



# 11<sup>th</sup> International Penguin Congress

Viña del Mar, Chile. 4-9 September 2023

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ABSTRACT BOOK

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## Organizing institutions



### Local Organizing Committee

Alejandro Simeone (Universidad Andrés Bello)  
Juliana Vianna (Pontificia Universidad Católica de Chile)  
Guillermo Luna-Jorquera (Universidad Católica del Norte)

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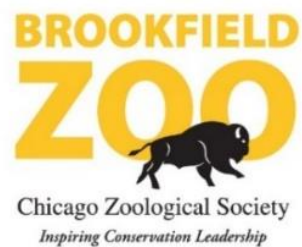
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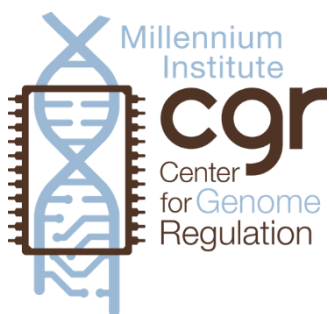


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RESERVA  
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# Keynote presentations



**Magellanic penguin**  
*Spheniscus magellanicus*

## Keynote speakers



**Dr. Juliana Vianna** is an associate professor at the Faculty of Biological Sciences and the UC Institute for Sustainable Development, principal investigator of the Lili Millennium Nucleus and the BASE Millennium Institute, and alternate director of the CRG Millennium Institute, in addition to directing other research projects in Chile. Her line of research is population genomics and phylogeography, addressing aspects such as the adaptation of penguins to climate change. Due to her research, she has been on the cover of prestigious magazines such as Science and PNAS for her findings on genes related to longevity and the origin of penguins, respectively.



**Dr. Rory Wilson** is a professor of Aquatic Biology and Sustainable Aquaculture in the Department of Biosciences, Swansea University, United Kingdom. Rory's work involves developing and using new methods, particularly animal-attached tags, to study the behavioural ecology of enigmatic animals that are, otherwise, difficult to study. He was bestowed a Rolex Award for Enterprise in 2006 for his animal tag developments. He was chief scientific consultant for Nat Geo's highly acclaimed 7-part series, 'Great Migrations', is listed in the top 50 conservationists in the BBC power list and is a Fellow of the Learned Society of Wales. He has published over 350 research articles and has been an editor for four peer-reviewed journals.



**Dr. Heather Lynch** works in the Department of Ecology & Evolution in the Stony Brook University in New York, USA. She applied her expertise in the statistical analysis of complex ecological datasets to a range of problems including patterns of survivorship in mammals, biodiversity patterns in dendritic ecological networks, and the effect of reproductive asynchrony in defining the geographic range of the bagworm. Her current research revolves around a large-scale vessel-based breeding bird survey program called the Antarctic Site Inventory, a project she manages in partnership with the non-profit research organization Oceanites. Her work to understand the complex spatiotemporal dynamics of Antarctic penguins, in particular their response to climate change on the Antarctic Peninsula.



**Dr. Andrea Raya** is an independent CONICET researcher at the Austral Center for Scientific Research (CADIC, CONICET), Ushuaia, Argentina. During the last 20 years, together with WCS, she has studied the ecology and conservation of seabirds at Austral latitudes of South America. She carries out long-term monitoring of the reproduction, feeding and spatial ecology of seabirds in the region. Her research helps to identify the critical points and the main threats in the marine ecosystem for the reproduction and feeding of seabirds in the southwestern Atlantic Ocean and the Beagle Channel and contributes to the creation and management of protected areas and guidelines for better practices for the conservation of seabirds and oceans.



## **A decade of genetics studies contributing to penguin knowledge and conservation**

**Juliana Vianna<sup>1,2,3,4</sup>**

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In the last years, an increasing number of studies have contributed to the understanding of the penguins' evolutionary history, adaptation, and population structure in a temporal and spatial context. Phylogenomic studies revealed that penguin crown-group originated during the Miocene in New Zealand and Australia, and that Aptenodytes is the sister group to all other extant penguin species. Penguin species have introgressed throughout much of their evolutionary history, mainly taxonomic groups such as Eudyptes. The use of microsatellite data or SNP from genomic data has revealed two patterns: 1) low or reduced population structure between penguin breeding colonies for most penguin species (Emperor, king, adelia, chinstrap, Humboldt, Magellanic, African, Galapagos penguin), with subtle differentiation is associated environmental variation; or 2) moderate to high population structure (gentoo penguin, southern and northern rockhopper penguins) associated with species ecology and philopatric behavior. Studies on genomic adaptation have revealed genes under selection during diversification and those allowing local adaptation. With the increasing of populational studies along the geographic distribution, new information and contributions to taxonomic clarification and species delimitation in several cases became available. Genomic data have supported for example: 1) three different rockhopper penguin species, southern, eastern, and northern rockhopper penguins; 2) four different taxa for gentoo penguin; 3) Macaroni and Royal penguins as one species but different subspecies. Therefore, the number of penguin species has changed which should be accompanied by management and conservation actions.

## **From musing to marvelling: Inroads into understanding penguins at sea**

**Rory P. Wilson<sup>1</sup>**

<sup>1</sup> Swansea University, United Kingdom.

Just 40 years ago, I used to muse about what penguins did at sea because virtually nothing was known about this part of their lives, despite them being the most specialised bird for life in, and under, the ocean. But advances in methods and technology have fundamentally changed that now. This talk will look at how we have advanced our approaches to study penguins at sea, what we have learnt about penguin behavioural ecology, and how it can help us understand how fickle prey and the cold, dark sea have shaped these birds to be the incredible creatures that they are. Finally, I will muse over what I believe are critical issues that we should address for the penguins of the future.

## **The Antarctic Penguin Biogeography Project and the Penguinindex provide models for data curation and exploration with opportunities for expansion to all penguin species**

**Heather Lynch<sup>1,2</sup>, Emma Talis<sup>2</sup>, Christian Che-Castaldo<sup>2</sup>**

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The Antarctic Penguin Biogeography Project is an effort to collate all public information about the distribution and abundance of Antarctic penguins and to make such data available to the scientific and management community. This database, which is continuously updated as new information becomes available, provides a unified and comprehensive repository of information on Antarctic penguin biogeography that contributes to a growing suite of applications of value to the community. One such application is the Mapping Application for Antarctic Penguins and Projected Dynamics (MAPPPD; [www.penguinmap.com](http://www.penguinmap.com)), a browser-based search and visualization tool designed primarily for non-specialists, and *mappdr*, an R package developed to assist the Antarctic science community. This dataset currently contains records of *Pygoscelis adeliae*, *Pygoscelis antarctica*, *Pygoscelis papua*, *Eudyptes chrysolophus*, *Aptenodytes patagonicus*, and *Aptenodytes forsteri* annual nest, adult, and/or chick counts conducted during field expeditions or collected using remote sensing imagery and made available through either published or unpublished sources. These data have been used to construct a version of the Living Planet Index, called the Penguinindex, which has identified key eras of change among Antarctic penguins. In this talk, we present these findings and make the case that this approach to data curation and exploration could be feasibly expanded to all penguin species, and though a significant effort would be required, the long term benefits for penguin conservation would be considerable. Not only would such a platform facilitate easier collaboration among scientists but it would ensure that global assessments of biodiversity incorporate the changes being observed for penguins globally.

## Protecting penguins and preserving oceans: Conservation efforts in Tierra del Fuego and Southern South America

**Andrea Raya Rey<sup>1,2,3</sup>**

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<sup>3</sup> WCS Representación Argentina, Amenábar, CABA, Argentina

Tierra del Fuego, located at the southern tip of South America, is a region of immense ecological importance, boasting a diverse array of wildlife and pristine marine ecosystems. The region is home to several penguin species. Penguins, as charismatic and iconic species, are an integral part of the rich biodiversity of Tierra del Fuego. However, these populations face numerous challenges, ranging from habitat degradation to climate change impacts.

Over the course of time, our objective has been to illuminate the pivotal matters concerning the conservation of penguins and the preservation of the ocean within this distinctive and ecologically significant region. In the current talk, I aim to present diverse approaches that we have pursued over the years, transitioning from research focused on conservation to the implementation of strategies for management and protection.

It is evident that despite the exponential growth in penguin research over the years, there persist gaps that hinder our comprehensive examination of certain facets necessary to address specific inquiries. Are the amassed data sufficiently robust to establish the vulnerability of these species to climate change and other stressors? Or do we possess the requisite data but necessitate more collaborative analysis to enhance our comprehension?

This talk will explore relevant dataset to assess penguin status, emphasizing the importance of long-term research, interdisciplinary and international collaboration. At the end protecting the penguins is preserving the ocean for us and future generations to come.



# Oral Presentations



King penguin  
*Aptenodytes patagonicus*



## 1. At the zoo, King penguins live longer, but age faster: methylation patterns reveal the cost of a sedentary life for an active bird

**Robin Cristofari<sup>1</sup>, Leyla Davis<sup>2</sup>, Trucchi Emiliano<sup>4</sup>, Maria Elena Figueroa<sup>3</sup>, Flavia Fernandes<sup>4,7</sup>, Elodie Paciello<sup>7</sup>, Gaël Bardon<sup>5,7</sup>, Sören Franzenburg<sup>6</sup>, Sandrine Zahn<sup>7</sup>, Britta Meyer<sup>8</sup>, Céline Le Bohec<sup>5,7</sup>**

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<sup>8</sup> University of Hamburg, Hamburg, Germany.

King penguins are famous for their outstanding metabolic abilities. In the wild, they can fast for up to 100 days a year, withstand hypoxia during deep foraging dives, actively swim for thousands of miles, and switch between acute obesity and extreme underweight several times year without visible adverse effect. Yet at the zoo, King penguins switch to a much more sedentary lifestyle: they don't dive, feed as much as they like, barely exercise - but hey have medical care and don't need to fret about predators. As a result, their lifespan can extend from 2 to nearly 4 decades. But this comes at the cost of a thorough life history reprogramming, including a much lowered reproductive success. Here, we take advantage of a known-age, known life-history population of electronically-monitored King penguins in the Crozet Archipelago, matched with known-age King penguins across European zoos, to understand the changes incurred through this radical lifestyle transition. Using whole-genome methyl-sequencing, we investigate the role of genome-wide methylation in adapting from wild to sedentary conditions. We find that paradoxically, lifespan extension at the zoo involves clear epigenetic age acceleration: sedentary birds live longer, but age faster. Our results underline the central role of epigenetic mechanisms in rapidly matching a common genomic background to widely divergent environmental challenges, and open the way for a finer understanding of the non-genetic determinants of adaptation in avian ecology.

## 2. Genetic analysis of HPAIV H5N1 clade 2.3.4.4b is Humboldt penguins, Chile 2023

**Naomi Ariyama<sup>1</sup>, Gabriela Muñoz<sup>1</sup>, Rodolfo Reyes<sup>2</sup>, Mauricio Ulloa<sup>2</sup>, Carlos Navarro<sup>2</sup>, Magdalena Johow<sup>3</sup>, Victor Neira<sup>1</sup>**

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<sup>3</sup> Servicio Agrícola y Ganadero, Chile.

In December 2022, HPAI H5N1 clade 2.3.4.4b emerged in Chile. The virus was first detected pelicans and gulls. After, cases on different avian species have been detected. Penguins has been found deceased or stranded in proximity of other positive animals and some of them have been confirmed positive to HPAIV H5N1. This study aims to investigate the genetic diversity of the highly pathogenic avian influenza virus (HPAIV) H5N1 detected in penguins in Chile. Between January and March 2023, 131 cases of suspected penguins were submitted for HPAIV diagnostic, involving the sampling of 270 animals. Positive samples were sequenced using MINION ONP technologies for whole genome sequencing and phylogenetic analysis. From the total cases, only four cases were positive for influenza detection, all of them HPAIV H5N1 positive. The positive samples were successfully sequenced, obtaining complete or near-to-complete genomes. Both were collected from different locations, one from Iquique and another from the Coquimbo Region. The sequences were identified as A/Humboldt penguin/Chile/238744-2/2023(H5N1) and A/Humboldt penguin/Chile/239590/2023(H5N1), which will be deposited in GenBank (In Process). The phylogeny was constructed with 90 reference sequences collected during the HPAIV H5N1 outbreak in Chile, from different hosts, to evidence the genetic relationship between them. The penguin sequences presented 99.5% identity on the HA segment between them. The phylogeny suggests that the penguin viruses belonged to different subclusters, highlighting the genetic diversity of the virus in the penguin population. These findings provide important insights into the genetic diversity of HPAIV H5N1 in penguins and can help improve our understanding of the transmission and evolution of the virus.

### 3. Genomes of banded penguins suggest islands of differentiation during ecological speciation

**Fabiola León<sup>1,2,3,4</sup>, Eduardo Pizarro González<sup>1,2,3</sup>, Patricia Parker<sup>5</sup>, Daly Noll<sup>1,2,3</sup>, Marcela Espinaze<sup>6</sup>, Guillermo Luna-Jorquera<sup>7</sup>, Alejandro Simeone<sup>8</sup>, Esteban Frere<sup>9</sup>, Gisele Dantas<sup>10</sup>, Robin Cristofari<sup>11</sup>, Omar Cornejo<sup>12</sup>, Rauri Bowie<sup>13</sup>, Juliana Vianna<sup>1,2,3,4</sup>**

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Biodiversity is the result of evolutionary processes, often generated by natural selection. Recently divergent species and inhabitants of contrasting environments are ideal models for recognizing genomic characteristics related to speciation processes that arise in response to adaptation to discordant environmental pressures. We sequenced 113 complete genomes of the four banded penguin species of the *Spheniscus* genus throughout their entire distribution range in South America and Africa. Three different methodological approaches, based on  $F_{st}$  (OUTFLANK, PCAadapt, and GWDS), allowed us to recognize genomic islands of differentiation in a limited number of loci with key functions that characterize early speciation and suggest strong divergent selection on genes related to reproductive isolation, especially for the Galapagos and Humboldt penguin species. This could have been primarily mediated as an adaptive response to high temperature oscillations and effective size constraints imposed by the insular habitat, while among the Magellanic penguins and the African penguins it is marked by genome-wide selection. Among the genes with signals of divergent selection are those related to spermatogenesis, oxidative stress, plumage diversification, osmoregulation, visual and olfactory abilities, and immunology. Signals of hybridization were detected between sympatric colonies and subtle or null population structure within the species. Galapagos penguins show genomic diversity, low effective population sizes, and ongoing declines that support the vulnerability of this species.

#### 4. Major Histocompatibility Complex (MHC) and mate choice in the Magellanic penguin, *Spheniscus magellanicus*

**Gisele Dantas<sup>1</sup>, Mariana Dornas Flôres<sup>1</sup>, Ana Milliones<sup>2</sup>, Esteban Frere<sup>2</sup>**

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Some hypotheses predict that the Major Histocompatibility Complex (MHC) drives the mating preference among vertebrate species. Females choose their partners for olfactory clues, color, or morphological characteristics, and studies have shown a correlation between these characteristics and MHC. Many bird are considered monogamous, but recent studies have shown a high extra-pair paternity rate (EPP). Several hypotheses were formulated to explain this, such as the increase in genetic diversity in the offspring, the selection of good genes, or the search for optimal heterozygosity. In a previous study, Magellanic penguin showed a high rate of EPP, but no relation between EPP and the increase in genetic diversity. Thus, we investigated the relation of EPP with the choice of patterns based on MHC. For this, we sequenced MHC class II DR $\beta$ 1 gene (343pb) at 20 breeding pairs from Puerto Deseado, Argentina. We identified MHC alleles for each reproductive pair; estimated the genetic diversity compared between EPP breeding pairs and without EPP breeding pairs; and compared the coding region of exon 2 from MHC II between EPP pairs and without EPP pairs. We found high allelic diversity, and lower genetic diversity in EPP pairs than in those without EPP pairs. The breeding pairs that were involved in EPP showed more similar MHC protein than those without EPP pairs. This study brings a new perspective to understanding the partner's choice in the Magellanic penguin, which can be very important to apply in conservation ex-situ. It is necessary to investigate this theme in other penguin species.

