formulation, hence may justify this route of administration.

Antibiotic course length is an important consideration in the successful treatment of osteomyelitis. In this study, only 12% of yellow-eyed penguin cases were treated with more than four weeks of antibiotics, despite osteomyelitis being present in 39% of cases. There is no consensus in the medical community for the appropriate duration of antibiotic coverage in human osteomyelitis treatment (Peters et al., 2020). A study of adults with chronic osteomyelitis treated with parenteral antibiotics for 4-6 weeks demonstrated a 60-90% cure rate, although this figure was reduced with the isolation of *Pseudomonas spp.* (Garwood & Kim, 2015). Recent reviews of human osteomyelitis treatment have shown no statistically significant benefit to extending antimicrobial therapy beyond six weeks, and that adverse outcomes increased by 300% when courses were extended to twelve weeks (Peters et al., 2020; Lipsky & Uçkay, 2021). Length of treatment is best guided by clinical assessment of the patient, blood parameters (including white cell counts) and repeat radiographs (Garwood & Kim, 2015), as was used in this study. C-reactive protein and erythrocyte sedimentation rate are also commonly used to determine antibiotic duration in human osteomyelitis treatment (Garwood & Kim, 2015). Erythrocyte sedimentation rate is not a useful diagnostic test birds, due to the lack of rouleaux formation of avian erythrocytes (Burton et al., 1966), and C-reactive protein has not been validated in birds, but could potentially be a useful test to guide treatment in the future.

Human osteomyelitis research mainly focuses on distal osteomyelitis as a complication of diabetes mellitus, rather than originating from a traumatic inoculation of bacteria via a predator wound. When considering antibiotic therapy provided to human shark attack victims, similar antimicrobial agents are used to those in penguins, including amoxicillin-clavulanic acid or a combination of a second-generation cephalosporin and metronidazole (Woolgar et al., 2001). Usually these are utilised as a prophylactic against infection, as medical advice is generally sought promptly before significant infection is established. In some cases, antibiotics are not prescribed (Woolgar et al., 2001).

Primary closure at admission is commonly performed on human shark attack injuries, after wound irrigation. This is usually due to the extensive nature of the injuries, with very deep lacerations through skin and muscle. Wounds are often left to close by secondary intention healing when there is a large deficit making closure difficult, or skin grafts are otherwise used to cover large soft tissue deficits (Woolgar et al., 2001). Occasionally suction drainage is used in very deep wounds (Woolgar et al., 2001). By contrast, there was a variable approach to the management of penguin wounds. Whilst approximately one third of wounds were managed as open wounds, and two thirds were closed at some point during their treatment, with a staged wound closure approach being the most common approach. Human and penguin shark-bite wounds are likely to differ at presentation due to the high prevalence of infected wounds at hospital admission in penguins, compared to human patients. Drains are not effective in the management of infected wounds in birds, as avian pus is usually solid due to the absence

of oxidative activity from the lack of myeloperoxidase, catalase and alkaline phosphatase in avian heterophils (Daimon & Caxton-Martins, 1977; Maxwell & Robertson, 1998; Genovese et al., 2013; Howerth, 2019).

3.4.5 Outcomes of treatment

There are very few reports of veterinary hospital survival rates of wild penguins, and no other studies detailing these figures in yellow-eyed penguins. The hospital survival rate for yellow-eyed penguins in this study was similar to survival rates seen in rehabilitated African penguins, whereby juvenile birds had an average release rate of 70% (54-86%) (Vanstreels et al., 2019b), however those individuals were admitted for a wider range of reasons (e.g. oiling, injury, moult, debilitation). Survival rates were higher in little penguins treated and rehabilitated for oil spill contamination in New Zealand, with 90.6% of admitted penguins for veterinary treatment and rehabilitation released (Sievwright et al., 2019a; Sievwright et al., 2019b).

Post-release outcomes in penguins can be highly variable, due to abundant threats in the marine and terrestrial environment (Section 1.3), leading to significant interannual variation (Gownaris & Boersma, 2019). Survival of penguins can also be highly variable between sites (Sherley et al., 2014), sexes (Gownaris & Boersma, 2019) and age groups (Marchetti & Price, 1989; Sherley et al., 2014; Stein et al., 2017; Hinke et al., 2020). Despite this, the information gleaned can still provide some indication of how individuals fare post release, when interpreted in this context. Approximately 50% of released birds were resighted more than a month later. This post-release survival rate was similar to those seen in another study where survival for little penguins after the Rena oil spill was 52% in the six months subsequent to release, with no significant difference between rehabilitated and control birds (Sievwright et al., 2019a)

Juvenile survival is often much lower than adult survival unless food availability is high (Sherley et al., 2014), likely due to inefficient foraging by a lack of experience (Marchetti & Price, 1989), and hence in this study these age classes were considered separately. Post-fledging survival to adulthood is low in yellow-eyed penguins, with an average survival rate of 17.2% (Stein et al., 2017). Juvenile survival in this study was comparatively good, with 33% of birds resighted again at least one month later. Despite juvenile resighting post-release being slightly higher than adult resighting in this study, there were no significant differences between the two age classes and post-release outcomes. Another study of Magellanic penguins (98% of which were juveniles) that received veterinary treatment for a range of presentations had an average resighting rate of 12.3%, although this was highly variable depending on the year with one season having a rate of 16.3% and the following year 1.8%, suspected to be related to poor food availability in the latter year (Bhering et al., 2022).

Approximately 50% of yellow-eyed penguins in this study were found surviving post-release, 22% of these were resighted as non-breeders, and 28% of these made a breeding attempt (equating to 44% and 56% of resighted birds respectively. This is comparable to the little penguins from the Rena oil spill where 39% of resighted

rehabilitated birds were seen breeding the following season (Sievwright et al., 2019b). Despite the yellow-eyed penguin monitoring program surveying breeding sites on a triannual basis, we cannot be confident that 100% of individuals are sited each year. Therefore, post-release survival rates of yellow-eyed penguins treated for marine predation injury may be higher. Breeding success in yellow-eyed penguins is complicated by an incongruent sex ratio of 2:1 males to females in adult yellow-eyed penguin populations (Richdale, 1957), and the availability of food resources (van Heezik & Davis, 1990; Crawford et al., 2006; Sherley et al., 2013). Only breeding attempts, not overall measures of breeding success (such as clutch size, hatch rate, fledging rate), were examined in this study.