## 5. Species delimitation beyond phylogenomics: integrative approaches reveal gentoo penguin speciation

**Daly Noll<sup>1,2,3,4</sup>, Jane Younger<sup>5</sup>, Luis R. Pertierra<sup>6</sup>, Michelle Greve<sup>6</sup>, Eduardo Pizarro G.<sup>1,2,3</sup>, Fabiola Leon<sup>1,2,3</sup>, Debora Y. C. Brandt<sup>7</sup>, Joshua Tyler<sup>8</sup>, Gemma Clucas<sup>9</sup>, Hila Levy<sup>10</sup>, W. Brian Simison<sup>11</sup>, Julie McInnes<sup>5</sup>, Pierre Pistorius<sup>12</sup>, Céline Le Bohec<sup>13,14,15</sup>, Francesco Bonadonna<sup>15</sup>, Phil N. Trathan<sup>16</sup>, Andrés Barbosa<sup>17</sup>, Andrea Raya Rey<sup>18,19,20</sup>, Gisele P.M. Dantas<sup>21</sup>, Rauri Bowie<sup>22</sup>, Elie Poulin<sup>2,4</sup>, Juliana A. Vianna<sup>1,2,3</sup>**

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One of the great challenges that must be faced in the climate change scenario is to be able to determine the adaptive potential of species. Species that show low signs of genetic diversity will be less likely to respond to future changes in their local environments and will therefore be at greater risk of extinction. In recent years, high levels of genetic divergence have been detected among gentoo penguins from distant regions, recording four evolutionary lineages distributed throughout the Southern Ocean. In this work, the complete genomes of 64 penguins were resequenced, and the degree of differentiation of the genomes was considered to answer different evolutionary questions. As we expected, high levels of neutral and adaptive differentiation were found between the genomes of penguins from distant colonies, suggesting that the 4 lineages have followed their evolutionary course in isolation and adapted to the climate and ecological conditions of their local habitats, identifying genes that favor their persistence in different environments of the Southern Ocean. Furthermore, our results suggest that the four gentoo penguins have different adaptive potential in response to the future change of their local environments. The results of this work provide consideration for IUCN to locally assess the threats and potential risk of extinction of some of the gentoo penguin lineages.



## 6. Uncovering population structure in the endangered Northern rockhopper penguin (*Eudyptes moseleyi*) across islands in the southern Indian and Atlantic Oceans

**Heather Ritchie-Parker<sup>1</sup>, Alex Ball<sup>1</sup>, Trevor Glass<sup>2</sup>, Charly Bost<sup>3</sup>, Antje Steinfurth<sup>4</sup>**

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The Northern rockhopper penguin (*Eudyptes moseleyi*) is an 'Endangered' species (IUCN Red List) as populations have been in rapid decline for several decades. The species has a transoceanic distribution with breeding colonies found on five islands in the South Atlantic Ocean (Gough, Nightingale, Inaccessible, Alex, and Tristan da Cunha) and two islands in the southern Indian Ocean (Amsterdam, and St Paul) with seemingly no intermediary populations. Outside of breeding seasons the birds do forage widely in the southern oceans and vagrant individuals have been recorded in South Africa, Kerguelen Islands, Australia, and New Zealand. The origin of these vagrant birds is largely unknown and it remains unclear to what extent birds move between islands and oceans. Understanding the connectivity, or lack thereof, of these (sub)populations is essential for developing appropriate conservation strategies. This is especially pertinent as some (sub)populations may be at greater extinction risk than others and source-sink dynamics may occur. Genetic data can provide insights into the movement of Northern rockhopper penguins and aid in the development of conservation strategies. Here we use a combination of mitochondrial DNA markers (CR and ND2) and a ddRAD sequencing approach to uncover population structure across the species' distributional range. Using this genetic data we; 1) investigate historic and contemporary patterns of gene flow, 2) estimate the extent and direction of migration between islands, and 3) overlay these patterns with ecological and environmental factors to better understand the driving forces shaping population structure.

## 7. Effects of ectoparasites on the foraging behaviour of and Antarctic penguin

**Virginia Morandini<sup>1</sup>, Josabel Belliure<sup>2</sup>, Carlos de la Cruz<sup>3</sup>, Roger Colominas-Ciuró<sup>4</sup>, Andrea Bueno<sup>1</sup>, Andres Barbosa<sup>1</sup>**

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The presence and distribution of ticks in Antarctica have been recently reported to be widely present along the Antarctic Peninsula in the three species of *Pygoscelis* penguins. The presence of ticks affects penguins by reducing survival, transmitting blood parasites (i.e. *Babesia* sp.), diseases (i.e. Lyme borreliosis caused by *Borrelia burgdorferi* s.l.), and increasing both immune response and oxidative stress. In general, the effects of ticks on foraging capability of hosts have been little studied and, to our knowledge, have never been tested in penguins. We found differences in the density of ticks in a chinstrap penguin rookery, and we analyzed whether it effects the performance of penguins during their foraging trips comparing high (HTD) and low (LTD) tick density areas. The study was conducted during the austral summer of 2017/2018 in the Vapour Col penguin rookery at Deception island, South Shetlands (63° 00'S 60° 40'W). Chinstrap penguins (12 in LTD and 16 in HTD areas) breeding with two chicks were captured at nest and GPS-TDR were deployed for five days. Penguins nesting in HTD areas traveled more distance and used more pelagic habitats ( $\delta^{13}\text{C}$ ) to forage than individuals in LTD areas, but no trophic differences ( $\delta^{15}\text{N}$ ) were found. Therefore, individuals in HTD areas forage in most distant areas because of the energy value of krill or a competitive displacement by LTD area individuals.

## 8. Finding the causative agents of infectious diseases affecting hoiho (yellow-eyed penguins) in New Zealand

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Hoiho/yellow-eyed penguins (*Megadyptes antipodes*) are predicted to become extinct on mainland Aotearoa New Zealand in the next few decades, with disease being a significant contributor to their decline. Diphtheritic stomatitis (DS) has resulted in significant mortality and in 2022 was identified in over 75% of all monitored chicks. Although *Corynebacterium* species have been inconsistently isolated from DS oral lesions, an alternate primary causative pathogen is suspected. In addition, a new disease termed respiratory distress syndrome (RDS) causing lung pathology has been identified in very young chicks with a high mortality within the first week of life, however no causative pathogens have been identified. This project aimed to identify possible pathogens responsible for diseases impacting hoiho. Using a metatranscriptomics approach, we discovered a novel and abundant gyrovirus in tissue samples from chicks with RDS, sharing only 40% amino acid identity within other gyroviruses discovered in diseased birds. Due to its high abundance and absence of other pathogenic organisms, it is highly likely that this novel gyrovirus is associated with RDS. In oral and cloacal samples taken from chicks with DS, we identified a novel and highly abundant picornavirus, most closely related to other penguin megriviruses. Evaluation of pre-symptomatic, symptomatic and antibiotic-treated penguins found no other obvious pathogens associated with the disease, although variable bacterial abundances were identified that could contribute to opportunistic secondary bacterial infections. Overall, we show how metatranscriptomics can provide a rapid, genomics-informed diagnosis for wildlife disease investigations and identify likely viral candidates that require further scrutiny.

## 9. Lab-In-A-Suitcase: Rapid, field-based portable device for wildlife disease surveillance in the field

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With the increasing threat of infectious diseases threatening the survival of Antarctic wildlife, there is increasing need portable genomic sequencers such as the MinION and PCR machines has greatly increased our ability to assess the presence of infectious agents in the field in real-time. Our study tested the ability of Oxford Nanopore's MinION device to rapidly detect microbes and infectious pathogens in Penguins and flying seabirds from sub-Antarctic and Antarctica. In laboratory experiments samples were inoculated with Avian Cholera and Avian Influenza to test the ability of the MinION to detect known pathogens using a shot-gun DNA/cDNA metagenomics approach. Both pathogens were detected. Analysis pipelines and databases have been developed for offline identification of pathogens has been developed for both diseases for use in areas with limited internet access.

Field-based DNA/RNA extractions and MinION sequencing was conducted onboard Quark Expeditions World Explorer during the 2022-23 season from samples collected from Penguins. Internet access and speed were the biggest challenges for species identification and analysis in the field. To overcome these limitations offline databases and pipelines have been developed and tested to allow offline identification of the microorganism's present including RNA viruses, parasites, and bacteria.

## 10. Population health evaluation and monitoring of Humboldt penguins (*Spheniscus humboldti*) at Punta San Juan, Peru from 2007-2023

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The marine protected area at Punta San Juan (PSJ), Peru safeguards critical habitat for endangered Humboldt penguins (*Spheniscus humboldti*), which face threats from poaching, habitat loss, introduced predators, commercial fishery practices, and El Niño climatic events. Since 2007, the health of 569 individual penguins has been evaluated through veterinary examination and biological sample collection. Serial sampling over time has also been possible with some birds. The PSJ Program maintains a continuous biologist presence at the site, allowing for identification tagging, biologging, census counts, reproductive monitoring, and other correlative data.

Population reference ranges have been established for complete blood cell counts, plasma biochemistries, and environmental toxicants (elemental and organic). Seroprevalence was identified for avian influenza (9.6%), avian avulaviruses (paramyxoviruses or APMV) types 1 (18.5%), 2 (20.3%), and 3 (5.4%), *Salmonella pullorum* (2.2%), *Chlamydia psittaci* (36.4%), and aviadenovirus type 1 (33.3%). No serologic evidence was present for West Nile virus, Marek's disease, avian orthoreovirus (reovirus), avian tremovirus A (avian encephalitis virus), siadenovirus A (hemorrhagic enteritis), *Mycoplasma gallisepticum*, and Eastern and Western equine encephalitis viruses. Assay validity for avian infectious bursal disease and Venezuelan equine encephalitis was not clear. No clinical disease has been associated with any of these infectious diseases, excluding the 2022-2023 ongoing outbreak of highly pathogenic H5N1 avian influenza virus. Comparison to data from PSJ in 1992-94 demonstrates increased prevalence for aviadenovirus 1, influenza, and APMV, while the prevalence for *C. psittaci* decreased.



## 11. The influence of biotic and abiotic factors on the bacterial microbiome of gentoo penguins (*Pygoscelis papua*) across the Scotia Arc

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The microbiome is a key factor in the well-being of vertebrates. To identify geographic and biotic factors that may affect the microbiome of wild birds in polar environment, we studied pygoscelid penguin populations in the Scotia Arc. By integrating wide geographic sampling with bacterial 16S and eukaryotic 18S sequencing of over 350 faecal samples, we were able to test for associations between the host microbiome and a suite of potential geographic and ecological factors. The most dominant associations with microbiome differences were host species and colony identity. There was also a relationship between the proportion of host DNA in the sample and the microbiome, which may reflect gut passage time. The dominant effect of colony on the gentoo penguin microbiome indicates that local factors play an important role in the microbiome structure of these polar seabirds. This may reflect the local transfer of microbes either via faecal-oral routes, during chick feeding, or other close contact events. Other factors including diet and host species also play significant roles in structuring the faecal microbiome, and in at least some locations, the microbiome composition varied considerably between individuals. The importance of colony as a factor may also indicate the potential for disruption of microbiome associations at a local scale which could influence host success in the changing polar environment. The implications of such changes need to be considered in the wider context of regional climate change, and microbiomes may be a useful indicator of change in the future.

## 12. Unique composition and neutral process characterize the bacterial communities in multiple body sites of the Magellanic and king penguins

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Animal hosts live in continuous interaction with bacterial partners, yet we still lack a clear understanding of the ecological drivers of animal associated bacteria, particularly in seabirds. Here, we investigate the effect of body site in bacterial communities' structure of two seabirds in the Strait of Magellan, the Magellan and the king penguins. We used 16S sequencing to profile bacterial communities associated with chest, back, foot, and Magellan penguin nest soil. Our results highlight the predominance of the genus *Psychrobacter* in all penguin body sites, and species-specific trends in bacterial communities' ecological properties. Overall, alpha diversity was similar across penguin body sites, yet, in king penguins it differed between foot and chest, whereas in Magellan penguins, phylogenetic diversity differed between back and nest. Similarly, weighted unifrac distances followed by PERMANOVA statistical tests revealed structure in all body site bacterial communities in king penguin, whereas Magellan penguin body sites had a similar composition among all body sites, yet all body sites bacterial composition differed from the nest. Furthermore, bacterial abundance in penguin body sites fits well, particularly in king penguin, highlighting the role of stochastic process in bacterial body site microbiota assembly of penguin body sites. Our results represent the first report of body site bacterial communities in seabirds specialized in subaquatic foraging. Thus, we believe it is useful information that will aid in environmental monitoring programs to survey ocean health.

### 13. Temporal trends of Hg in emperor penguin eggs over a 10-year period

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Mercury (Hg) is a pollutant of major concern, which reaches remote environments, including polar systems. Hg biomagnifies along marine food webs and top predators such as seabirds are therefore good bioindicators of Hg contamination in the ocean. They allow determining the degree of Hg contamination and its temporal variations. In this study, we used Emperor penguin (*Aptenodytes forsteri*) eggs to monitor the temporal trend of Hg contamination in Adélie Land over the period 2010-2021. Hg was analysed on six eggs each year and for seven years over the period. Albumen had the highest Hg concentrations over other egg compartments and contained the majority of the total Hg quantity in the egg (*i.e.*,  $70 \pm 8\%$ ). Yolk, shell and egg membrane showed lower Hg concentrations, and they accounted for  $27 \pm 7\%$ ,  $1.7 \pm 0.7\%$  and  $0.8\% \pm 0.3\%$  of the total egg Hg, respectively. Hg concentrations increased by 4.9% per year in egg white over the period (from 0.35 to 0.59  $\mu\text{g}\cdot\text{g}^{-1}$  dry weight). In contrast, no significant changes were observed in the yolk and shell membrane, suggesting these compartments are not suitable for monitoring temporal trend of Hg contamination in emperor penguins. Variation of Hg concentrations in emperor penguin eggs reflects variations in the metal accumulated by the females, suggesting an increase in the bioavailability of Hg in the high-Antarctic marine environment. Hg eliminated in eggs is less than 2% of the amount that is excreted in feathers during the annual moult, thus indicating that females have no significant advantage over males to depurate Hg.

## 14. Circumpolar assessment of mercury contamination: the Adélie penguin as bioindicator of Antarctic marine ecosystems

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Mercury (Hg) is a global pollutant of major concern, that even reaches remote polar oceans. In contrast to the Arctic Ocean, studies documenting Hg contamination in the Southern Ocean are extremely sparse and large-scale monitoring is urgently needed. Here, we used Adélie penguins (*Pygoscelis adeliae*) from 24 colonies all around Antarctica to identify large-scale variation of Hg contamination in Antarctic marine food webs. Mercury analyses were performed on body feathers collected on adults (n=485) and chicks (n=48), between 2005 and 2021. To investigate the role of feeding ecology on spatial variation in feather Hg contamination, carbon- ( $\delta^{13}\text{C}$ ) and nitrogen- ( $\delta^{15}\text{N}$ ) stable isotopes, which are proxies of feeding habitat and trophic position, were also analysed. Overall, feather Hg concentrations were below toxicity thresholds for seabirds ( $<1.62 \mu\text{g.g}^{-1}$ ). In adults, Hg contamination was driven by both site location and feeding ecology. The highest Hg concentrations were observed in the Ross Sea, probably because of a higher consumption of fish in the diet compared to other sites (krill-dominated diet). Because of their shorter bioaccumulation period, chicks exhibited lower mean Hg concentrations ( $0.22 \pm 0.08 \mu\text{g.g}^{-1}$ ) than adults ( $0.49 \pm 0.23 \mu\text{g.g}^{-1}$ ). This study provides a unique, comprehensive assessment of Hg transfer to predators all around Antarctic marine ecosystems. Such large-scale assessments are critical to assess the effectiveness of international regulatory treaties such as the Minamata Convention on Mercury. Adélie penguins could be exceptional circumpolar bioindicators of future temporal trends of Hg contamination in the Southern Ocean, especially in the context of climate change.

## 15. Faecal hormone analysis as a non-invasive tool for assessing stress in the Pōhatu Kororā (*Eudyptula minor*) colony, Aotearoa

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Marine and terrestrial environments are under increasing anthropogenic threat globally. In Aotearoa, one family that exists at the boundary of these two systems and thus experiences threats from both environments is our penguins. Penguins are exposed to a variety of stressors, such as human disturbances, predation, and food scarcity. When penguins are exposed to these stressors for prolonged periods, it can lead to chronic stress, which is characterised by high levels of corticosterone in their blood, and can lead to a decrease in body condition and fitness. Understanding how penguins respond to stressors can provide insight into the overall health and resilience of penguin populations. The kororā, little blue penguins (*Eudyptula minor*) at Pōhatu, Banks Peninsula, were monitored weekly over the 2022-23 breeding season. During routine monitoring, faecal samples were collected opportunistically. This pilot study aims to explore the potential of non-invasive faecal samples for investigating corticosterone levels of local kororā across the season. In this talk, I will review the benefits and limitations of non-invasive sampling and discuss the challenges of unravelling stressors and their impacts in a complex environment. By improving our understanding of the physiological impacts of stress, we can better protect these charismatic birds through informed conservation efforts.

## 16. Paralytic shellfish poisoning of Magellanic penguins and other seabirds and marine mammals at Península Valdés, Argentina, in 2022

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Península Valdés, northern Argentinean Patagonia, harbours a large community of seabirds and marine mammals, including large colonies of Magellanic penguin (*Spheniscus magellanicus*). A harmful algal bloom was recorded in Península Valdés from September to November 2022, causing substantial mortality of right whales, sea lions and seabirds. Moribund seabirds were occasionally seen ashore or at sea with neurological signs (disorientation, inability to stand, walk or swim). Carcasses of 42 Magellanic penguins and 40 other birds (grebes, cormorants, gulls, terns, swan, shearwater) were opportunistically collected and necropsied. Cause of death was not determined for five penguins, and the death of three penguins was attributed to malnutrition and helminth hyperinfection. The death of the remaining 34 penguins (65% juveniles, 35% adults; 38% males, 59% females, 3% sex not determined) was attributed to paralytic shellfish poisoning (PSP). Most PSP cases had regular to good body condition, drowning (marked lung congestion with pleural edema, white foam in bronchi), and recent vomiting (empty stomach with abundant mucus, mild superficial gastritis). Two penguins choked while regurgitating squid, and in two penguins the asphyxia seemed to result from respiratory muscle paralysis (without drowning). PSP toxins were detected in 5/9 samples from 3 penguins analyzed, with the highest concentrations in stomach contents, further toxicological analyses are under way. Concurrently, PSP toxins were detected in plankton, shellfish and right whale (gut contents and other fluids and tissues). This event illustrates that PSP harmful algal blooms can cause of mortality events of penguins and other marine fauna in the Patagonian Sea.

## 17. Camera logger footage highlights the unique foraging behaviour of King penguins breeding in Bahía Inútil, Tierra del Fuego, Chile

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About one decade ago a King Penguin *Aptenodytes patagonicus* breeding colony has established at Bahía Inútil, Strait of Magellan, Tierra del Fuego, Chile, with first egg laid in 2010 and first chick fledging in 2015. Since, breeding pair numbers and chicks fledged have slowly increased. In February 2023 we counted 40 breeding pairs, 29 of which were rearing chicks. This breeding site differs from all others in nearly every aspect since it is located in a confined environment about 300 km away from the open seas in the Atlantic and Pacific Oceans. Three high-utilization foraging areas were identified: One just off the colony in Bahía Inútil in waters ca. 50 m deep, another one at the entrance of the bay in waters ca. 200 m deep and a third one in the Magellan Strait between Punta Arenas on the mainland and Porvenir on Tierra del Fuego in waters ca. 330 m deep. As a result, foraging dive depths of King Penguins from this colony are significantly shallower than those of conspecifics from elsewhere. In addition, they feed mainly on Fuegian sprats *Sprattus fuegensis* and Patagonian squid *Doryteuthis gahi*, whereas elsewhere their diet is dominated by myctophids. We deployed underwater camera loggers on the birds to visualize their feeding behaviour in this environment. As a result, we collected exceptional video footage from birds feeding on swarms of Fuegian Sprats, dead sprats, and Patagonian squid, often together with conspecifics, Magellanic Penguins *Spheniscus magellanicus* and even Slender Tuna *Allothunnus fallai*.

## 18. Changing diets over time: knock-on effects of marine megafauna overexploitation on their competitor *Spheniscus magellanicus* in the South-Western Atlantic Ocean

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This study compares the  $\delta^{15}\text{N}$  values and the trophic position of Magellanic penguin (*Spheniscus magellanicus*) throughout the Late Holocene in three regions in the South-Western Atlantic Ocean to assess the hypothesis that the decimation of megafauna lead to changes in the trophic position of mesopredators. Modern and ancient mollusc shells were also analysed to account for changes in the isotopic baseline through time. Results revealed that modern Magellanic penguins have higher  $\delta^{15}\text{N}$  values than their ancient conspecifics in the three regions, after controlling for changes in the isotopic baseline. Such temporal variability might be caused by three non-mutually exclusive processes: decreased availability of pelagic squat lobster resulting from decreasing primary productivity through the Late Holocene, increased availability of small fishes resulting from the sequential depletion of other piscivores (South American fur seal and sea lion and Argentine hake) since the late 18<sup>th</sup> century and modification of the migratory patterns of Magellanic penguins. Although disentangling the relative contribution of all those processes is impossible at this time, the results suggest that the ecology of Magellanic penguins has undergone major changes since the Late Holocene.



## 19. Chasing the fish with little penguins: spatial and temporal variability in relation to environmental conditions

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Little penguins are resident top predators foraging on small pelagic fish. Over 20 years of monitoring off Phillip Island, Australia, penguin tracking data revealed that their core foraging areas are patchy in time and space. In particular, they differ in years of high and low breeding success and between sub-colonies, age classes and stages of breeding. Environmental conditions such as sea surface temperature, primary productivity, waves, ocean current and thermocline presence have been linked to little penguin foraging performance in time but not in space. Although these oceanographic conditions were averaged across the entire foraging zone, they were not fine-scale enough to be directly linked to the penguin foraging hotspots. Combining 12 years of tracking data from 2010 to 2022 on 636 monitored penguins, we investigated the time and space variability in their foraging grounds in relation to environmental conditions. Using little penguin average presence and variability maps, we first tested for the existence of persistent and occasional hotspots. Second, we ran a Spatial Density Model to explain the effect of environmental conditions on the penguin at-sea distribution. We used several oceanographic variables such as bathymetry, primary production, surface temperature, marine heat wave, current, wave, thermocline depth and persistence, oxygen and oxycline. We aim to better understand prey availability by connecting foraging places with the local environmental conditions. This study will provide crucial baseline information to guide a study of prey distribution in the foraging zone of little penguins using acoustic surveys from a sailing drone.

## 20. Compensating for harsh conditions at sea: plasticity of king penguin foraging strategies facing an experimental increase in workload

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In the context of the current climate crisis, particularly marked in marine ecosystems of polar areas, understanding the plasticity and resilience of penguin responses to environmental changes is critical to accurately model and predict population trajectories. Ideally, modelling should rely on experimental data testing whether causal effects of environmental changes or climate forcing on individuals occurs directly by acting on individual energetics and performance, or indirectly via, for example, integrative effects mediated through the food web.

Here, we investigated the effects of harsh foraging conditions at sea on individuals' physiological and reproductive performances in king penguin (*Aptenodytes patagonicus*). We experimentally created sub-optimal foraging conditions for 20 breeding pairs (compared to 20 controls) by decreasing the foraging benefit/cost ratio by increasing travelling costs relative to energy acquisition. Such conditions may be encountered in years of low resource accessibility.

Treated adults performed longer trips, but foraged in the same areas as control birds, and showed overall decreased swimming speed, especially ascent and descent rates. However, treated birds performed more foraging dives that were overall 12% shallower than control birds. We found little effects of our experiment on individual physiological state or reproductive performance. Our results suggest that king penguins plastically adjust their foraging behavior to mitigate the effects of increased workload, indicating some scope for coping with harsh conditions at sea.

## 21. DNA metabarcoding of faecal matter informs on African penguins' diet in South Africa

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The endemic African penguin *Spheniscus demersus* was classified as endangered in 2010 due to a rapid decline in the global population and continuing threats, including food shortages, oil and noise pollutions, diseases and extreme weather events. Due to the worsening status of the species, non-invasive dietary methods need to be employed to decrease the impact of stomach content long-term monitoring. We used DNA metabarcoding of faecal matter to determine the diet of African Penguins at eight major colonies along the South African coast in 2020/2021. The amplification of a highly conserved region in metazoans (18S rDNA) indicated that 93% of faecal samples contained food DNA, and most sequences were identified as Actinopterygii (bony fish) DNA. Fish taxa were then determined with a second primer pair amplifying a region of the 16S rDNA gene. Overall, 21 fish taxa were identified. Sardine *Sardinops sagax* DNA clearly dominated in both relative read abundance and frequency of occurrence in samples recovered from the Eastern Cape colonies, where diet varied very little between breeding stages (incubation versus chick-rearing) or age (adults versus chicks). By contrast, DNA recovered in chick samples from Western Cape colonies was much more varied with mackerel *Trachurus* sp., redeye round herring *Etrumeus whiteheadi*, and anchovy *Engraulis encrasicolus* DNA identified at most colonies. Ultimately, this study showed the value of using DNA metabarcoding to assess the diet of endangered species. Importantly, it revealed the extent of potential overlap between African penguins' diet and commercial fisheries.

## 22. Does age matter? Foraging behavior and stress of known-age breeding Magellanic penguins *Spheniscus magellanicus* at Matillo Isl., Argentina

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Life history theory states long-lived individuals compromise between current reproduction and survival over successive breeding attempts in order to maximize their fitness. Seabirds must forage efficiently to breed successfully, and the cost of reproduction may be reflected in higher stress levels of the breeding individuals. Known-age breeding Magellanic penguins *Spheniscus magellanicus*, between 4 and 20 years old, with less than 20-day old chicks were equipped with GPS devices (IgotU) and TDR – accelerometers (DVL-400, Little Leonardo, Japan) for one foraging trip on the same day (n = 10). Upon recovery of devices, a drop of blood was extracted from their tarsal vein and blood smears were made *in situ* and stained in the laboratory with Giemsa stain. Foraging distance increased ( $F = 9.98$ ,  $p = 0.01$ ), and path sinuosity decreased with age of breeders ( $F = 5.49$ ,  $p = 0.04$ ). Heterophil to lymphocyte ratio, used as a proxy of stress, also decreased with age ( $F = 7.66$ ,  $p = 0.02$ ). Older individuals went further and in straighter paths and had lower stress levels than younger breeders, which may indicate individuals continue adjusting their foraging behaviour on successive breeding attempts and lower stress levels may be reflecting more efficient behaviors as individuals age. Life-long fitness of individuals is defined as the number of offspring produced given the accumulative costs of reproduction and the current study on Magellanic penguins suggests these costs seem to reduce as individuals age.

## 23. Fishery-penguin conflict: more than just spatial overlap

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Competition between commercial fishing and seabirds foraging on a mutual prey source has been evident since the 1970s, but it is often difficult to disentangle the true impacts of fishing from other factors (e.g. climate change). A unique field experiment off South Africa has provided the opportunity to study African penguins *Spheniscus demursus* with and without fishing activity within 20km of breeding colonies.

Using GPS data from breeding African penguins and vessel location data acquired from Global Fishing Watch, we investigated spatial and temporal overlap during the breeding season when adults are more constrained in their foraging grounds. In addition, we used penguin GPS-Time-Depth data to assess the impact of fishing on the foraging behaviour of penguins.

By comparing overlap during the breeding season, we concluded that spatial competition was occurring on a very small scale (< 5% of the penguin foraging area also contained fishing activity). However, the presence of the fishery resulted in significantly longer foraging dives and surface intervals, and a reduced proportion of forage to search dives in penguins.

To conclude, we found that regardless of the apparent lack of spatial overlap, the presence of the fishery around the breeding colony resulted in penguins carrying out more energetically costly foraging dives. Fishing pressure within 20km of breeding colonies appears to be one of multiple stressors contributing to the decline of this endangered seabird.

## 24. Foraging behavior, personality, and nutritional condition of breeding chinstrap penguins from Deception Island, South Shetlands, Antarctica

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As the ability to acquire energy determines the resources an individual can allocate to reproduction and self-maintenance, foraging behavior is a key trait to better understand the mechanisms underlying these differences. We analyzed Chinstrap penguins foraging movements from Vapour Col penguin rookery in Deception Island, South Shetlands (63° 00'S 60° 40'W), during the austral summer of 2022/2023. An amount of 30 breeding adults with two chicks were captured at nest and equipped with GPS-TDRs during five days. Individual personality plays a role in determining shifts in seabirds' foraging behaviour and habitat use, thus we included a standardized test to measure the response to a novel object (bold-shy test). However, previous studies showed how poor nutritional status increases risk taking in ecological contexts involving predation, novelty, and exploration. This reveals nutritional condition as a potential explanator of individual behavioral responses. To include the role of nutritional condition as a confounding factor when studying behavioral responses using a typical bold-shy test, we used biochemical markers of blood to quantify nutritional status (i.e., urea, uric acid and beta-hydroxybutyrate concentrations) and examine the associations between foraging behavior, sex, colony position, nutritional status and behavioral responses of breeding chinstrap penguins.

## 25. Foraging strategies of Magellanic penguins from a central Patagonian colony during the incubation period

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Incubating Magellanic penguins (*Spheniscus magellanicus*, MP) must balance nest attendance with foraging performance. Few studies focused on MP's behavior during incubation probably due to logistical and technological constrains. We studied foraging behavior of 17 MP (11 males and 6 females) during incubation (2019 and 2022 breeding seasons) using GPSs in Cabo dos Bahías (44°54'50"S; 65°32'37"W), Argentina. Additionally, we determined trip duration of 25 and 21 breeding pairs in 2019 and 2022 respectively by recording nests attendance of micro-chipped penguins. Two foraging strategies were determined: 14% breeding pairs alternated trips of 3-5 days and 86% alternated only two trips. Although 65% of males made an additional trip ( $9.3 \pm 3.3$ h; total distance:  $25.1 \pm 9.5$ km) before the second egg was laid. Males foraged first during  $22.3 \pm 4$  days, followed by females that traveled for  $14.6 \pm 6.0$  days; showing differences in trip duration between sexes ( $F=21.9$ ;  $p<0.0001$ ). Males and females moved northwards to foraging areas related to thermal fronts. There was no differences between sexes in maximum distance from the colony  $503.8 \pm 71.2$ km ( $F=0.2167$ ;  $p=0.6511$ ) and total distance traveled  $1,545.9 \pm 153.2$ km ( $F=1.920$ ;  $p=0.197$ ). These results may indicate that females adjust their trips during incubation to return to the nest and feed the chicks when they first hatch. MP incubation trips may have a critical effect on breeding performance, being influenced by food availability and presence of the mate in the nest. Further studies to elucidate foraging strategies during incubation will fill a knowledge gap on this species.

## 26. Inter-annual consistency in the phenology and trophic niche of the Southern rockhopper penguins from Isla de los Estados, Tierra del Fuego, Argentina

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Phenological and trophic niche consistency are known to impact the ability of species to respond to climate change. Understanding these ecological variables is key to assessing the potential adaptability of endangered species like the Southern rockhopper Penguin (*Eudyptes chrysocome*). To study their phenology and colony attendance we deployed time-lapse cameras from 2013 to 2017 at Isla de los Estados (Argentina) and processed the images using the citizen science project Penguin Watch. We also collected adult feather samples to study their pre-moult foraging niche and trophic position (TP) using stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis. To estimate their TP, we used previous information of baseline's stable isotope values of the food web around the colony. We found phenological consistency between years with little variation in arrival ( $\sim$ October 1<sup>th</sup>) and departure dates ( $\sim$ March 30<sup>th</sup>) for the breeding season as well as in departure ( $\sim$ February 1<sup>th</sup>) and arrival ( $\sim$ February 20<sup>th</sup>) for the pre-moult trip. Nonetheless, we found significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between years, with 2014 and 2017 having the lowest values for both isotopes for the pre-moult period. Regarding TP, we found no differences between years. Results indicate that rockhoppers are consistent in their phenology and their TP across years, with differences in isotope composition possibly related to variation in foraging areas. This data provides key information for evaluating their adaptive capabilities, resilience and their risk of phenological mismatch, all important in order to assess their vulnerability to future environmental changes.