In the context of this study, 24% of rehabilitated juveniles made attempts to breed post-release. Comparatively on average only 12.3% of fledgling yellow-eyed penguins between 1981-2003 attempted to breed (Stein et al., 2017). This apparent improvement in breeding success may be an artefact since in this study, as a juvenile bird was regarded as one that was post-fledge up to 2 years of age, so it is possible that some birds were close to breeding age when they were injured, and not truly representative of post-fledge breeding rate. Juvenile mortality is believed to be highest in some penguin species in the first few weeks after fledging (Hinke et al., 2020), so our data may have missed this critical time-period for birds.

A 5% recrudescence rate of latent osteomyelitis was deemed relatively low. Chronic osteomyelitis cases in humans have a long-term recrudescence rate of 20-30% (Garwood & Kim, 2015). It is highly likely that only a small proportion of birds with injury recrudescence were found in a timely manner to facilitate treatment, due to the seasonal occurrence of monitoring and high potential for birds with such conditions to be unnoticed or not sighted. It is therefore difficult to make conclusions about the true prevalence of injury recrudescence, but these results indicate that treatment of osteomyelitis is generally successful. All eight birds that re-presented with disease relating to the original injury were deemed to be chronic injuries at initial presentation.

Repeated marine predator attacks were seen in more than 15% of penguins admitted to wildlife hospitals. One bird that was included in this study was attacked in three separate marine predator events. A second bird was known to be attacked a third time, being admitted into a veterinary wildlife hospital for the latest injury in January 2020, although only two events were counted due to the date restriction on the data. To the best of our knowledge, this is the first time that there have been multiple documented attacks of marine predators on the same individual penguins, demonstrating the continued threat of marine predation which does not just threaten inexperienced birds.

3.4.6 Prognostic factors

Several injury presentation factors had a significant effect on hospital survival of predated yellow-eyed penguins. Birds with tendon/ligament damage, nerve injury and osteomyelitis had a much lower prognosis for survival to release.

Accurate diagnosis of tendon or ligament damage in a live patient is difficult unless ruptured tendon ends are visible in the wound. More commonly, ruptured tendon ends retract from the wound edges. Nerve damage is usually determined by persistent neurological deficits on physical examination and ruling out the presence of structural tendon or ligament damage, or based on a response to treatment indicating only partial or reversible nerve damage, such as inflammation. A neurological examination by a veterinarian, including assessment of superficial and deep pain is valuable. The use of additional diagnostic imaging such as ultrasound and MRI may be useful in quickly identifying tendon or ligament damage (Johnson et al., 2019). The diagnosis of nerve damage may be aided by the use of electromyography (EMG), which has been used in avian species previously to determine neural deficits in peripheral limbs (Holland & Jennings, 1997).

Injury grade based on the modified pododermatitis scale, was highly predictive of hospital survival. Grade 4 injuries had the best prognosis, likely due to bite location on the upper leg or back where there was increased muscle coverage. Grade 6 and 7 injuries had the poorest prognosis, as they often involved damage to underlying tendons/ligaments, nerves or bone. The modified Cierny Mader osteomyelitis and chronicity scales were unhelpful predictors of prognosis. However chronicity was helpful to predict reoccurrence, with birds presenting with chronic injuries at admission more likely to present again for a recrudescent illness.

Several treatment factors were associated with hospital survival. Antibiotic duration predicted hospital survival, with courses of less than one week in length associated with mortality, and those of 4-5 weeks duration associated with survival to release. There are likely confounding factors affecting these results though, as treatment length and duration were not randomly selected. Wound management technique also had a significant effect on hospital outcome. Staged closure was more likely to have a favourable outcome than a completely open or closed approach. This was an unexpected result, as it was predicted that wounds that were managed with primary closure to have been the least serious, and hence to be a better prognosis. Due to the retrospective nature of this study and that clinical judgement was used to determine the best treatment approach for each presenting wound, selection of wound management approach was also not random, and therefore it is difficult to make conclusions about the best wound management approach.

Length of time in care was an interesting indicator of survival to release. The majority of non-survivors died within the first few weeks of care. This is probably due to animals being euthanised early, or dying in the initial stages of treatment due to the extent of their injuries. Surviving birds stayed an average of 51 days in hospital and rehabilitation. Length of stay was commonly extended by commencement of moult, as injuries usually occurred just prior to the moulting period. One bird that was held in captivity for more than 300 days, survived and was released. Length of time in care did not affect post-release outcome.

3.4.7 Necropsy review

The necropsy review captures information about yellow-eyed penguins attacked by marine predators, which either died in the wild or in human care. The true prevalence of yellow-eyed penguin mortality from marine predation is unknown, as the data does not capture the birds that died at sea or are undiscovered on land. The subset of birds examined at necropsy was more likely to have been biased towards birds that had severe injuries with secondary complications.

Due to the close proximity of tendon and bone to the skin on the dorsal aspect of distal limb of penguins, localised infection and damage to these structures was common in the most severely injured birds. Nearly half of all birds that were euthanised or died due to their marine predator injuries had osteomyelitis. Tendon damage or tenosynovitis was most common on the dorsal aspect of the foot, in particular involving the extensor digitorum longus and cranialis tibialis. Due to presence of a deep fat pad (corpus adiposum plantare profundum) and superficial fat body (corpus adiposum plantare superficiale) between the skin and the flexor tendons on the plantar aspect of the foot, significant injury or infection to flexor tendons was rarely observed.