## 27. Key foraging areas for Adélie penguins from a declining colony in the Western Antarctic Peninsula

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Fildes Peninsula, in King George Island/Isla 25 de Mayo, represents a logistic hub for human activities in the Antarctic Peninsula, and is one of the areas with the largest human footprint in the continent. Ardley Island, just off-shore of the Fildes Peninsula, is an ASPA and one of the few areas where the three Pygoscelid penguins breed sympatrically. Adélie penguins breeding parameters have been monitored since the 1980s. Since then, the number of breeding pairs declined by more than 30%, reaching a minimum of 184 in 2022/2023. Since 2019 we are studying their breeding and foraging ecology. By integrating spatial location, dive and accelerometry data of 57 individuals, we characterized their foraging behavior and identified foraging areas regularly used by the colony. Both changes throughout and across seasons were observed. During the late guard stage, trip duration and distance traveled were longer, individual foraging areas larger, frequency of dives lower and dive efficiency higher, likely reflecting changes in energetic demands of chicks. We also found significant differences between years in trips duration, distance and area explored, probably reflecting variation in prey availability, but not in parameters often used to infer foraging effort and success, such as dive duration, bottom time or number of wiggles. Furthermore, during the 3 seasons, most of the foraging activity of the colony was restricted to an area within 10 km from the colony, inside Maxwell Bay, an area intensely used by logistic and touristic vessels during the breeding period.

## 28. Longitudinal, full-annual cycle study of Adélie penguin foraging behavior reveals within-individual changes with age

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Age-related variation in foraging performance can result from both within-individual change and selection processes. These mechanisms can only be disentangled by using logistically challenging long-term, longitudinal studies. Coupling a long-term demographic data set with high-temporal-resolution tracking of 18 Adélie penguins (*Pygoscelis adeliae*, age 4-15 yr. old) over three consecutive annual cycles, we examined how foraging behavior changed within individuals of different age classes. Evidence indicated within-individual improvement in young and middle-age classes, but a significant decrease in foraging dive frequency within old individuals, associated with a decrease in the dive descent rate. Decreases in foraging performance occurred at a later age (from 12-15 yr. old to 15-18 yr. old) than the onset of senescence predicted for this species (9-11 yr. old). Foraging dive frequency was most affected by the interaction between breeding status and annual life-cycle periods, with frequency being highest during returning migration and breeding season. Overall, foraging dive frequency was highest for successful breeders during the chick-rearing period, even as food became less available. To mitigate food availability, successful breeders dived deeper than non-breeders or failed breeders. As failed breeders also exhibited lower dive descent rates throughout the annual cycle, our results suggest potential differences in 'individual quality' between successful and failed breeders. Females performed more foraging dives per hour than males. This longitudinal, full annual cycle study allowed us to shed light on the changes in foraging performance occurring among individuals of different age classes and highlighted the complex interactions among drivers of individual foraging behavior.

## 29. Resources, risks and refugia: assessing the spatial overlap between yellow-eyed penguin foraging distribution, prey, commercial fisheries, and marine protected areas

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Conservation of yellow-eyed penguins (*Megadyptes antipodes*) focuses on managing terrestrial threats, while little is being done to understand and prevent marine threats. More specifically, it is unknown if penguin foraging areas overlap with resources (i.e., prey), risks (i.e., commercial fisheries interactions, bycatch), and areas of refugia (i.e., marine protected areas, MPAs). First, we determined if suitable penguin foraging habitat, predicted by maximum entropy or Maxent species distribution models (SDMs), also supports a high diversity of seven key prey species using stacked Maxent SDMs. Then we created a novel index to predict areas of likely interactions between penguins and commercial gillnet/trawl fisheries. Lastly, we established a baseline measure of overlap between penguin foraging distribution, current MPAs, and the proposed South-East Marine Protected Area (SEMPA) network. In general, areas along the middle of the continental shelf had the highest probability of penguin presence, prey diversity, and overlap with gillnet and trawl fisheries. However, less than 1% of the total range of yellow-eyed penguins overlaps with a marine reserve or MPA, and the proposed SEMPA network will only protect 3.6% of their range. By assessing threats to yellow-eyed penguin survival, these findings can be used to inform ongoing conservation management decisions and marine spatial planning aimed to prevent the predicted localised extinction of this species.

### 30. Seeing the sea through the eyes of Humboldt penguins - how do things look in the face of growing anthropogenic threats?

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The Humboldt Archipelago in Northern Chile is a population stronghold for Humboldt penguins. Recently, two major mining projects have been proposed in the vicinity of the islands. In 2022, we studied the at-sea distribution and foraging ecology of Humboldt penguins breeding on Isla Choros, an island situated in the centre of the archipelago. GPS dive logger data confirmed that some birds foraged immediately adjacent and most within 10km downstream of potential pollution associated with the proposed mining ports and major shipping lanes. PenguCam loggers revealed new insights into the foraging behaviour of Humboldt penguins. The productive waters near the coast often had a green tinge with comparably poor visibility at depth. Some areas contained high quantities of South American sea nettle, a jellyfish that penguins routinely checked for small commensal fish hiding within the tentacles. This may be a promising strategy in the face of climate change and ocean acidification. Half of the penguins foraged solitary and exclusively pelagically near the surface for individual fish or small/juvenile swarm fish and squid, remaining penguins foraged in groups of sometimes more than 50 individuals herding demersal fish. This latter behaviour leads to increased risk of bycatch mortality, since a single net could catch a group of birds hunting together. Their surprisingly flexible foraging strategies may enable Humboldt penguins to adapt to global change if other anthropogenic threats are being reduced. Our work illustrates the importance of obtaining robust data to inform marine spatial planning to mitigate anthropogenic impacts on this threatened species. This study was supported by SPHENISCO e.V. (Germany).

## 31. Sex, but not size, is related to foraging success and efficiency in Magellanic penguins

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Body size can affect species' foraging success and efficiency which consequently influence survival and/or reproductive success. Males of most seabird species (74%) are larger than females and threatened species are more likely to have skewed sex ratios than non-threatened species. Recent studies of declining seabird colonies found that female mortality is higher than male mortality and suggest this is because larger males have higher foraging success than smaller females, however few have quantified the foraging success of males and females and/or of large and small conspecifics. We used automatic weigh scales and linear mixed models to quantify the foraging success of sexually size dimorphic Magellanic penguins at Punta Tombo, Argentina between 2015 and 2020 (n foraging trips = 986, n individuals = 158). We found males brought back 50% more food than females even though males are only 18% larger in mass than females (female foraging success =  $0.40 \pm 0.06$  kg, male foraging success =  $0.60 \pm 0.06$  kg). Size did not have an influence on foraging success (i.e. large males did not bring back more food than smaller males). Males were more efficient foragers than females, bringing back 180% more food per hour than females (female foraging efficiency =  $0.005 \pm 0.004$  kg/hr, male foraging efficiency =  $0.014 \pm 0.001$  kg/hr). Preliminary results show trip duration and trip location were similar for males and females. These results suggest it is not size but other sex-specific foraging behaviors that are driving differences in foraging success and potentially mortality rates.

## 32. Stable isotope ecology of two declining sub-Antarctic penguins: the erect-crested penguin and the Eastern rockhopper penguin

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New Zealand's sub-Antarctic penguins are chronically understudied, with the erect-crested penguin (*Eudyptes scalaris*; ECP) being the least studied penguin in the world. The Tawaki Project's Bounty-Antipodes Expedition 2022 aimed to assess the population trends and foraging ecology of both the ECP and the Eastern rockhopper penguin (*Eudyptes filholi*; ERHP). Our preliminary results found drastic population declines on Antipodes Island, but more stable conditions on the Bounties. To understand why, we are investigating their foraging ecology with GPS data loggers and stable isotope analysis of blood and feathers. We deployed data loggers on breeding females (25 ECP and 5 ERHP) on Antipodes Island. Our data indicates that ECP forage primarily within a 20 km radius of the island with minimal overlap between northern and southern colonies. However, the southern birds may exert higher foraging effort than their northern counterparts with deeper dives and longer distances travelled. ERHP foraged at a similar distance but exhibited more variable trajectories. Finally, we collected blood (red blood cells and plasma) and feathers from both the Bounties (30 ECP) and Antipodes (30 ECP and 30 ERHP). We will analyze the stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) to compare trophic dynamics and niche between the two species and islands during breeding and the pre-molt. By combining tracking and stable isotope data, we will characterize the foraging behavior of both species and aim to identify differences between species, colony, and island group. Ultimately, we hope to elucidate potential causes for the sharp declines on Antipodes.

### 33. Videos indicate that Adélie penguins catch more prey under ice, does acceleration data tell a different story?

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Animal-borne video loggers are an important tool in the investigation of animal behavior; however, their utility is limited by time-consuming post processing. Combining motion sensing devices with video loggers improves interpretation by providing visual confirmation of behaviors. This study pairs time, depth, and tri-axial acceleration, with video to study foraging behavior of Adélie penguins (*Pygoscelis adeliae*) and apply a machine learning approach to detect prey capture from accelerometry data. Devices were deployed on chick-rearing adults at Cape Crozier, Antarctica, capturing 39 foraging trips across two seasons. Manual video annotation produced a dataset of habitat and behavioral observations across 4,766 individual dives. Birds spent 61% of video periods actively diving, with roughly a third of dives reaching depths where light levels were insufficient to identify behaviors. We observed 7,625 individual prey captures, 1,937 of which were identifiable to prey type. Krill was the predominant prey type followed by fish, and squid. Foraging dives occurred primarily in open water habitat, but individuals foraged under the sea ice at depths less than ten meters in 4% of dives. Prey capture rates were highest for shallow ice-associated dives with a mean of 11.3 prey captures per minute compared to 0.7 for non-ice dives. Prey capture rates for dives outside the video period will be investigated using a machine learning approach to interpret prey capture behaviors from acceleration data. Successful models would provide a new method for investigating prey capture in relation to fine-scale habitat features and improve on current methods that exclude shallow-water dives.

## 34. Climate and human impacts on global penguin hotspots: current assessments for conservation

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Penguins are considered as one of the most threatened groups of seabirds and deserve conservation priority. However, there is a lack of a reliable, comprehensive, and systematic effort aimed at providing quantitative, spatially-explicit assessments on the multiple threats that the world's 18 penguin species are facing. That is what we have done in this work, by combining the most spatially-resolved information on the global penguin distributions (based on > 800,000 penguin occurrences available in GBIF) with that for their main threats: climate-driven environmental changes at sea, industrial fisheries, and human disturbances on land. Our analyses reveal that the areas that accumulate more threats for penguins concentrate in coastal regions within temperate areas; particularly in the coast of Peru, the Patagonian shelf, the Benguela upwelling region and the Australian and New Zealand coasts. When weighting these potential pressures with species-specific vulnerabilities, species such as *Spheniscus humboldti*, *Spheniscus demersus* and *Pygoscelis antarcticus* emerge as the ones under the highest pressure. However, all these potential threats are unevenly distributed spatially, even within species distribution ranges, so contrasting pressures are expected among populations within species. Our approach explicitly differentiates between climate and human pressures, since the more achievable management of local anthropogenic pressures (e.g., fisheries and land-based threats) may provide suitable means for ameliorating cumulative impacts and maintaining penguins within a "Safe Operating Space", where they may remain resilient to global processes such as climate change.



## 35. Conservation success and failure: How human disturbance shaped the fate of penguins

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Changes in species distribution can create new conservation challenges due to potential exposure to new or intensified threats. An example is the range expansion of the Magellanic penguin in the northernmost sector of the Atlantic, which has established new colonies. We report the different outcomes of two such colonies based on the effectiveness of their conservation measures. Fifteen years ago, we discovered the Pedral colony with only six penguin pairs settled. However, the colony was suffering from various forms of human disturbance such as poaching, garbage disposal, all-terrain vehicle traffic, dogs, and intentional fires. Urgent measures were implemented, including designating Pedral as a Wildlife Refuge, developing a management plan, and fostering the creation of a public and private protected area. Stricter regulations to reduce habitat degradation were also put in place, along with intense education campaigns. Ecotourism operations were supported, providing jobs and incomes, and a regular presence on the colony was secured. As a result, Pedral thrived with over 4,000 pairs of penguins. In contrast, the Punta Pozos colony settled and peaked at 24 nests, and no effective measures could be taken to reduce human disturbance from unregulated visits, leading to complete abandonment. In conclusion, the success of these colonies depended on effective conservation measures taken to mitigate human disturbance during the earliest stages of settlement. Urgent and strategic action can provide a healthy and safe habitat for penguins to thrive, while inaction can lead to colony abandonment.

### **36. Exploring threats: changes in a declining Humboldt penguin population and its association with fishing activity and environmental conditions inside the species' foraging range**

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In Peru, world-leading industrial fisheries and recurrent environmental oscillations have historically impacted marine resources and seabird populations. In Punta San Juan (PSJ), one of the most important reproductive sites for Humboldt penguins (HUPE) in Peru, prey limitation is hypothesized to be a primary driver of recent breeding declines. This study aims to evaluate the local effect of industrial fishing and environmental conditions on the HUPE breeding population at PSJ from 2012-2022. We used GPS data collected during brooding from 2012-2016 (N=40) to estimate an inter-annual HUPE foraging range. Subsequently, using public data we estimated the monthly accumulated fishing hours (Global Fishing Watch) and average SST (MODIS) inside the HUPE foraging range from 2012-2022. We applied a change point analysis on SST to identify "warm" and "cold" periods. Additionally, we used HUPE counts from April-August (1st breeding season) to estimate monthly breeding population (adults) and reproductive success (chick/adult) values. Fishing effort ranged from 1.5-53.4 hours with no evident trends. The SST trend was divided into four distinct periods: 2012-2014 (17.91°C), 2015-2017 (19.27°C), 2018-2020 (18.11°C), and 2021-2022 (17°C). Similarly, reproductive success decreased after 2015, increased after 2018, and peaked in 2021-22. In contrast, the breeding population gradually decreased from 2015-2020 and did not recover. Results suggest that environmental fluctuations are the main driver behind the breeding decline at PSJ. Additionally, the long-term impacts of the prolonged 2015-2017 warming highlight the complex dynamics that affect HUPE population trends and the need to study and protect this species at an ecosystem scale.

## 37. Humboldt penguin status and conservation plan: A report on the 2019 PHVA, Lima, Peru

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In October, 2019, Population & Habitat Viability Analyses (PHVA) were hosted in Lima, Peru, to assess the range-wide status of Humboldt penguins (*Spheniscus humboldti*) and develop strategic actions related to conservation. Using census data and other best estimates of demographic rates, the PVA suggested that overall population could be decreasing on average by 7% per year. Hundreds of iterations of the baseline model calculated mean time to extinction of 59 years; if additional data confirm these analyses, then Humboldt penguins meet IUCN Red List criteria for moving from current threat category of “Vulnerable” to “Endangered.” During the PHVA, sensitivity testing projected trends in future population size resulting from potential conservation actions aimed at impacting the most important demographic traits. Based on discussion and prioritization of threats, working groups assembled around fisheries and fishing practices, population biology and demography, communications and education, and human disturbance. The resulting plan recommended key goals, objectives, and actions, along with timetables for their implementation. Of 14 conservation goals, the top-ranked are: 1) Educate and engage public about threats and solutions; 2) Reduce predation impacts and human disturbance; 3) Census consistently across the range; 4) Promote spatial and temporal regulation of the gill net fishery to minimize overlap with peak penguin foraging needs. Examining all goals shows that key skills are needed: 13/14 require communications & networking; 10/14 require research or investigation; 7/14 involve policy or management activities; and 8/14 require financial support. We will present PHVA results and call-to-conservation-action and will describe progress to date.

## 38. Insights on Galápagos penguins from a 50+ year study

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Galápagos penguins are classified as endangered by IUCN because of their restricted range and small population size. They breed mainly in the western part of the Galápagos Archipelago, Ecuador on Fernandina and Isabela Islands. Although penguins are seen on other islands, breeding elsewhere is unlikely because of introduced predators such as rats, cats, and dogs.

The precise number of Galápagos penguins in the population is unknown, as they are difficult to see when they are breeding or while foraging in the ocean. During El Niño (e.g. 1997 & 2015), when upwelling along the Equator is blocked by warm surface waters, penguins are in poor body condition and often abandon their nests and few juveniles are present. Conversely, during strong La Niña events when upwelling is strong and food abundant (e.g. 2021 & 2022), penguins are in good body condition and many juveniles are seen.