Surprisingly more than half of the deceased birds presented for necropsy examination after a period in captivity had some form of secondary complication likely due to the captive environment. Mycotic lower respiratory tract infections were the most common complication, representing 18% of all captivity complications. Fungal cultures were not performed in the majority of cases, however it was suspected that the likely causative agent was *Aspergillus spp.,* considering the frequency of isolation in other penguin species (Appleby, 1962; Xavier et al., 2011; Reed et al., 2020), based on the finding of characteristic fungal hyphae on histopathology cases and limited testing of respiratory isolates when available. Fungal pathogens, such as *Aspergillus*, are ubiquitous in the environment. Infection usually occurs due to the inhalation of a high spore load, or underlying immune deficiency of the host (Doneley, 2016). Fungal respiratory infections are very commonly seen in captive penguins (Appleby, 1962; Xavier et al., 2011; Itoh et al., 2020; Krol et al., 2020; Trumpp et al., 2021), but are rarely seen in wild birds (Ewbank et al., 2021). Predisposing factors include inadequate ventilation, immunocompromise from stress (e.g. frequent handling or transportation of individuals between facilities) or concurrent diseases (Fischer & Lierz, 2015).

Preventative measures against *Aspergillus* infection are multifactorial and should include adequate ventilation, reducing stress for optimal immunological function, and prophylactic antifungal medication for all captive penguins. Adequate air handling systems to turnover air at 7-8 cycles/hour and the use of non-high-efficiency particulate absorption pleated filters (e.g. Airguard Type DP40 filters) can reduce environmental *Aspergillus* load in indoor facilities (Rivas et al., 2018). Penguin handling and movement between facilities should be kept to a minimum, to reduce stress and subsequent immunosuppression (Silva Filho et al., 2015). Prophylactic oral antifungals were used for almost all penguins admitted to veterinary wildlife hospitals in this study, but these results suggest that this was ineffective at preventing aspergillosis. Recent pharmacological studies indicate that to reach therapeutic steady state plasma

concentrations in Humboldt penguins, doses of 8.5mg/kg PO BID or 20mg/kg PO SID of commercial itraconazole capsules may be required (Bunting et al., 2009). Conversely, a prophylactic dose of itraconazole (Sporonox) 5mg/kg PO once daily seemed adequate in the prevention of aspergillosis in seabird species after an oil spill event, with *Aspergillosis* identified as the cause of death in only 1/428 birds; where nearly 400 of the birds rehabilitated were little penguins (Gartrell et al., 2019). Compounded itraconazole used at a dose of 15mg/kg orally once daily given to Magellanic penguins was found to be only slightly protective against the development of aspergillosis (Silva Filho et al., 2015). However there is also evidence to suggest that a generic compounded itraconazole powder was unable to maintain therapeutic plasma concentrations, compared to the commercial capsule formulation (Sporonox) (Bunting et al., 2009). It is unknown how well the human capsule formulation (Itrazole), used by one veterinary wildlife hospital in this study, is absorbed in birds.

Alternatively antifungal resistance to itraconazole may have reduced the efficacy of preventative measures. *Aspergillus* strains can show variable resistance to antifungal agents (Yerbanga et al., 2022). Resistance to itraconazole has been reported in 100% of *Aspergillus fumigatus* strains cultured from archived samples from a series of zoological collections in the UK. There was, however, 100% susceptibility of the same strains to terbinafine and voriconazole in vitro (Reed et al., 2020). There is reasonable evidence to suggest that terbinafine at a dose of 15mg/kg PO SID (Bechert et al., 2010) reaches therapeutic levels in African penguins for the treatment of aspergillosis infections. Voriconazole may also be a feasible future option for the prevention of mycotic infections in the future, with studies indicating that a dose of 5mg/kg PO SID may be safe and effective (Hyatt et al., 2017), however individuals should be closely monitored as toxicities have been reported in several penguin species (Hyatt et al., 2015). In future cases of mycotic pneumonia in yellow-eyed penguins, the organisms involved should be cultured and/or sequenced for identification and determination of antifungal sensitivities.

The second and third most prevalent hospital complications were bacterial respiratory infections and tracheal stenosis, representing 17% and 12% of captivity complications respectively. Bacterial pneumonia and air sacculitis were attributed to a range of causes including septicaemia or aspiration pneumonia. Tracheal stenosis was seen approximately 7-14 days post-intubation under general anaesthesia. It is suspected that in most cases the tracheal mucosa sustained trauma, likely due to the placement of an oversized endotracheal tube into the airway or excessive movement of the tube (Doneley & Raidal, 2010; Jankowski et al., 2010). In some cases, it may have otherwise been caused by acidic irritation of the tracheal mucosal lining by the aspiration of small amounts of regurgitated material. Over several weeks, the damaged tissue exhibits an excessive response depositing granulation tissue in the lumen of the airway, such that the bird eventually asphyxiates. In all cases, soft silicon Cole endotracheal tubes, designed for birds, were used to intubate the penguins. Substantial care should be taken when repositioning intubated patients to reduce the risk of trauma to the trachea, including disconnecting the bird from the anaesthetic circuit before changing position, and ensuring to use appropriately sized endotracheal tubes.

Seven individuals had gastrointestinal ulceration or perforation, accounting for 11% of observed hospital complications. The lesions were all centred around the proventriculus, with one case also involving the oesophagus, and another involving the ventriculus and duodenum. Some of these cases were on meloxicam, a non-steroidal anti-inflammatory, which can cause gastrointestinal ulceration (Summa et al., 2017). Recent pharmacokinetic studies suggest a prolonged half-life of meloxicam in penguins, recommending an oral dosing regimen of 1mg/kg every 48 hours (Morrison et al., 2018). Most yellow-eyed penguins treated for marine predator wounds were given meloxicam at a dose of 0.5-1mg/kg PO BID, in accordance with recommended parrot doses, with the lower dose more commonly used historically (Wilson et al., 2005) and higher doses adopted based on more recent publications (Molter et al., 2013; Montesinos et al., 2017), before pharmacological information was available for penguins. Other potential causes of gastrointestinal ulceration include stress, bacterial overgrowth within the proventriculus or ventriculus, penetrating foreign bodies or direct trauma from the marine predator attack. Pre-emptive gastroscopy could potentially be performed of penguins under the initial general anaesthetic, to investigate presence of gastrointestinal ulceration on admission, and repeated for any birds displaying regurgitation or melaena during treatment, to further investigate causes.

Avian malaria was considered a potential secondary hospital complication due to its much higher prevalence in yellow-eyed penguins located in rehabilitation centres (65.9%) compared to the wild mainland population (6.8%), indicating a possible risk factor for the spread of disease (Kay, 2021). Our necropsy survey revealed avian malaria to be present in 3/50 (6%) individual captive penguins, similar to the prevalence in wild populations, suggesting it was not over-represented in captive individuals undergoing hospital treatment.

3.5 Conclusion

Uplifting wild animals for treatment in veterinary hospitals or rehabilitation centres is a major undertaking, not without risk for the individual animal and the entire population (Alden et al., 2021). Many species encounter considerable stress in an unfamiliar environment with regular handling, and nosocomial infections are possible. There are frequent conspecific and interspecific interactions directly and indirectly in veterinary hospitals, thus there is also the potential for disease transmission (Morley, 2002; Weese, 2012; Burgess & Morley, 2015).

This study indicates that the majority of wild yellow-eyed penguins with marine predation injuries uplifted for veterinary care had significant injuries that warranted treatment. Wounds were often polyclonally infected, and rates of antimicrobial resistance were high in bacterial isolates tested. The majority of injured yellow-eyed penguins survived to release, with more than half of these individuals resighted again and 28.4% of all released individuals attempting to breed post-release. Injury recrudescence rates were relatively low, and multiple predator attacks to the same individuals were common. The presence of secondary complications from the initial wounding such as osteomyelitis, tendon damage or nerve damage were the most significant predictors of survival. The most common complication of hospitalisation observed in necropsied yellow-eyed penguins were mycotic respiratory infections, therefore efforts should be targeted to reduce the risk of these infections through a multifaceted approach.

By examining complications affecting yellow-eyed penguins brought into captivity for treatment, risk factors were identified, preventative measures examined, and knowledge gaps were addressed to increase the likelihood of successful treatment, rehabilitation and release of individuals. The high prevalence of severe injuries in hospitalised birds and good post-release survival indicates that treatment of these birds is justifiable on ethical and conservation grounds.

CHAPTER FOUR

Discussion

4.1. Key findings

Definitive proof of predation events in the marine environment is difficult to obtain, and studies with this information are rare. It is therefore difficult to understand the interactions that occur between penguins and their predators, including the magnitude of potential increased predation to yellow-eyed penguins, and whether these events are partially responsible for current decline in the northern population of this species. This study has investigated potential marine predators, by the identification of possible shark species from which a tooth was isolated from a penguin leg wound. It has also involved a pilot study examining the dentition in several species of teleost fish and New Zealand sea lions, by measuring tooth dimensions and interdental distances, and visualising possible bite impressions from these predators.

This study has demonstrated a substantial increase in the numbers of yellow-eyed penguins seen by veterinary clinics following marine predation since 2014. In some years, more than 10% of mainland yellow-eyed penguins are taken into captivity for wound treatment, often during the chick rearing phase. Two parent birds are usually required to successfully rear a chick prior to post-guard stage (Marchant & Higgins, 1990), therefore any disruption to this process, such as uplifting a bird for medical treatment, usually results in any chicks being uplifted for hand-rearing. A review of necropsy reports has also supported the increased trends seen by veterinary clinic data in the last decade.

Using the modified pododermatitis grading scale, injuries can be categorically assessed by severity, based on the presence of infection or necrotic tissue, involvement of underlying structures or loss of function in the limb due to nerve or tendon damage. Approximately 84% of yellow-eyed penguins brought into veterinary care had Grade 3 injuries or above, indicating that most uplifted individuals would have benefited from veterinary treatment in hospital.

There was an alarming amount of antibiotic resistance displayed by the bacterial isolates cultured from yellow-eyed penguin wounds in this study. Due to this being a retrospective analysis, our findings are likely influenced by biased case selection. Culture samples were not collected routinely by all veterinary wildlife hospitals. Some individuals were sampled after a lack of initial response to antibiotic treatment. Despite this, one alarming finding was the high prevalence of multi-drug resistant strains of bacteria present in these wounds.

Hospital survival was closely related to the severity of initial injury, indicated by the modified pododermatitis scale, as well as the presence of tendon or ligament damage, nerve damage and osteomyelitis. Penguins with tendon or ligament damage were nine times less likely to survive to release. Those with nerve damage were seven times less likely and those with osteomyelitis were three times less likely to survive. Hospital survival was independent of chronicity, which was surprising, as some complications (such as osteomyelitis) can be prevented with rapid treatment, including antibiotic coverage and appropriate wound care. Chronicity was predictive of injury

recrudescence, such that one priority should be the prompt identification and uplifting of injured penguins.

Post-release monitoring information of wildlife is rarely obtained, particularly for marine species where long-term monitoring can be expensive and time-consuming. By utilising ecological data available through ongoing population monitoring, and combining it with veterinary information, we were able to follow individuals post-release, and correlate their outcomes with clinical, pathological and treatment factors. Post release outcomes appeared favourable for birds that received veterinary treatment for their wounds, however, due to the retrospective nature of this study, and without a control group comparing treated and untreated birds with post-release outcome, it is very difficult to make strong conclusions about the effect of treatment for long-term survival and breeding. These are also highly dependent on a range of other external factors, as the species faces a substantial number of major threats affecting their survival and breeding success, independent of physical injuries due to marine predation.