Sea level is rising globally due to global warming, resulting in a loss of nesting habitats. For example, one nest on Fernandina that was dry and fledged chicks in the 1970's had both eggs smashed by a high tide in the 2000's. To counter this loss of habitat, we built 120 nest sites in 2010. In September 2022 86 nests were still usable and 33 were occupied, sometimes within 6 months, although 2 nests on Fernandina took more than a decade to be occupied. The success of our nest project provides a key step towards conservation of this Equator-adapted penguin.

## 39. IUCN SSC Penguin Specialist Group – member feedback and way forward

**Lauren Waller<sup>1,2,3</sup>, P Dee Boersma<sup>3,4</sup>, Charly Bost<sup>3,5</sup>, Andre Chiaradia<sup>3,4,6</sup>, Megan Dewar<sup>3,7</sup>, Susie Ellis<sup>3</sup>, Pablo Garcia Borboroglu<sup>3,4,8,9</sup>, Tom Schneider<sup>3,10</sup>, Phillip J Seddon<sup>3,11</sup>, Alejandro Simeone<sup>3,12</sup>, Phillip N Trathan<sup>3,13</sup>, Barbara Wienecke<sup>3,14</sup>**

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The IUCN Penguin Specialist Group (PSG) is a recently established organization, and we recognize the importance of building a strong community to enhance our effectiveness. To this end, we are actively working to foster collaboration and networking among members. By sharing knowledge and experiences, we can capitalize on the collective expertise of the group, leading to more effective conservation efforts. Our goal is to build a thriving community that is dedicated to the long-term protection and preservation of penguin species worldwide. In 2020, we sent a survey to our membership to: update the member database; ensure that all penguin species are represented within the PSG; assess the skill base that members have access to; assess any skill gap in our current membership; obtain input on the PSG's future role; recruit new members; and use the feedback received to draft the strategic plan. The steering committee subsequently met over a period of months to compile a draft of the strategic plan which was distributed to the membership prior to the 11<sup>th</sup> IPC. In 2023, we sent another survey to determine how members' expertise can best aid global penguin conservation. This talk will provide feedback on the first member survey, provide an overview of the draft strategic plan and propose ways in which the PSG membership can best be engaged. Feedback from the membership on both the strategic plan and member engagement will be sought to scale up the contribution that this specialist group can make to global penguin conservation.

## 40. Magellanic penguins as a keystone species in Patagonian coastal systems

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The ecological importance of penguins on land remains unknown. To address some of these aspects, we investigated a Magellanic penguin (*Spheniscus magellanicus*) colony at Cabo dos Bahías (~7,000 pairs), central Patagonia, Argentina. We used camera traps (CT), from September/2022 to January/2023, to compare the activity of vertebrates within (CT=8; 18,384h) and outside the colony (CT=10; 14,928h). Additionally, we employed direct observation (135h), monitored penguin carcasses (CT=8; 1,050h), collected arthropods from them, and performed forensic analysis to identify species interacting with penguin carcasses. Species richness [species=22: including aerial predators as brown skua (*Catharacta antarctica*) and kelp gull (*Larus dominicanus*)] and activity were higher within the colony (records/h=0.140) than in the surroundings (species=11; records/h=0.068). Even when considering the species common to both sites [mostly herbivores as guanaco (*Lama guanicoe*), hare (*Lepus europaeus*) and lesser rhea (*Rhea pennata*)], the general activity remained higher within the colony (records/h=0.105). Penguins were depredated and/or scavenged by skua (*Catharacta antarctica*; eggs/carcasses), kelp gull (*Larus dominicanus*; eggs/chicks/carcasses), armadillo (*Chaetophractus villosus*; eggs/chicks/carcasses), Geoffroy's cat (*Leopardus geoffroyi*; chicks/adults), vulture (*Cathartes aura*; carcasses), and caracara (*Caracara plancus*; carcasses). The carcasses were also used by arthropods (families=12) for consumption and/or metamorphosis. Additionally, kelp gull and dolphin gull (*Leucophaeus scoresbii*) interacted indirectly with penguins by consuming adults' regurgitates. Magellanic penguins may be a keystone species in coastal Patagonia; as they are an important food resource that influence the spatial use of marine and terrestrial species, benefiting the functioning of the ecosystems where they breed.

## **41. "Safe Operating Space for Penguins (SOSPEN)" initiative: a global effort towards the IUCN-Penguin Specialist Group vision of "penguins in perpetuity"**

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Thirteen of the world's 18 penguin species are under conservation threat in the IUCN Red List. Penguins deserve conservation priority. A crucial step towards the IUCN Penguin Specialist Group (PSG) vision of "penguins in perpetuity" is improving their resilience to climate change by identifying key marine areas for penguins. Efforts for managing and mitigating the impacts on penguins are directed towards an IUCN PSG initiative for a Safe Operating Space for Penguins (SOSPEN).

Based on broad partnerships, SOSPEN aims to (i) assemble available tracking datasets for penguins; (ii) incorporate tracking data to identify key marine areas for individual penguin species; (iii) further develop the analyses of the spatial distribution of the major climate and human threats facing penguins; and (iv) incorporate this information within an operational Marine Ecosystem Model, to simulate past and future scenarios based on climate projections under the IPCC scenarios.

The SOSPEN initiative will build on penguins' tracking datasets provided to the Retrospective Analysis of Antarctic Tracking Data project and acknowledges the contributions of the data providers. We seek colleagues to join us in this initiative by providing their penguin tracking data and by becoming partners in the analysis and publication process. SOSPEN partners will retain full ownership of their datasets, which will be used solely to pursue the research objectives of SOSPEN. This initiative aims to be at the forefront of collaborative efforts in creating effective conservation strategies.



## 42. The catalytic role of ESG investment in resolving the current fisheries – penguin impasse in South Africa

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We use the South African small pelagic (sardine and anchovy) fishing sector as a case study to explore the role investors may be able to play in contributing to resolving the current impasse in South Africa between the small pelagic fishing sector and those involved in African penguin conservation. In so doing, this would also contribute to the implementation of ecosystem-based fisheries management (EBFM).

Management oversight and regulation of fisheries usually fall within a country's government structures. However, the requirements of various stakeholders, which include fishing industries as well as those impacted by fishing activities, can often be conflicting, leading to difficult decision-making processes for the government. In some cases, much-needed decisions, and their implementation, within an EBFM framework, are delayed or watered down as a result of this conflict. With the threats and pressures that ocean ecosystems are currently under, these delayed or ineffective decisions can have disastrous ecological consequences for these systems.

In this talk, we provide a brief overview of the challenges faced in ensuring adequate food availability for the endangered African penguin in South Africa. We then discuss the role that investors in fishing companies can play in addressing these challenges. We will give feedback on our progress in addressing this within the context of Environmental and Social Governance (ESG) reporting, where investors require fishing companies to report on ESG indicators that are relevant to the impacts of their extractive activity.

### **43. To count or not to count: comparing metrics of reproductive success in Adélie penguins**

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To understand population growth, individual performance, and factors driving changes in penguin populations, we need to measure reproductive success. However, defining a "successful" breeding attempt is complex, especially for colonial species like Adélie penguins that have unique life-history traits where juveniles enter a crèche before reaching independence. At Cape Crozier, a colony of ~300,000 pairs of Adélies, a long-term demographic study allowed us to compare productivity measurements of known-age individuals. For a subset of six breeding seasons, we compared three methods to estimate reproductive success: 1) chick presence on median crèche day, 2) chick size at last sighting, and 3) outcomes of marked chicks followed through crèche. We also compared these metrics to estimates of mean colony productivity obtained from ground counts at ~2 weeks after median crèche date over 20 years. By improving the accuracy and repeatability of productivity estimates, we can gain deeper insights into factors driving changes in penguin populations and inform conservation strategies.

## 44. Tracing seal predation back to the source colony of their penguin prey: a trace element and stable isotope analysis

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There has been much uncertainty surrounding the population status of little penguins (*Eudyptula minor*) in Australia and the predatory impacts of the recovering long-nosed fur seal (*Arctocephalus forsteri*). Identifying penguin colonies that are experiencing seal predation helps contextualise the threat of a native predator relative to other pressures experienced by penguins to inform conservation management. Here, we present a novel application of stable isotope and trace element techniques that identify the source colony of little penguins preyed on by long-nosed fur seals. Chemical 'feather-prints' from six major breeding colonies across south-east Australia were differentiated and compared with feathers from preyed penguins recovered from seal scats. Feeding trials of captive seals confirmed that digestion of penguin feathers did not compromise Stable Isotope (d13C, d15N) or Trace Element (Al, Ti, Sr, Mg) signatures. The resulting chemical 'feather-prints' provided robust baselines for comparison with scat feathers with source colony feathers being correctly classified at the local (78%) and regional scales (85%). Differentiated 'feather-prints' were likely driven by industrial inputs from land, colony-specific penguin foraging patterns and proximity to oceanographic systems. We determined that 46-70% of preyed feathers were assigned to 'local' penguin colonies. We expect that the regional penguin abundances and the proximity of their colonies to seal sites, as well as demographic-specific foraging patterns may shape their contribution to seal diet at local, regional and inter-regional scales. This diagnostic tool is powerful and non-invasive, having broad applications towards identifying seabird colonies at greatest risk to seal predation and informing targeted, site-specific, conservation effort.

## **45. Creating spaces for Humboldt penguin conservation in Ica, southern Peru**

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Humboldt penguin population in Peru has experienced an undeniable decline. To cope with this situation, ACOREMA launched initiatives to sensitize the different stakeholders. One of these, implemented since 2010, is Creating spaces for Humboldt penguin conservation, aimed to conservation of the species through involvement of the local population in critical areas. The geographic scope of the project is the Ica region, southern Peru, where the four marine protected areas in the country converge. An important component of this project are the workshops directed to children (3 to 12 years old) with parents involved in the fishing activity at the ports of San Andrés (Pisco) and Tambo de Mora (Chincha). Other components were oriented to several publics: school children and teachers, fishermen cooperatives, local guides and tour operators, youth groups, universities, and the general public. The participation of 55,000 people and 98 institutions is estimated to have been reached using a wide coverage of strategies and tools: workshops, conferences, puppet shows, parades, presentations at public spaces, fairs, etc. The work also involved the production of awareness materials, as well as the creation of a character PISKO, the Humboldt penguin. Among the results of the project a book produced by children to promote Humboldt penguin conservation was published, as a complement of the multiple materials produced by them and other community groups.

## **46. Waddling to success: Using little penguins as a model for business strategy**

**Jessica McKelson<sup>1</sup>**

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Phillip Island Nature Parks (Nature Parks) relies on the success of its tourism operations to fund ongoing significant social, environmental, conservation, research and education outcomes. The most popular attraction is the Penguin Parade, where visitors can witness these seabirds returning home from the ocean to their burrows on any night of the year. This attraction is the primary revenue source for the Nature Parks, and underpins our conservation and research operations that support the population of 40,000 seabirds.

COVID-19 significantly impacted our sustainable funding model, which had previously enjoyed 60% of all visitation from international markets, and resulted in an average annual investment of \$4.1 million into environmental and scientific outcomes. In response to the pandemic and the closure of our attractions, the Nature Parks innovatively live-streamed the penguins to an online global audience. This effort resulted in the Penguin Parade being a continued destination of choice for domestic visitors as the International tourism market recovered and enabled the ongoing support of our conservation efforts

Since the reopening of the Penguin Parade, the domestic market has accounted for 80% of all visitation. Although the market is steady, the uncertain tourism landscape underscores the importance of conducting visitor research and adapting to changing markets. This research continues to inform and drive the Nature Parks tourism business strategy where Little Penguins are the primary focus. Our penguins are intrinsically linked to our tourism success, and the conservation values and outcomes of the penguin colony would not be possible without this tourism support.

## 47. Conservation of the Humboldt penguin in Chile: are we doing enough?

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The Humboldt penguin occurs along the coasts of Peru and Chile in the Southeastern Pacific Ocean. By the 1850s the species was to be found by the hundreds of thousands, but the exploitation of the guano deposits removed most of its breeding habitat. This, coupled with constant El Niño events and the start in the 1950s of a large-scale fishery targeting on the Peruvian Anchovy (its main prey), set in a complex process of population decline that is still going on today. To conserve the species, in the last 40 years, Chile has: 1) created national parks/reserves to protect the main islands where Humboldt penguins breed, 2) created marine protected areas around the islands where penguins forage, 3) classified the Humboldt Penguin as Vulnerable, 4) developed a national management plan. Is this enough to adequately protect the Humboldt Penguin? A recent census (2021-2022) revealed the breeding population in Chile probably does not exceed 3,000 pairs and major colonies have declined in the last two decades. Although breeding has been reported at nearly 40 islands along the Chilean coast, 32 contain <50 pairs each and 6 concentrate >90% of the breeding population. In 2019, a PVA suggested that the overall population could be decreasing on average by 7% per year and predicted a mean time to extinction of 59 years (range 35-85 years). While management decisions seem to be the right ones, they are not being effective. We may not be doing enough to adequately protect the Humboldt Penguin. We dedicate this presentation in loving memory of Gabriel Knauf. This study was supported by SPHENISCO e.V. (Germany).

## 48. Birds of a feather flock together? Winter dispersion of Southern rockhopper and Magellanic penguins

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We studied the non-breeding dispersion of Magellanic (*Spheniscus magellanicus*, MP) and Southern Rockhopper (*Eudyptes chrysocome chrysocome*, SRP) penguins. We analyzed spatial movement characteristics and phenology and studied spatial-, environmental- and trophic-niche overlap: a) among three MP colonies located along the breeding distribution of Argentina (n=1 year & 31 penguins), and b) between MP and SRP breeding in close proximity (25 km apart, n=3 years & 31 penguins). For it, we used geolocators and stable isotopes from blood samples. We found similar trip lengths and dispersal distances among MP colonies, all heading northwards. This resulted in the northern and the central colonies facing similar oceanographic features and exploiting comparable trophic niches (anchovy domain) in contrast to individuals breeding further south that remained in the sprat domain. In fact, the overlap between central and northern colonies was 44%, between central and southern colonies was 38%, and between northern and southern colonies was 19%. Between SRP and MP, both also heading northwards, there were no differences in the maximum distance reached but in total distances traveled ( $\bar{x}_{SRP}=1220\pm36\text{km}$ ,  $\bar{x}_{MP}=1135\pm66\text{km}$ ). On average, MP departed 12 days earlier than SRP and arrived 21 days before, dispersing ~9 days less than SRP. The interspecific spatial overlap was 43% while isotopic niches did not overlap. SRP was situated on lower trophic levels and expressed a more pelagic diet/habitat. Our results indicated space and trophic niches segregation during the non-breeding period among MP colonies and between penguin species, avoiding competition and making extensive use of the Patagonian Shelf.

## 49. Sex-specific migratory behavior in Magellanic penguins results in more risks for females

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Protecting migratory animals requires knowledge of their distributions throughout the year. Spatial or temporal segregation of females and males during part of the annual cycle complicates conservation measures and can lead to sex-biased mortality. Females and males of many seabird species use separate areas during the nonbreeding season and sex-biased bycatch in fisheries is common. We satellite tracked eight female and eight male post-breeding adult Magellanic penguins *Spheniscus magellanicus*, including seven mated pairs, at Punta Tombo, Argentina, during their fall northbound migration, April-July 2022. Females stayed significantly closer to shore than males ( $62 \pm 43$  km vs  $109 \pm 51$  km). On average females and males went to similar latitudes from northern Argentina to southern Brazil. Females tended to stay farther north, however, with some males headed south by June. Body size did not influence how far offshore a penguin migrated, when controlling for sex. On Average, females left the colony 2 days before their mates and did not encounter them at sea. The mean distance between mates at sea was more than 350 km. Females are likely exposed to fishing gear and pollution more than males because of the areas the females used. Migratory routes varied by individual as well as sex. The large area that penguins use during their nonbreeding season makes protection of the population difficult.