The majority of deceased yellow-eyed penguins which were necropsied by Wildbase Pathology died as a result of severe secondary complications of the original injury, or from a disease acquired in the captive environment. Due to the anatomy of penguin limbs, underlying structures such as nerves, tendons and bone are at risk of damage or infection when predators afflict bites. Examining the secondary complications of captive care allow major complications to be identified, and risk mitigation strategies to be implemented, in an attempt to reduce the mortality risk to individuals brought into care.

4.2. Scientific questions raised

The true numbers of marine predation attacks on yellow-eyed penguin populations are unknown, due to the lack of evidence remaining after some predation events (i.e. the penguin is consumed by the predator, or the penguin dies at sea before it can return to shore). It is difficult to quantify the extent that changing monitoring effort and interventions may contribute to an increase in frequency of yellow-eyed penguins receiving veterinary treatment, and thus get an accurate understanding of the magnitude of the increased predation pressures.

With marine predation believed to be a driving factor behind population declines in other penguin species worldwide (Ryan & Kerr, 2012; Horswill et al., 2014; Weller et al., 2016; Morrison et al., 2017; Colombelli-Négrel et al., 2022), it is important not to dismiss this as a threat. There is substantial evidence to suggest that tens of thousands of species are changing their distributions worldwide as a result of climate change (Lenoir et al., 2020), which may alter the frequency of predator interactions or prey availability, potentially causing a predator to prey-switch to penguins or consume penguins as a higher proportion of their diet. Expanding on this investigation to include other penguin species in New Zealand presenting with similar wounds may be instructive for understanding the ecological interactions occurring.

Larger sample sizes and visiting wild penguin sites at concurrent times of the year, would have helped gain a better understanding of the spatial distribution of attacks. Regardless, this study has shown that 50% of mainland birds have scars or active wounds suggestive of a marine predator attack at some point during their life. Investigation of the subantarctic population of yellow-eyed penguins for scars and active wounds indicative of marine predation would be useful to indicate latitude restrictions of predators attacking yellow-eyed penguins. This could also be important to establish a baseline for the southern population, in case a novel predator becomes an emerging threat to this populations in the future.

Further work could be undertaken to investigate potential predators of yellow-eyed penguins in New Zealand. Bite impressions could be explored further using jaws from common New Zealand shark species, using both flat sections of modelling clay, and clay moulded to replicate a life-size yellow-eyed penguin model with representative dimensions. This may help correlate bite wound characteristics seen in yellow-eyed penguins with potential predators specific to New Zealand. Measuring the interdental distances for a larger sample size of possible predatory teleost fish, sharks and New Zealand sea lions, may also help identify likely species. Wound depth has the potential to rule out certain predators from some wounds, and should be routinely measured along with length, width and spacing.

To definitively correlate wound pathology with predator species, direct evidence is required. PCR testing of swabs collected from bite wounds may be instructive for the predator species involved. Alternatively, GoPro footage currently being used to assess yellow-eyed penguin diet may inadvertently capture footage of a marine predator attack, assuming the camera is retrievable from the penguin afterwards, otherwise predation attacks closer to the coastline may be observed by eyewitnesses, although this would need to be appropriately documented for expert verification of species involved.

A review of treatment information did not provide clear evidence for optimal treatment approaches to penguin wounds; presumably because wounds vary significantly in depth, severity and structures involved. One of the most debatable decisions in the treatment of osteomyelitis in humans is appropriate duration of treatment. Excessively long courses of antibiotics promote antimicrobial resistance (Lodise et al., 2007; Costelloe et al., 2010; Llewelyn et al., 2017), but insufficient doses of antibiotics may lead to recrudescent infection. Research investigating the validation of C-reactive protein in yellow-eyed penguins as a diagnostic tool to indicate the reduction of inflammation, alongside repeat white blood cell counts and radiographs, could be useful in the future.

4.3. Conservation implications and field applications

The northern population of yellow-eyed penguins is under increasing pressures from a range of processes, threatening their survival on the mainland of New Zealand. Management interventions are necessary, to support the survival of this species in future decades. There are substantial challenges associated with monitoring yellow-

eyed penguins, due to their low population density over a wide stretch of New Zealand coastline. However, it is believed that ongoing financial investment in monitoring and researching this species is essential to their persistence on the New Zealand mainland (Hoiho Governance Group, 2019). Technology, such as motion sensor cameras, could be utilised on some of the more commonly used penguin tracks at major breeding sites, to quickly identify injured or unwell birds, so that timely interventions can occur.

It is hoped that this study will provide more clarity regarding the different types of injuries seen in marine predation events of yellow-eyed penguins, and a deeper understanding of the severe complications that can occur as a result of the injury. Our findings indicate that foot injuries can be severe and have detrimental effects on the individual, depending on the extent of injury or infection and structures involved. This research can be used by ecologists, researchers and rehabilitators assessing penguins in the wild to guide and support their decisions in uplifting birds for veterinary assessment and treatment. In particular, the visual depiction of the grading scale may prove a useful tool for assessing wounds in the field. At the same time, people should be aware of the limitations of this scale, such that the diagnosis of osteomyelitis, tendon and nerve damage is likely to require veterinary assessment and diagnostic imaging. Therefore any injuries that are likely to be classed as Grade 3 or above (i.e. active injuries that aren't merely superficial and non-infected) should be assessed by a veterinarian on the basis of animal welfare grounds. Any bird shown to be dragging a limb should be immediately uplifted and taken to a veterinarian.

This study provides evidence that a reasonable proportion of yellow-eyed penguins with marine predator injuries can be successfully treated and released, despite intensive wound management, at times long antibiotic courses, and the requirement to house them in temporary captive care. It is also encouraging that a reasonable proportion of penguins contribute to the wider population through breeding attempts post-release.

With antimicrobial resistance being identified as one of the top ten threats to human health in 2019 (World Health Organisation, 2019a), all antibiotic use should be considered judiciously. To minimise the risk of antibiotic resistance, it is our recommendation that microbial culture samples are collected routinely on all patients prior to starting antibiotic therapy, and antimicrobial therapy adjusted based on culture and sensitivity results. Deep tissue or bone biopsies collected under general anaesthesia are considered the most optimal samples, to reduce the risk of contamination from skin flora that is more likely by swabbing wounds (Conterno & Turchi, 2013; Heravi et al., 2019; Senneville et al., 2020). Ideally both aerobic and anaerobic bacterial culture would be undertaken, and potentially fungal cultures if indicated by cytology. Cytology is also recommended for all wounds, including performing a gram stain while awaiting bacterial culture and sensitivity results. Repeat culture and sensitivity testing should be performed on any wounds that are not responding as expected, as additional organisms may have colonised during hospitalisation, or antimicrobial resistance may have been acquired by existing organisms. Whilst routine culture and sensitivity testing will increase the cost of treating individual birds considerably, it is regarded as gold standard practice, and may reduce hospitalisation times by avoiding treatment delays using

inappropriate first-line antibiotics when resistance is present. Additional funding of veterinary treatment of yellow-eyed penguins may be required to facilitate this testing. Various diagnostic imaging modalities may be further explored to diagnose secondary injury complications more rapidly. Johnson et al. (2019) showed that tendon ruptures could be readily diagnosed using MRI or ultrasound in deceased yellow-eyed penguins. This may facilitate the exploration of further treatment options for tendon and ligament repair in yellow-eyed penguins, or alternatively earlier euthanasia may be implemented in cases with extensive damage to major tendons. Radiographic delay in imaging osteomyelitis also provides challenges for assigning appropriate treatment to patients. For cases of uncertainty, MRI or a combination of radionucleotide bone and labelled white blood cell scanning may prove useful in diagnosing osteomyelitis in these cases (Lipsky et al., 2012). Alternatively if these diagnostic techniques are unavailable, the use of serial radiographs several weeks later is recommended.

Alternative treatment options to systemic oral or intravenous antibiotics could also be investigated by veterinarians. Topical wound treatment solely, or more frequent use of antibiotic impregnated beads in wound sites may be useful in limiting whole microbiome exposure to antimicrobials. The use of regional limb perfusion for antibiotic or anti-inflammatory delivery, could also be explored. This technique has been documented in a domestic chicken (*Gallus gallus domesticus*) for the treatment of a unilateral cellulitis and deep pododermatitis. In this case a tourniquet was placed on the affected leg and a single dose of intravenous antibiotic and an anti-inflammatory were delivered into the medial metatarsal vein, flushing with heparinised saline in between, and the tourniquet was removed 15 minutes later. An assessment of treatment response made several weeks later suggested substantial clinical improvement based on improved ambulation, reduced surface skin temperature and limb diameter, and reduced white cell count and total protein (Ratliff & Zaffarano, 2017).

This epidemiological, clinical and pathological review of marine predation in yellow-eyed penguins highlights the usefulness of exploring ecology and veterinary medicine as two intertwined disciplines for the conservation of wildlife. It is hoped that the information in this study can be used by ecologists, researchers, wildlife rehabilitators and veterinarians to appraise the current interventions that are being undertaken in the northern population. Further research is required to more thoroughly understand marine predator interactions with yellow-eyed penguins, and to help determine drivers behind potential increased predation events. With ongoing medical developments, in both the human and veterinary fields, new diagnostic and treatment measures can be explored to improve clinical outcomes and reduce complications from captivity.

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Appendices

Appendix 1: Initial haematology results of individual yellow-eyed penguins hospitalised at veterinary wildlife hospitals for marine predator injuries.

Subject #	Day of Hospital	Chronicity at Presentation	Hospital Survival: Died (0), Released (1)	PCV (%)	Estimated WCC (x10^9)	Heterophils (x10^9)	Heterophils (%)	Eosinophils (x10^9)	Eosinophils (%)	Basophils (x10^9)	Basophils (%)	Lymphocytes (x10^9)	Lymphocytes (%)	Monocytes (x10^9)	Monocytes (%)
1	2	Acute	1	51											
2	12	Chronic	1	24											
4	8	Acute	1	36											
7	9	Acute	1	20											
8	20	Acute	0	44											
9	6	Acute	1	45											
10	3	Chronic	0	27											
11	14	Chronic	1	23											
12	2	Acute	1	25											
13	1	Acute	1	54											
15	1	Chronic	0	44											
17	6	Chronic	1	48											
18	2	Chronic	1	46											
20	1	Acute	0	24											
23	1	Chronic	0	20											
24	33	Chronic	1	37	21.8	17.4	80	0	0	0	0	4.4	20	0	0
26	3	Acute	1	46											
30	1	Chronic	1	48											
34	3	Chronic	1	58											
35	5	Acute	0	16											
37	1	Chronic	1	43											
40	15	Chronic	0	54	19.6	11.4	58	0	0	0	0	7.6	39	0.6	3
41	4	Acute	1	35											

42	2	Acute	1	36											
53	11	Acute	1	26											
54	2	Chronic	1	46											
57	1	Acute	0	38											
60	16	Acute	1	35											
63	3	Acute	0	36											
64	3	Chronic	0	26											
65	2	Acute	1	57											
70	10	Acute	1	42	14.8	12	81	0	0	0	0	2.7	18	0.1	1
71	2	Acute	1	44											
79	2	Acute	0	20											
80	4	Acute	1	30											
86	U	Acute	1	32											
89	2	Acute	1	42											
90	6	Chronic	1	22	27.8	18.3	66	0.3	1	0	0	7.2	26	2	7
96	12	Chronic	1	40											
99	10	Chronic	0	30											
100	5	Acute	1	55											
101	1	Acute	0	29											
106	1	Chronic	1	30	7	3.4	48	0	0	0	0	3.3	47	0.3	5
107	10	Chronic	1	45											
111	4	Chronic	1	35											
112	2	Acute	1	28											
113	2	Acute	1	58											
118	11	Acute	1	39											
121	8	Acute	1	52											
122	4	Chronic	0	62	34.6	17.3	50	0	0	0	0	16.6	48	0.7	2
126	5	Acute	1	55											
127	16	Acute	1	23											
128	6	Acute	1	23											
131	9	Chronic	1	64											
132	10	Chronic	0	37	27.8	11.9	43	0	0	0.3	1	15	54	0.6	2
133	9	Chronic	0	55	40.5	36.9	91	0	0	0.8	2	2.4	6	0.4	1