## 50. Disparate dispersal behavior of fledgling Adélie penguins from two colonies on Ross Island

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Several studies have implicated post-fledging survival as an important driver of Adélie penguin population dynamics. Juvenile seabirds, including penguins, are particularly difficult to study as they do not return to breeding colonies for several years post-fledging. As a result, this important life history phase remains understudied. We present initial results from the first tracking study of Adélie penguin fledglings in the Ross Sea. In late summer 2023, we deployed newly-invented, transmitting GPS-TDR tags on 54 fledgling Adélie penguins at two colonies on Ross Island, Cape Crozier (~270K breeding pairs) and Cape Royds (2.4K breeding pairs). Data from the first 30 days showed that penguins from the two colonies undertook contrasting dispersal patterns. Those originating from Cape Crozier moved rapidly in a northeasterly direction, covering an average of 61 km per day. In contrast, penguins from Cape Royds stayed near the Victoria Land coast and moved much more slowly (25 km per day). Diving data indicated that fledglings made an average of 483 (SE  $\pm$  52) dives before their first foraging dive was recorded. Fledglings performed fewer and shallower foraging dives compared to adults over the same period but started to approximate adult dive behavior by the end of the first 30 days at sea. The rate of tag disappearance was higher for Cape Royds fledglings over the first 30 days (70% lost) compared to Cape Crozier fledglings (40% lost), suggesting higher early mortality rates of fledglings from the much smaller Cape Royds colony.

## 51. Going with the flow: Adélie penguins adjust to sea-ice movement during winter migration

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Communities of upper trophic level vertebrates in polar regions are tied to sea ice, but how their movement within dynamic sea-ice fields is affected by ice movement is little known, particularly during the non-breeding season. In the southern Ross Sea, Antarctica, Adélie penguins undergo the longest winter migration known for the species, potentially assisted by the Ross Gyre, which drives ocean circulation and the large-scale movement of sea-ice in this region. While Adélie penguins spend much of their wintering period in areas of high sea ice cover, little is known about what areas or biophysical features may provide important foraging habitat. Adélies need access to at least some open water to feed. Polynyas, which are areas of open water surrounded by ice, with heightened spring productivity, provide important open-water habitat for many species in summer, and may also serve as important feeding grounds during winter. Using remotely sensed ice data and geolocation-based penguin movement data, we test the hypotheses that penguins use sea-ice movement to aid their migration and that polynyas serve as foraging hot-spots during the non-breeding period. Penguins traveled greater distances when their movement vectors aligned with those of sea ice and the amount of ice support received depended on which route a penguin took. Our findings show the importance of ocean/sea-ice circulation patterns to wildlife movement and life history within the Ross Sea. Changes in sea ice may thus have consequences not only to energy expenditure, but to the ecological interactions that have defined this region.

## 52. Spatial assignment of winter migration of Magellanic penguin (*Spheniscus magellanicus*) using predator-based isotopic landscapes

**Juliana Gonzalez<sup>1,3,4,5</sup>, Melina Barrionuevo<sup>2</sup>, Esteban Frere<sup>3</sup>, Pablo Yorio<sup>5</sup>, Noela Sanchez-Carnero<sup>4,5</sup>, Javier Ciancio<sup>5</sup>**

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Studying animal movement can provide valuable insights into migration habits and space utilization, which are crucial for developing accurate conservation policies. Techniques based on electronic devices such as dead reckoning or telemetry (like GPS and GLS), have been used to track the movement of migratory Magellanic penguins (*Spheniscus magellanicus*). These methods have generated extensive individual tracking data, but they have limitations when it comes to following long distance migrations: while self-recording GPS batteries do not last long, solar rechargeable tags or satellite transmitter service are expensive and the GLS tag geographic accuracy is low. In recent years, the use of isotopic landscapes for retrospective geolocation has emerged as complementary method, and has proven useful in elucidating migration habits of pelagic sea birds. In this work,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isoscapes of the Atlantic Patagonian Shelf were used to infer location of Magellanic penguins feeding at their wintering grounds. The isoscapes used were based on blood samples of breeding Magellanic penguin, and showed a strong latitudinal gradient with minimal error, which is requisite for their use to assign location. Subsequently, blood from just arrived individuals were sampled in one colony and maps of probability of spatial use were developed from the isoscapes and contrasted with same individual GLS data to evaluate the assignment accuracy. Our study provides valuable information for the understanding of penguin use of space in non-breeding season.

## 53. A Multi-UAV approach to surveying large penguin colonies

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As the pace of global change accelerates, there is an increasing need for rapid assessment of related impacts on populations. In this context, UAVs offer several advantages over traditional aerial surveys, including lower cost, ease of deployment, reduced risk to participants, and reduced disturbance to wildlife. In this study, we tested the feasibility of using a multi-UAV system to conduct regular surveys of large penguin colonies in Antarctica and developed an automated workflow to increase the speed, efficiency, and precision of processing data from UAV surveys. To address the first objective, we designed a multi-UAV path-planning method optimized to make the best use of limited flight time. We demonstrated the algorithm's capabilities by using a team of drones to conduct multiple photographic surveys at three Adélie penguin colonies on Ross Island, Antarctica. The largest colony, which contains ~300,000 pairs and spans over 2 km, was surveyed in about 3 hours. In contrast, previous human-piloted single-drone surveys of the same colony required over 2 days to complete. To address the second objective, we constructed a workflow that generates a mosaicked image of the penguin colony, uses a trained convolutional neural network model to predict the number of penguins in the image, and applies post-prediction statistical modeling to generate a breeding population estimate. We provide initial estimates of the number of breeding pairs at two colonies and compare them to other censusing methods.

## 54. Association between molt and breeding phenology helps explain the recent decline in breeding Humboldt penguins at Punta San Juan, Peru

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Punta San Juan (PSJ) in Peru is an important historical reproductive site for Humboldt penguins (HUPE). Since 2015 a decline in HUPE population at PSJ has been observed. However, to date, reasons behind it are not clear. Changes in phenology can reflect population response to climate change that affect reproduction and migration patterns. This study explores if there is a detectable relationship between breeding and molting phenology at PSJ between 2010-2022 to help explain the decline. Number of molting penguins (MP), active nests (AN) and brooding nests (BN) were collected in PSJ through weekly routine surveys between 2010 to 2022. Change point analysis was used to evaluate breaking points in mean abundance of MP, AN and BN; permitting up to four segments. Finally, linear models were run between maximum values, peak dates and the difference in days between peak MP, AN and BN.

We detected one significant change point for each MP, AN and BN; that split each time series into period 1 (P1) and period 2 (P2). P1 always included years of higher abundance and P2 lower abundance. P1 included years 2010 to 2016 or 2017, and P2 from thereafter. An inverse relationship was found between the number of days of peak dates of MP and peak dates of BN. Therefore, results suggest that an increase in the bouts between molt and peak nesting impacted the number of HUPE brooding nests produced in years of warmer ocean conditions (2016-2018) contributing to the PSJ decline.

## 55. Cape Royds penguin colony trends revisited

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The longest, continuous Southern Ocean biological time series is the annual counts of the Cape Royds Adélie penguin colony beginning 1955. Factors affecting colony size have varied over time, with direct and indirect influence from human activity. Herein we present evidence that the 67 years of demographic trends can be divided into the following 5 periods. First, an initial decrease due to uncontrolled tourism from McMurdo Station/Scott Base, until recovery with designation as Antarctic Specially Protected Area in 1975. Second, colony further increase in response to 'competitive release' from removal of ~16,000 trophically competing Minke Whales from IWC Areas V and VI (colony wintering area). Third, the 1982 whaling ban and subsequent whale recovery exerted a negative competitive effect but one countered by increased winds facilitating a more persistent McMurdo Sound Polynya (MSP; easier ocean access during nesting), and the removal to 1984 of ~2000 Weddell Seals (to feed sled dogs). Fourth, 2001-2005 colony decrease when the B15 mega-iceberg opposed the wind effect to increase sea ice cover thus to retard colony access. Lastly, recovery plateaued at a lower historical level due to wind-generated Ross Gyre velocity retarding penguin arrival despite increased MSP; seal recovery and high nest predation by South Polar Skuas had additional negative influence. The colony's lack of full recovery from the B15 event differs starkly from the dramatic increases in neighboring colonies. This history demonstrates the value of long time series, as well as regional time series, toward understanding penguin population dynamics.

## 56. Individual identification of Humboldt penguins using neural networks

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The accurate identification of individuals is essential for behavioural studies and population dynamics. For many species, external markers such as rings are used for identification purposes. However, for penguins, the use of flipper rings has been shown to have negative effects on both survival and reproductive success. While RFID identification partially solves the problem, its main drawback remains the short reading distance. To address this challenge, we present a vision-based method of individual recognition for Humboldt penguins, achieving an impressive 98% accuracy rate.

Our model not only achieves a high accuracy rate for known individuals, but it is also capable of detecting with great precision when an individual is 'unknown,' i.e., not present in the labelled dataset. Additionally, our method can work with images containing multiple individuals, segmenting the image into several smaller images, adjusting their size appropriately, and identifying the individual on each picture, all automatically.

We have compared our method with other existing similar approaches using different metrics to demonstrate its superiority. Furthermore, we have found that our approach is transferable to other species and taxa, as long as they exhibit persistent skin patterns. To facilitate the implementation of our method, we provide a detailed protocol.

Overall, our image-based method of individual recognition for Humboldt penguins offers a non-invasive and highly accurate alternative to traditional methods of individual identification. Its transferability to other species with persistent and stable skin patterns suggests broad potential applications in behavioural and population studies.

## 57. Divided home, divided fate: The mystery behind divergent populations trends of Erect-crested penguins on subantarctic islands

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The Erect-crested penguin (ECP) is the least known penguin species. It is endemic to New Zealand's subantarctic Bounty and Antipodes Islands, the latter generally considered the species' stronghold. There is only patchy information about population trajectories and even less is known about ECP's ecology. Despite being one of four penguin species classified as 'endangered' by the IUCN red list, ECP have received surprisingly little scientific attention. This ranking is based on the assumption that ECP numbers have declined by 50-75% since the 1970s. However, a partial drone survey of the Bounty Islands in 2019 and a review of historic data cast doubt on the veracity of this assumption. In November and December 2022, we conducted a complete survey of the world breeding population of ECP using drones on the Bounty and Antipodes Islands. Our data indicate that the species has remained stable at around 26,000 pairs on the Bounty Islands at least since the 1990s, and that the reported population decline for the archipelago probably resulted from extrapolation errors. Meanwhile, on the Antipodes Islands located 200 km south of the Bounties, penguin numbers seem to be in free fall. Preliminary results from ground counts and comparison of drone and satellite imagery suggest a decline of up to 50% on the island in the past 15 years putting the current population size at less than 25,000 pairs. We will present original data and discuss our current hypotheses which may (or may not) explain why the trajectory of two practically neighbouring penguin populations seem to be heading in opposite directions.



## 59. First estimates of male and female survival for the rare and endangered Galápagos penguin

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Galápagos penguins (*Spheniscus mendiculus*) are rare and endangered, and studies of their survival offer insights into their population dynamics, responses to environmental change, and can support their conservation. Studying Galápagos penguins is challenging due to their asynchronous breeding schedules, lack of colonial breeding, and geographic remoteness. Survival rates of Galápagos penguins have therefore not been robustly estimated, despite the population's conservation status. We built a Bayesian mark-recapture model to estimate survival rates of adult male and female Galápagos penguins using data from 484 individuals captured in Galápagos, Ecuador between 2010 and 2022. Because the breeding location and status of captured individuals were often unknown but likely influenced their detectability, we estimated detection probabilities for two latent classes that we assumed represented 1) a highly detectable class of individuals that breed within the study area, and 2) a less detectable class of transient individuals that were captured while passing through the study area. We estimated semiannual survival to be 0.84 (95% CI=0.78-0.92) for males and 0.85 (95% CI=0.77-0.93) for females. Survival was lowest between 2015 and 2016, coinciding with an El Niño, during which adults were in poor body condition and did not breed. Overall, survival was lower than expected for Galápagos penguins, and we discuss possible biological and methodological reasons for these low survival estimates. Our results illustrate the challenges in determining survival rates of rare and remote seabird populations while highlighting the ability of hierarchical mark-recapture models to confront these challenges to better understand population dynamics under climate change.

## 60. How a rover should approach penguins to get scientific data without disturbance

**Yvon Le Maho<sup>1</sup>, Nicolas Durr<sup>2</sup>, Victor Planas-Bielsa<sup>3</sup>, Julien Courtecuisse<sup>1</sup>, Mathieu Brucker<sup>1</sup>**

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RFID avoids the impact of flipper-bands and allows automation of data collection. Its main limitation is the short reading distance. To prevent human presence and resulting disturbance, we pioneered a few years ago the use of remote-controlled rovers. The behavioural and physiological responses of penguins to a rover approach also enable to determine the cues that make them perceive this intruder as threatening or not, depending on their breeding status. The rover induced in incubating king penguins a similar increase in heart rate and territorial defence than a passing fellow breeder, whereas incubating emperor penguins prudently retreated. However, emperors expressed no fear but curiosity when a fake small chick camouflaged the rover (Le Maho et al., *Nature Methods* 2014). We will now present data obtained with a prototype of a fake adult emperor penguin in the natural environment. With more space, it will allow fitting additional instrumentation to provide new data such as images and vocalizations that would be almost impossible to get otherwise. We have also investigated the effect of how the rover is approaching i. e. speed and method of progression. For this, we focused on captive Humboldt penguins in a zoo setting which provides an ideal environment for controlled experiments. By combining in-captivity and in-the-wild tests, our goal is to identify the warning signs that shape penguins' response to an intruder, based on their innate or learned through experience perception. Ultimately, in addition to a better understanding of penguin biology, it will enable refining study protocols.

## 61. Penguins and ARGOS satellites telemetry: A long story of migration monitoring

**Sophie Baudel<sup>1</sup>**

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Argos is the main satellite telemetry system used by the wildlife science research community, environmental agencies, NGOs, foundations, charities, parks, reserves, etc. for animal tracking and animal behavioral data collection all around the globe, to observe, to analyze, to understand large-scale migrations.

Argos tracking data collected from hundreds of animal species among birds, land animals, marine animals, fishes (more than hundred thousand of animals have been tracked since the beginning of the Argos constellation in 1986) make available a high-precision mapping of migratory corridors, feeding and foraging areas, breeding, and birth areas, and the fine description of seasonal, interannual and other time-scale dependent behavioral factors.

The community of biologists studying the penguins has been faithfully using the Argos system since the beginning, having collected (figures from 2007 to present) a rich archive of Doppler locations and other sensor data, like temperature, depth.

The talk will illustrate the Argos-tracked penguin species by figures, global and regional maps, and time animations.

It will also introduce the future of the Argos satellite constellation with the perennial and long-term support of the 4 institutional space agencies launching the Argos satellites (namely Cnes, Noaa, Eumetsat, Isro) and the launch by the end of 2023 of the new and innovative Kineis nanosatellites constellation, fully compatible with the Argos institutional one. Kineis will offer decisive functional and technical steps: a new downlink capacity, an improved revisit time with more than 30 satellites, an improved Argos Doppler positioning, new modulation frequencies, etc.

## **62. Prey-mediated environmental effects on little penguins: using sailing drone to monitor the marine ecosystem**

**Claire Saraux<sup>1</sup>, Lilia Guillet<sup>1</sup>, Jonas Hentati-Sundberg<sup>2</sup>, Andre Chiaradia<sup>3</sup>**

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Scientists largely agree that climate effects on individuals and populations operate not only directly by modifying individual physiology and behavior, but also indirectly through changes in habitat or food supply. Yet, it is difficult and expensive to monitor the oceans resulting in the absence of data on prey or in a mismatch between the required and available spatio-temporal resolution of these data. These limitations make it hard to study prey-predator interactions and their consequences on population dynamics. Here, we propose to develop a holistic approach integrating prey population dynamics within the study of environment-predator relationships in one of Earth's most rapidly changing marine environments, the Bass Strait, Australia. We are using an innovative prey survey method, a sailing drone equipped with an echosounder (200kHz) to test the hypothesis that the key factor to which predators respond is not prey biomass but prey accessibility. It will provide for the first time a continuous index of prey biomass and spatial distribution in the entire little penguin foraging grounds at a fine spatio-temporal resolution throughout multiple little penguin breeding seasons. Results from the trial deployment of the drone in 2023 show promising possibilities, as we were able to detect plankton layers and fish schools along transects. The simultaneous monitoring of oceanographic variables (sea surface temperature, salinity, oxygen and chlorophyll a) will enable us to build fish habitat models. Developing automated monitoring systems of prey using sailing drones coupled with detailed seabird monitorings could help comparative analyses across ecosystems.