134	13	Acute	0	40	38.5	32.7	85	0.8	2	3.1	8	0	0	1.9	5
135	74	Chronic	1	44	34.1	24.9	73	0	0	1	3	5.5	16	2.7	8
137	48	Acute	0	47	17	12.4	73	0	0	0	0	3.6	21	1	6
138	22	Chronic	1	44	52	45.8	88	0	0	0	0	6.2	12	0	0
140	27	Acute	1	49	26.5	22.3	84	0	0	0.3	1	2.9	11	1	4
141	2	Chronic	0	26	8.9	6.4	72	0.5	5	0	0	1.5	17	0.5	6
142	4	Chronic	1		15.2	13.4	88	0	0	0	0	1.8	12	0	0
143	5	Chronic	0	13											
145	8	Acute	0	37	17.1	12.8	75	0.4	2	0	0	2.2	13	1.7	10
146	7	Acute	0	37	14	6.6	47	0.3	2	0.4	3	6	43	0.7	5
148	2	Acute	1	27											
149	6	Acute	1	30	32.1	26	81	0	0	0	0	3.2	10	2.9	9
150	2	Chronic	1	56	18.3	16.7	91	0.5	3	0	0	1.1	6	0	0
152	22	Chronic	0	12	87	79.2	91	0.9	1	0.9	1	4.3	5	1.7	2
153	5	Chronic	0	40											
154	5	Acute	1		46.6	30.3	65	0	0	0	0	16.3	35	0	0
155	12	Acute	1	50											
156	2	Acute	0	20	29.3	25.2	86	1.2	4	0	0	2.6	9	0.3	1
157	3	Chronic	0	24	19.2	14.4	75	0	0	0	0	4.6	24	0.2	1
158	29	Chronic	1	37	37.8	35.5	94	0	0	0	0	0.4	1	1.9	5
159	107	Chronic	1		14.7	8.9	61	0	0	0	0	5.8	39	0	0
162	42	Chronic	1	36	12.4	10.4	84	0	0	0	0	2	16	0	0

Appendix 2: Initial biochemistry results of individual yellow-eyed penguins hospitalised at veterinary wildlife hospitals for marine predator injuries.

Subject #	Admission #	Day of Hospital	Chronicity at Presentation	Hospital Survival: Died (0), Released (1)	TP g/L	AST U/L	CK U/L	UA umol/L	Glu mmol/L	Ca mmol/L	Phos mmol/L	Ca:P Ratio	K+ mmol/L	Na+ mmol/L
3	1	7	Chronic	0	43	610	241	934	8.2	2.43	1.41	1.72	5.3	153
4	1	8	Acute	1	50									
7	1	9	Acute	1	39	372	358	1217	14.5	2.39	2.35	1.02	5.6	146
8	1	20	Acute	0	69	HAEM	1291	>2975	>38.9	2.42	4.24	0.57	HAEM	151
11	1	14	Chronic	1	44	168	255	361	10.2	2.57	1.06	2.42	5.4	152
12	1	2	Acute	1	46									
13	1	1	Acute	1	64	324	2142	332	14.6	2.64	1.08	2.44	4.6	148
15	1	1	Chronic	0	64									
16	1	3	Chronic	1	48	226	146	171	12	2.7	0.83	3.25	4.7	148
21	1	2	Chronic	1	55	189	426	653	12.7	2.78	0.88	3.16	4.2	149
24	1	33	Chronic	1	63	389	288	171	11.3	2.61	0.95	2.75	4.7	142
30	1	1	Chronic	1	52	173	303	174	19.2	2.39	0.31	7.71	4	150
31	1	2	Acute	1	56	776	1594	260	13.8	2.75	1.46	1.88	4.7	151
34	1	3	Chronic	1	63	>2000	>14000	536	12.9	2.78	2.24	1.24	11.8	168
40	1	15	Chronic	0	56	253	894	259	14.9	2.85	1.15	2.48	4.1	155
53	1	11	Acute	1	57									
70	1	10	Acute	1	51	331	518	178	13.7	2.37	0.74	3.20	4.6	145
86	1	U	Acute	1	54									
90	1	6	Chronic	1	50	283	396	148	12.4	2.96	1.58	1.87	4.1	147
104	1	2	Acute	1	58	HAEM	389	1377	12.5	2.59	2.76	0.94	HAEM	144
106	1	1	Chronic	1	48	372	584	209	15.7	2.42	1.03	2.35	3.9	150
112	1	2	Acute	1	54	190	220	171	10.8	2.68	1.17	2.29	5.1	148
118	1	11	Acute	1	34									
121	1	8	Acute	1	50									
122	1	4	Chronic	0	65	399	831	68	13.6	2.75	1.01	2.72	3.2	150
127	1	16	Acute	1	50									
128	1	6	Acute	1	45	210	294	76	11.7	2.47	1.86	1.33	5	151
132	1	10	Chronic	0	73	121	866	734	12.8	2.77	1.54	1.80	3.9	144
133	1	9	Chronic	0	74	286	421	994	16.3	2.85	0.68	4.19	2.8	153
134	1	13	Acute	0	67	174	376	390	16.3	2.73	1.14	2.39	3.1	148
135	1	74	Chronic	1	64									
137	1	48	Acute	0	66									
138	1	22	Chronic	1	66	1402		428	13.2	2.85	1.15	2.48	6.5	145

140	1	27	Acute	1	74	255	80	364	11	2.99	0.69	4.33	4.6	147
141	1	2	Chronic	0	62	273	658	196	15.2	2.77	1.09	2.54	3.1	152
142	1	4	Chronic	1	43	352	952	295	14.2	2.47	0.53	4.66	4.4	151
143	1	5	Chronic	0	54									
145	1	8	Acute	0	55	312	206	204	14.3	2.68	0.74	3.62	3.8	145
146	1	7	Acute	0	36	389	842	553	12.4	2.44	1.41	1.73	5.4	144
147	1	55	Acute	0		224	433	156	12.3	2.58	0.42	6.14	2.6	146
148	1	2	Acute	1	61	394	1167	124	10.6	2.7	0.83	3.25	4.7	146
149	1	6	Acute	1	74	289	626	160	13.9	2.96	0.85	3.48	3.7	146
150	1	2	Chronic	1	52	367	1193	105	14.4	2.56	1.51	1.70	3.7	147
152	1	22	Chronic	0	70									
153	1	5	Chronic	0	50	859	714	906	12.4	2.22	1.13	1.96	5.1	149
155	1	12	Acute	1	42	460	1271	307	17.8	2.77	1.85	1.50	4.7	148
156	1	2	Acute	0	56	238	660	353	13.8	2.82	0.65	4.34	3.5	149
157	1	3	Chronic	0	21	365	1576	896	13.9	2.13	1.11	1.92	4.8	148
158	1	29	Chronic	1	70	385	1699	358	15	2.53	1.12	2.26	5.1	151
162	1	42	Chronic	1	62	261	495	501	12	2.76	1.57	1.76	4.7	145

Appendix 3: Bacterial isolates and antibiotic sensitivities for all initial samples collected from marine predator wounds.
AMX (amoxycillin-clavulanic acid), CLI (clindamycin), CET (cephalothin), ENR (enrofloxacin), TMS (trimethoprim sulphonamide), AMK (amikacin), CIP (ciprofloxacin), CAZ (ceftazidime), GEN (gentamicin), AMP (ampicillin), MTZ (metronidazole), NEO (neomycin), PMB (polymyxin B), TOB (tobramycin), TIC (tilcarcillin), DOX (doxycycline), CEF (cefovecin).

Subject #	Organism #	Organism	Acute/ Chronic @ presentation	Day of Hosp	Amount of Growth	AMX	CLI	CET	ENR	TMS	TET	AMK	CIP	CAZ	GEN	AMP	MTZ	NEO	PMB	TOB	TIC	DOX	CEF
2	1	*Bacillus thuringiensis*	Acute	1	Moderate	I	S	I	S	S							R	S	S				
2	2	*Clostridium perfringens*	Acute	1	Light																		
35	1	*Escherichia coli*	Acute	3	Heavy	I	R		S	I				S	S		R						
35	2	*Kocuria rhizophila*	Acute	3	Heavy	S	S		R	S				R	S		R						
35	3	*Azoarcus sp BH72*	Acute	3	Heavy	R	R		R	R				R	R		R						
35	4	*Clostridium perfringens*	Acute	3	Heavy																		
40	1	No bacteria isolated	Chronic	15	None																		
58	1	*Pantoea agglomerans*	Acute	4	Light	S	R		S	R				S	S		R						
58	2	*Edwardsiella hoshinae*	Acute	4	Heavy																		
60	1	*Streptococcus canis*	Acute	6	Heavy	S	R		S	S				S	S		R						
60	2	*Proteus mirabilis*	Acute	6	Heavy	S	R		S	S				S	S		R						
120	1	*Staphylococcus sciuri*	Chronic	3	Moderate	S	S		S	R				S	S		R						
120	2	*Clostridium perfringens*	Chronic	3	Heavy																		
122	1	No bacteria isolated	Chronic	3																			
132	1	*Escherichia coli*	Chronic	12	Scant	R	R	R	R	R	S	S	S	S	S								
132	2	*Pseudomonas aeroginosa*	Chronic	12	Light	R	R	R	R	R	R	S	R	S	S								
132	3	*Enterococcus*	Chronic	12	Scant	S	R	R	M	R	S	M	R	R	S								
135	1	*Clostridium perfringens*	Chronic	16	Scant																		
137	1	*Staphylococcus aureus*	Acute	51	Scant	S	S		S	S	S		S	R									
137	2	*Clostridium perfringens*	Acute	51	Scant																		
145	1	*Pseudomonas*	Acute	8	Scant	S	R	S	S	S	S					R							

146	1	*Staphylococcus Coagulase -*	Acute	16	Scant	I	R	S	R	R	R	S	R		R	R				R	R		
147	1	*Escherichia coli*	Acute	55	Light	R			R	R	M	S		S									
147	2	*Enterococcus*	Acute	55	Light	S			R	R	S	R		R									
147	3	*Klebsiella*	Acute	55	Light	R			R	R	S	S		S									
147	4	*Staphylococcus Coagulase +*	Acute	55	Scant	S			S	S	S	S		R									
149	1	*Staphylococcus aureus*	Acute	5	Heavy	S	S	S	S	S	S	S	S		S	S				S	S		
150	1	No bacteria isolated	Chronic	4																			
156	1	*Staphylococcus intermedius*	Acute	2	Heavy	S	S	S	S	S												S	S
156	2	*Escherichia coli*	Acute	2	Heavy	S	R	M	S	S												S	S
158	1	*Corynebacterium*	Chronic	29	Moderate	S			S	S	S	S		S									
158	2	*Escherichia coli*	Chronic	29	Moderate				M	S	S	S		S									
158	3	*Edwardsiella tarda*	Chronic	29	Moderate	S			M	S	S	M		R									
158	4	*Enterococcus*	Chronic	29	Moderate	S			M	S	S	M		R									
159	1	*Escherichia coli*	Chronic	118	Heavy	M	R		S	R	M											M	
159	2	*Enterococcus*	Chronic	118	Heavy	S	R		S	S	S											S	
159	3	*Pseudomonas maltophilia*	Chronic	118	Heavy	S	R		M	S	S											S	
159	4	*Corynebacterium*	Chronic	118	Heavy																		
159	5	*Clostridium perfringens*	Chronic	118	Heavy																		