## 63. Re-establishing an African penguin colony at the De Hoop Nature Reserve, South Africa

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Re-establishing extinct seabird colonies is an effective conservation tool that has been used to restore seabird populations globally. This technique has been used predominantly for flying seabirds, with little focus on penguins. Establishing new or re-establishing extinct African Penguin colonies was identified as a potential conservation intervention for the species in the African Penguin Biodiversity Management Plan published by the South African government in 2013. Penguins established a small breeding colony in the De Hoop Nature Reserve, an area of high fish abundance on the southern cape coast of South Africa, in 2003 but the colony was abandoned by 2008 due to high levels of predation. In 2018, work was started to re-establish this colony. An ongoing predator monitoring programme was started and a predator-proof fence was constructed. Techniques from other seabird restoration projects have been used to attract penguins to the colony site, including artificial penguin decoys and call playback. Additionally, over 180 juvenile African Penguins have been released back into the wild from the site to encourage them to return to breed once they have matured. We present progress towards re-establishing the colony, including increasing numbers of moulting penguins using the site, the first record of breeding since 2008 and discuss the challenges faced and plans for further work.

## 64. The status and trends of Macquarie Island penguins

**Julie McInnes<sup>1</sup>, Penelope Pascoe<sup>1</sup>, Helen Achurch<sup>2</sup>, Ben Raymond<sup>2</sup>, Marcus Salton<sup>2</sup>, Noel Carmichael<sup>3</sup>**

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Penguin populations face a range of terrestrial and marine pressures that can lead to reduced breeding success and population changes. Understanding the status and trends of populations, and the environmental and anthropogenic drivers of change are critical for developing evidence-based conservation and management strategies. Macquarie Island is the breeding site for four penguin species: Gentoo (*Pygoscelis papua*), king (*Aptenodytes patagonicus*), rockhopper (*Eudyptes chrysocome*) and the endemic royal penguin (*Eudyptes schlegeli*). Population census frequency and survey methodology has varied between species due to differences in breeding phenology and nesting habitat. We provide an update on the population status and trends for each species where data is available, and highlight the challenges with long-term monitoring of some species. Population trajectories are variable. Gentoo penguin populations have declined 2% per annum over the past 34 years, with a 50% decline over three generations. King penguins are no longer increasing exponentially as they were in the 19<sup>th</sup> Century, with a 1% per annum decline in chick numbers over the last 15 years. Royal penguin numbers appear to be stable, however the large error range associated with density surveys has highlighted the need for more accurate data to understand population trends. Rockhopper penguin surveys are challenging due to the terrain and nesting habitat, yet there is a strong need for accurate methodologies given global population declines. We present preliminary research into correlations between counts and environmental conditions on land and at sea, and discuss future directions of research to fill data gaps.

## 65. Alarming prediction: Climate change effects on sympatric penguins of *Pygoscelis* genus

**Constanza Weinberger<sup>1</sup>, Carola Cañón<sup>1</sup>, Daly Noll<sup>1,2,3,4</sup>, Fabiola León<sup>1,2,3</sup>, Eduardo Pizarro<sup>1,2,3</sup>, Juliana A. Vianna<sup>1,2,3</sup>**

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The identification of environmental changes may be crucial to evaluate decision-making in the face of climate change. Phylogenetically close sympatric species, with different ranges of tolerances to environmental fluctuations, dispersion ability, and geographic range, could have different adaptive responses to the effects of climate change. *Pygoscelis adeliae* and *P. antarctica* are sympatric species in the Antarctic Peninsula, but differ in their foraging behavior and geographic range. Here, we evaluated the population structure among breeding colonies using ddRAD and estimated their historical and future (2100) spatial distribution through species distribution modeling (MaxEnt), using reported censuses. Based on the species' ecology, we selected the climatic variables from public databases (Chelsa and BioOracle). For the prediction of the future distribution, an intermediate-effect climate scenario (SPP2.4.5) was evaluated. We found high genetic diversity in both species, with low genetic structure in Adelie and none in the Chinstrap penguin. Chlorophyll concentration was the most significant predictor in determining the spatial distribution during the reproductive season for both species. Also, sea surface temperature had high predictive power for *P. adeliae* while the air temperature for *P. antarctica*. The 2100 prediction shows a noticeable decrease in the suitable area for both species, with risks of disappearing from the west-side of the Antarctic Peninsula for *P. adeliae* and of extinction for *P. antarctica*. Despite this serious forecast, evidence about their low genetic structure and high genetic diversity could contribute to the adaptive potential of these species, highlighting the importance of evaluating the effects on their survival.

## 66. Marine heatwaves in Western Australia affect breeding, diet and population size but not body condition of a range-edge little penguin colony

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Two marine heatwaves (MHWs) have developed along the Western Australian coast in 1999 and 2011. The extreme MHW in 2011 has been shown to have long term effects on the marine ecosystem. However, there have been few studies on the impacts of the this MHW on seabirds, and no biological impacts related to the severe 1999 MHW have been reported. Using data from 1986 - 2019 we investigated the impacts of these events on the ecology of little penguins on Penguin Island, located in temperate waters off Western Australia. Breeding outcomes but not body condition were negatively impacted by the MHWs. Diet composition changed during and after the 2011 MHW, with sandy sprat *Hyperlophus vittatus*, the typical major prey component, replaced by scaly mackerel *Sardinella lemuru*, a tropical fish species. Using an open robust design analysis for a single season, across six years from 2007 – 2019, we found the population during the austral spring decreased by 80% following the 2011 MHW, and the density of distribution on the island changed in relation to their foraging habitat. Finally, more penguins died from starvation or novel protozoal parasitic infections in 2011 and 2012. We show that the timing of MHW with respect to the birds breeding stage, as well as the timing of the return to more normal oceanic conditions influences the longevity of the impact of MHWs.



## 67. Record phenological responses to climate change in three sympatric penguin species

**Ignacio Juarez Martinez<sup>1</sup>, Alex Kacelnik<sup>1</sup>, Fiona Jones<sup>1</sup>, Jefferson Hinke<sup>2</sup>, Michael Dunn<sup>3</sup>, Andrea Raya Rey<sup>4,5,6</sup>, Heather Lynch<sup>8</sup>, Kate Owen<sup>3</sup>, Tom Hart<sup>1,7</sup>**

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The timing of breeding and its flexibility are important aspects of many species' realised niche, reflecting adaptations to synchronise with food supplies, dilute predation, avoid competition, and exploit abiotic seasonal fluctuations. In Antarctic Penguins these traits are typically studied through long-term monitoring of focal populations, making it difficult to characterize species-wide traits and trends. Here we use a network of one hundred time-lapse cameras to monitor sixty colonies across the Antarctic Peninsula and sub-Antarctic islands for a decade. This has allowed us to study some milestones of breeding phenology (season start, egg-laying and hatching) on three sympatric Pygoscelid species of penguins (Adélie, Chinstrap and Gentoo) at a sub-continental scale. We also explore a method to estimate breeding success from timelapse images and relate both to an unprecedented warming recorded at the study colonies. We found the breeding seasons for all three species advanced at record rates (10, 10, and 13 days/decade, respectively) showing great adaptive capabilities. Phenological change shows great intra- and inter-species variability and in all three species. Those advances are related to environmental warming in their respective colonies. The differences in response to warming between the foraging generalist Gentoos (local populations increasing) and the foraging specialists Adélies and Chinstraps (local populations decreasing) suggest recent changes selectively advantage the former while handicapping Chinstraps and Adélies. If these trends continue, they could lead to increased competition between species, a risk of trophic mismatch, and a reshape of community assemblages in the near future.

## 68. Sea ice concentration decline in an important Adélie penguin molt area

**Annie Schmidt<sup>1</sup>, Amélie Lescroël<sup>1</sup>, Simeon Lisovski<sup>2</sup>, Megan Elrod<sup>1</sup>, Dennis Jongsomjit<sup>1</sup>, Katie Dugger<sup>3</sup>, Grant Ballard<sup>1</sup>**

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Unlike in many polar regions, the spatial extent and duration of the sea ice season have increased in the Ross Sea sector of the Southern Ocean during the satellite era. Simultaneously, populations of Adélie penguins (*Pygoscelis adeliae*), a sea ice obligate, have been stable or increasing in the region. However, demonstrating links between Adélie penguin population growth and sea ice concentration has proved complex, with sea ice being implicated in different, sometimes contrasting, demographic patterns. Adélie penguins undergo a complete molt annually, replacing all their feathers in about 19 d shortly after the breeding season. Unlike most penguins, available evidence suggests that Adélies typically molt on sea ice, away from the breeding colonies, which makes this potentially vulnerable period particularly difficult to study. Here, we test the hypothesis that areas of high sea ice concentration provide important molting habitat for Adélie penguins. We analyzed data from 195 geolocating dive recorders deployed year-round on adult penguins at two colonies in the Ross Sea from 2017-2019. Remotely sensed data indicated that sea ice concentration during molt was anomalously low during the study and has declined since 1980. Further, estimated annual return rates of penguins to breeding colonies were positively correlated with sea ice concentration in the molt area over 20-years. Together these results suggest that sea ice conditions during Adélie penguin molt may represent a previously underappreciated annual bottleneck for adult survival, with important consequences for population dynamics.

## 69. Surviving the Heat: increasing ocean temperature and shifting breeding patterns of little penguins by the 22nd Century

**Andre Chiaradia<sup>1,2</sup>, Catriona McCallum<sup>2</sup>, Eleanor Moore<sup>2</sup>, Leanne Nguyen<sup>2</sup>, Vincent Blanc<sup>3</sup>, Richard Reina<sup>2</sup>**

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Climate change and its effect on marine biodiversity are vital to understanding how animals respond to the fast-changing marine system in the future. Sea surface temperature is critical in regulating a wide range of ocean processes. Understanding its impacts help in predicting the effects of global warming on marine life. We combined three separate studies, but all focused on the little penguins' response to the ocean temperature changes off the southern-east coast of Australia, where ocean temperature is increasing four times faster than the global average. We used 18 to 25 years of data to identify changes in the breeding timing, success, isotopic diet composition, chick survival and breeding phenology against ocean temperature, water stratification and marine heatwaves. Penguins advanced the timing of breeding by over a month in correlation to an increase in ocean temperatures. Individuals breeding before the mean egg-laying date were older and had higher reproductive success. Little penguins alter their foraging grounds in response to prey abundance and availability changes. They are coping with marine heatwaves by adjusting their phenology and foraging strategies to mitigate their impact on reproductive success. We forecasted our results until the year 2100 using IPCC scenarios. Little penguins will continue to advance the timing of breeding and slightly increase reproductive success until the end of the 21st Century. Their ability to adjust their life cycle to marine environmental changes makes them a valuable bioindicator for the ecological impacts of rising ocean temperatures and more frequent marine heatwaves.

## 70. Adaptive phenotypic programming to social density in king penguins

**Camille Lemonnier<sup>1,2</sup>, Quentin Schull<sup>1,7</sup>, Antoine Stier<sup>1,3</sup>, Rudy Boonstra<sup>5</sup>, Brendan Delahanty<sup>5</sup>, Emilie Lefol<sup>1,4</sup>, Laureline Durand<sup>1,4</sup>, Sylvia Pardonnet<sup>1,4</sup>, Jean-Patrice Robin<sup>1</sup>, François Criscuolo<sup>1</sup>, Pierre Bize<sup>6</sup>, Vincent A Viblanc<sup>1</sup>**

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Individuals living in different social environments often exhibit phenotypic differences. The cause of these differences may be genetic pre-adaptations or acquired adaptations dependent on the nature of the social environment. Penguins breed in colonies of varying sizes and densities where parents and offspring often face aggression from conspecifics. We used a large-scale cross-fostering experiment in king penguins (*Aptenodytes patagonicus*) to test for early adaptive programming of offspring phenotype to rearing density. We exchanged eggs just after laying within and between pairs breeding in high- and low-density colony areas, and assessed differences in parent and chick phenotypes (growth, metabolism, stress axis) and survival. Compared with parents breeding at low density, those at high density showed reduced resting behavior and increased aggression and vigilance; decreased stress response to handling, more pronounced hypometabolism during incubation, and increased maximum corticosterone binding capacity (MCBC). All suggested mechanisms to cope with a stressful social environment. Chick development was more influenced by the social environment of their foster than genetic parents. Those from low density areas, but reared at high density had increased weight gain and survival probabilities. Chicks reared at high density had lower baseline MCBC levels and higher baseline free corticosterone levels than chicks reared at low density. However, they had an attenuated acute corticosterone response to captures. Thus, offspring reared at high density were better equipped to handle the increased social demands of high densities. This ability is not intrinsic, but environmentally programmed after hatching to match their rearing environment.

## 71. Initial asymmetry: The effect within Magellanic penguin (*Spheniscus magellanicus*) broods in a cross-fostering experiment

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In seabirds, was suggested, that initial asymmetry (IA) within a brood only has an effect during the first days of the chick's growing period and are the parents who determine growth and survival of the chicks. In Magellanic penguins (*Spheniscus magellanicus*) usually two chicks hatch within 2 days. Our objective was to study the effect of IA in growth and survival of Magellanic penguin chicks at Isla Quiroga Santa Cruz from 2018 to 2022 with a cross-fostering experiment generating different IA and assess the feeding frequency during three stages of the chick's growth. We found that IA determine the asymmetry during the whole growing period ( $p < 0.001$ ), and last hatched chicks have a greater survival chance when the initial asymmetry with their older brother is smaller ( $p < 0.014$ ). The feeding frequency in different stages on the breeding season was different within seasons and periods ( $p < 0.001$ ), and the IA has an effect in which chick received food ( $p = 0.012$ ) and second chicks received less amount of food than its siblings when they were more asymmetrical. However, parents only have an effect in chick's growth ( $p = 0.029$ ). We found that IA affect the fate of chicks. Otherwise, the parental body condition only has an effect on the chicks' growth but not in their asymmetries. We conclude that parents fail to reverse the effect of initial asymmetry in a post-hatching stage, even in years of high reproductive success. But they could intervene in the survival within the brood would be through the establishment of an initial asymmetry.

## **72. Investigating the effects of early growth on little penguins' life-history traits**

**Justine Wintz<sup>1</sup>, Nicolas Joly<sup>1</sup>, Jenouvrier Stephanie<sup>3</sup>, Andre Chiaradia<sup>2</sup>, Claire Saraux<sup>1</sup>**

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Early-life conditions can play a strong role in juvenile development, often resulting in modifications of later life-history traits and population dynamics. Thus, the study of early development is of utmost importance especially to address potential conservation plans. Although faster-growing chicks may benefit from greater fledging success, they may also experience deleterious long-term effects on adult life-history traits due to the effects of growth on physiological functions through oxidative stress. We investigated the impact of early growth on later life-history traits in 1818 little penguin chicks from 1995 to 2020 at Phillip Island, Australia. We predicted that chicks growing at a faster rate should have a greater chance to fledge and reproduce early, but they could pay the price in the long run and die younger. Using clustering analyses on 11 growth parameters, we distinguished three different phenotypes: i) fast and heavy chicks: individuals growing fast and reaching high body masses (56% of the population); ii) slow but heavy chicks: individuals growing slowly but reaching high body masses (21%); and iii) slow and light chicks: individuals growing slowly to reach low body masses (23%). The fledging survival was significantly higher in fast and heavy (75%) and slow and heavy chicks (72%) than in slow and light chicks (47%). Current analyses investigate the effect of growth on juvenile survival, age at first maturity, adult survival and breeding success. Finally, we will link these life history traits to population dynamics using matrix models to understand the demographic consequences of early growth.

### 73. Patterns of skipped breeding and reproductive success in Magellanic penguins (*Spheniscus magellanicus*)

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Skipped breeding is a well-known phenomenon in seabirds. Using a 35-year dataset from declining colony of Magellanic penguins (*Spheniscus magellanicus*), we looked at how skipped breeding rates relate to lifetime reproductive success and environmental conditions. For males, a breeding attempt was the establishment of a territory, even if the individual did not get a mate; for females, a breeding attempt was if they laid at least one egg. On average, 13% of penguins a year skipped breeding. Females were twice as likely to skip as males: nearly 20% skip at least one year, compared to about 10% of males. Skipping rates also correlated with breeding outcomes in sex-specific ways. Where roughly the same percentages of males skipped breeding the following year regardless of whether their attempt failed at the egg stage, the chick stage, or a chick successfully fledged, females were more likely to skip breeding if the nest failed at either the egg or chick stage than if a chick fledged. We also considered environmental covariates with skipped breeding, including sea surface temperature, primary productivity, and the plume index at the Rio de la Plata estuary, where many penguins overwinter. Again, we found sex-specific differences in skipping rates, with females more likely to skip in years with poorer environmental conditions than males. Additionally, females that skipped more frequently had lower lifetime reproductive success. As climate change results in less favorable environmental conditions, a propensity to skip could have significant demographic consequences at a declining colony.

## 74. The neglected penguin: Reviewing the breeding of the Erect-crested penguin, *Eudyptes sclateri*

**Lloyd Davis<sup>1</sup>, Martin Renner<sup>2</sup>, David Houston<sup>3</sup>, Lei Zhu<sup>1</sup>, Wiebke Finkler<sup>4</sup>, Thomas Mattern<sup>5,6</sup>**

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Erect-crested penguins are the least studied of all penguins. They breed on two isolated subantarctic island groups, the Antipodes and Bounty Islands. Sporadic nest counts over the last half century indicate a dramatic decline in numbers. Here, we re-evaluate data from a study of erect-crested penguins undertaken in 1998 on Antipodes Island in the context of what we know about penguins 25 years later. Erect-crested penguins exhibit obligate brood reduction coupled with extreme reversed egg-size dimorphism. They arrive at the colony from their winter migration in a low state of reproductive readiness as evidenced by low rates of copulating, fighting, and low levels of testosterone in males. The laying interval is the longest of any penguin species with incubation being highly variable until clutch completion. Nests typically contain no nesting material and eggs are laid directly onto the ground. Most A-eggs are lost on or before the day the B-egg is laid, and none survive for more than a week after that. Males, with elevated testosterone levels, remain in attendance at the nest for up to 13 days after clutch completion despite females assuming most of the incubation duties. We examine possible explanations for the obligate brood reduction in this species and what it might mean for their resiliency. Finally, we discuss options for conservation marketing as a means of drawing attention to this enigmatic but troubled penguin species.



## 75. Unpacking the lifelong secrets of little penguins: Individual quality, energy allocation, and stochasticity in defining fitness

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Variability among individuals is one of the key factors in understanding evolutionary processes and demography. Yet, little is known about the relative importance of different life-history traits on interindividual variability. Here, we tested how individual quality, energy allocation trade-offs and environmental stochasticity can affect individual fitness over a lifetime. We monitored 150+ breeding little penguins (*Eudyptula minor*) over their entire life across 20 years. We investigated the interactions of 18 life-history traits and their relative effect on individual fitness using structural equation models. The penguins' fitness was highly variable and mainly driven by differences in the number of clutch per year, foraging capacities and above all early breeding timing. Positive covariation between traits might rely more on differences in individual quality than in breeding strategies. As phenology was by far the most important driver of penguins' fitness, we studied further why penguins breeding earlier displayed a higher fitness. Over 2500+ breeding events of ~ 500 penguins, delayed breeding was associated with reduced breeding outputs through less investment in chicks (shorter parental care, slower chick growth) and inefficient foraging (mass loss during breeding). Delayed phenology also induced important carry-over effects including less chance of breeding and lower success the next breeding season. With these results, we were able to establish the main ecological drivers of individual variability in little penguins' fitness at the lifetime scale and the main paths through which they affected breeding. We discuss the potential effect of such variability on population demography.

# Poster Presentations



**Southern rockhopper penguin**  
*Eudyptes chrysocome*

## 1. Antarctic weathervanes: penguin position in the nest sways with the wind

**Albert Palomino González<sup>1,2,3</sup>, Andrea Piñones<sup>1,2,3,4</sup>, Lucas Krüger<sup>3,5</sup>, Andrew D. Lowther<sup>6</sup>**

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During incubation and the beginning of chick rearing, when penguin chicks do not generate enough metabolic heat to survive independently, adults shelter their offspring from adverse weather. Specifically, strong winds increase heat loss rates and can ultimately affect breeding success. To minimize the effect of windchill, thus protecting their offspring, we expected adult penguins at exposed sites to lie backwards to the wind direction.

To test our hypothesis, we used generalized additive modelling to correlate the alignment to the wind of incubating and guarding penguins to wind exposure. We collected data at a chinstrap penguin colony in Nelson Island, South Shetland islands. We calculated penguin alignment as the angle between two vectors: wind direction and penguin position in its nest. We retrieved penguin position from orthomosaics derived from 60 m aerial photogrammetry. Wind speed and direction values were recorded at each subcolony. Finally, we extracted wind exposure values for each penguin using a Digital Elevation Model (DEM), also derived from aerial photogrammetry. In order to capture a wider range of wind conditions, this study included 4 subcolonies along an altitude gradient and overflow the colonies on three different days.

Wind exposure values extracted from the DEM show significant differences between three out of four subcolonies and reflect the differences in wind speed values recorded in the field. The selected model shows that penguins gradually improved their alignment to wind with increasing exposure, showing a steep rise in penguin alignment at exposures over 70%.

## 2. Circadian activity patterns of Magellanic penguins on land: the influence of light and temperature

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The activity pattern is evolutionarily defined by the interactions with physical and biotic variables, and may be modulated by the success in foraging, reproduction, and predation risk. We investigated the relationship between air temperature (recorded by camera traps) and light in the activity pattern of Magellanic penguins (*Spheniscus magellanicus*) on land. Data were obtained during 2022-23 breeding season by camera trapping (days=172; hours=4,200) in Cabo dos Bahías (44°55'S 65°33'W), Argentina. Activity patterns were defined regarding hourly distribution, temperature, and light categories (night=0, twilight gradient=1-4; day=5). Activity peaks were defined by grouping classes with  $\geq 10\%$  of the records of the most frequent class. We obtained 1,531 records from which 92.2% had a bimodal distribution peaking: 45.6% of the records from 3:00 (beginning of twilight) to 11:00h, mode and mean=07:00h (sunrise); and 46.6% from 15:00 to 23:00h (end of twilight), mode and mean=19:00h (sunset). Temperature varied between -2 and 43°C, whereas penguin activity was unimodal with 96.1% of records from 5°C to 28°C (mode=15°C; mean $\pm$ SD =15.7 $\pm$ 6.1). Circadian activity was not influenced by light or temperature separately, but responded to their interaction ( $R^2=0.589$ ;  $p=0.010$ ) increasing during twilight as temperature is lower and light starts to intensify. At night (19:00h-7:00h), activity increased with light ( $R^2=0.952$ ;  $p<0.001$ ) and at daytime (07:00h-19:00h) activity was negatively associated with temperature ( $R^2=0.379$ ;  $p=0.025$ ). Because daily light variation is more stable and limits nocturnal activity, changes in temperature can be critical and may change activity patterns (higher interannual variations), affecting the general behavior and therefore the breeding biology of penguins.

### **3. Deducing breeding success of the African penguin, *Spheniscus demersus*, from automated transponder reader data to reduce disturbance**

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The population of the endangered African Penguin is in serious decline with the total population falling at a rate of ca. 5% per annum. The need to carry out regular monitoring to collect demographic and phenological data to assist in the planning and implementation of conservation actions should be balanced against the disturbance that may be caused by such monitoring. Many African penguins are now fitted with Passive Integrated Transponders (PITs) that can be read remotely by ground readers at fixed locations in colonies. In this study we used data from these ground readers, combined with data collected during routine nest monitoring, to demonstrate that it should be possible to use data from the ground readers alone, to monitor breeding success in the future. We found patterns of movements that varied during the various breeding stages. Adults took shifts of several days during incubation; during the brood period the adults alternated each day with one remaining at the nest while the other went to sea. As the chicks grew large enough to be left alone, both parents usually foraged daily until the chicks fledged. In unsuccessful nests, adults stopped attending the nest shortly after it had failed. The findings of this research show that it could be possible to greatly reduce the potential impact of regular nest monitoring.

#### **4. Do Adélie penguins care about boundaries? Spatio-temporal consistency in the wintering behaviour of Antarctic sentinel species – implications for conservation**

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In polar regions, temporal variability in the sea ice may trigger yearly shifts in seabird movements and behaviour as an adaptation to changing environments. Thus, assessing this feature is crucial to evaluating possible threats. We evaluated the spatio-temporal consistency in wintering behaviour of Antarctic sentinel species within an ecologically relevant region proposed as a candidate Marine Protected Area (MPA). We tracked 62 Adélie penguins using geolocators with wet-dry sensor over 5 years from Ile des Pétrels (East Antarctica). Migratory movements were longitudinal and bounded by the 60°S latitude, reaching up to 1600 km westwards from the colony between July-August. Inter-annual overlap of wintering grounds indicated high spatio-temporal consistency (Bhattacharyya's affinity index: 0.82-0.96 among all years). Individual activity budgets varied throughout the winter, but foraging was mostly restricted to daylight. Daily time spent on water was the shortest around the winter solstice (June, 5.6±1 h), and increased with daylength towards the end of wintering period (September, 11.2±1.6 h). At a medium-temporal scale, individuals spent a relatively small proportion of the winter time within the proposed D'Urville Sea-Mertz (DUSM) MPA (12.3 ± 18.9 % of individual locations). Our results highlight the need to consider multiyear tracking datasets to properly assess the appropriateness of proposed MPAs, and call to extend the DUSM proposal to protect Adélie penguins in the critical winter period. More generally, the high inter-annual consistency found might imply species' vulnerability in the face of climate change. Overall, our work provides key knowledge to conservation and policymaking in the Southern Ocean.

## 5. Does haematology reflect at-sea movements in Magellanic penguins during the chick-rearing stage?

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Magellanic penguins (*Spheniscus magellanicus*) breeding at Cabo Dos Bahías (CDB; 44°55'S 65°33'W), Chubut, Argentina, show remarkable ecological plasticity with high inter-annual variability in foraging behaviour and breeding success. We hypothesized that individual differences in at-sea movements would be reflected in haematological parameters. We deployed GPS trackers on 27 adult Magellanic penguins (17 males and 10 females) at CDB during the early chick-rearing stage (chicks <30 days-old) of 2022. The tracker was recovered after a single foraging trip (trip duration, maximum distance to colony, total distance travelled) and blood was collected from the adult's tarsal vein (haematocrit, plasma total solids, leukocyte density, leukocyte proportions). Chick age upon device deployment was negatively correlated to plasma total solids ( $R=-0.503$ ) and positively correlated to the proportion of monocytes ( $R=0.531$ ). In turn, proportion of monocytes was different between the sexes ( $P=0.010$ ; males>females) and the correlation between this variable and chick age was only significant for adult males ( $R=0.582$ ). No significant associations were found between haematological and at-sea movement variables, except a negative correlation between total distance travelled and proportion of lymphocytes ( $R=-0.366$ ); however, this could be a spurious correlation as total distance travelled was also correlated to chick age ( $R=0.448$ ) and trip duration ( $R=0.589$ ). These results suggest that select haematological parameters in adult Magellanic penguins during the early chick-rearing stage are poorly reflective of demands from immediate at-sea movements, reflecting instead broader temporal patterns in adult haematology linked to the changing physiological demands associated with parental care as their chicks grow.



## 6. Examining the impact of food availability and nest structure on reproductive success of *Spheniscus humboldti* in Choros Island, Reserva Nacional Pinguinos de Humboldt

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Reproductive phenology is a key process in the survival of many species, and its synchronization with the surrounding environment is essential to ensure reproductive success. In this study on Choros Island (29°15' S, 71°32' W) in the Reserva Nacional Pinguinos de Humboldt, we examined how food availability and nest structure influence the reproductive success of *Spheniscus humboldti*. We monitored 50 nests for three breeding seasons (2019 to 2022), checking egg hatching and chick survival in each season. Additionally, we related this monitoring to food availability within a 50 km radius and nest architecture. As a result, we observed that a total of 86 eggs were laid with a hatching rate of 82.6%, of which only 56.3% of the 71 chicks survived. The highest egg-laying occurred one month after the highest level of marine productivity. The highest number of hatched eggs and chick survival was in nests that were completely covered by vegetation. In conclusion, we demonstrated that food availability and nest structure can significantly influence the reproductive phenology of penguins. These results emphasize the need for informed decisions on the management and conservation of Humboldt penguins, based on an understanding of their reproductive ecology and its relationship with the environment.



## 7. Fearless penguins, unfazed by *Felis catus*: Different behavioural and physiological stress responses of two populations of little penguins differing in levels of risk and disturbance

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Species that are constantly exposed to disturbances, such as human disturbance or nonlethal contacts with predators or conspecifics, can experience chronic stress. Within a species range, variation in the frequency and predictability of such disturbances can lead to population differences in stress responses. Here, we investigated the stress response of Little Penguins *Eudyptula minor* to an introduced predator and a conspecific at two South Australian colonies that differed in habitat, conspecific density, levels of human disturbance and predation risk (high, low). We used playback experiments of Cat *Felis catus* or little penguin calls and recorded the behaviour and physiological (heart rate) response of adults in relation to playback type (Cat, Penguin) as well as habitat characteristics (habitat type, nest type, nest visibility) and number of conspecifics present. Our results showed that individuals from the high-disturbance colony (also living in a mixed habitat with fewer neighbours) exhibited higher vigilance and heart rate responses than individuals from the low-disturbance colony (living in a closed habitat with a high number of neighbours). Our results highlight that guidelines for managing penguin species cannot be generalized across populations and need to be colony-specific.

## 8. Humboldt penguin behavioral responses reveals how to improve tourism guidelines in a marine protected area

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An important breeding site of Humboldt penguins was recently opened for tourism activity from boats in Peru. Ten visits simulating a regular tourist activity to Isla Guañape Norte were performed during August and October 2021 to determine the effects of approach distance, sound level and number of persons on board on the behavioral response of Humboldt penguins (alert or flee). Forty-six independent surveys indicate that behavioral responses are only related to the approach distance (Model selection AIC,  $w = 0.487$ ). Alert reactions occurred at  $\bar{X} = 70.15\text{m} \pm 16.98\text{ m}$  while flee were detected at shorter distances  $\bar{X} = 58.48\text{ m} \pm 16.95\text{ m}$ . Official tourism guideline for the island indicates 75 m as the minimum distance for boat approaches which is only 5 m away from the distance that triggers alert behavior. Increasing the minimum approach distance could assure a sustainable activity with less disturbance, considering that remaining stable on the sea is challenging. Replicating similar studies in other important sites for Humboldt penguins, could help for setting better regulations.

## 9. King penguin (*Aptenodytes patagonicus*) sightings and breeding attempts at Martillo Island, Tierra del Fuego, Argentina

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King penguins (*Aptenodytes patagonicus*) have a circumpolar distribution and inhabit subantarctic islands between 45°S and 55°S. Historical and archaeological records show this species used to inhabit the Fuegian region before their eradication by humans for commercial use in the 19<sup>th</sup> century. In recent years this species began to reappear in several areas of previous occupation. In 2013 a camera trap was placed facing a Gentoo penguin colony at Martillo Island, Tierra del Fuego and has been taking photos ever since. King penguins were registered, between 1 and 4 individuals at a time. Individuals were seen moulting, resting and even breeding. Breeding attempts such as displays, mating and incubation of rocks were observed the first years. In 2019-2020, a pair successfully produced a chick, for the first time, that lived 19 days. In 2020-2021 the chick lived 19 and in 2021-2022 only 9 days. In 2022-2023 adults stopped the reproductive event during incubation, abandoning the egg. Necropsies of the dead chicks did not reveal an apparent cause of death. Even if the early death of chicks produced by the breeding pair is overcome, breeding success will not occur until a larger amount of breeding pairs is recruited as chicks need to crèche during the winter months. The reoccurrence of species in previously vacated places may be due primarily to the lifting of anthropogenic impacts and may also indicate the environmental conditions are favourable for resettlement in this area.

## **10. King penguin locomotion on land: Biomechanical modeling and video footage analysis**

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Walking is a necessity for penguins because it allows them to get from the sea to their breeding ground in the wild. However, unique challenges specific to studying penguins have prevented a complete analysis of their motion on land. These challenges include insufficient anatomical data and time-consuming data collection and analysis techniques. We used CT scans of a King penguin to quantify its anatomical structure and thereby create the first biomechanical model of this species. We also used machine learning techniques to quickly extract kinematic data from penguin video footage. This information can be used to expand on existing knowledge about how penguins adjust their walking dynamics at different speeds and body sizes. In addition, it can be applied to the biomechanical model along with our accelerometry data for inverse kinematics analyses to show how joints that are not visible, such as the hip and knee, move as a penguin walks. Developing a more in-depth understanding of bipedal penguin motion can help us to understand why penguins walk the way they do; and even how changes in the environment, requiring them to walk longer distances, might affect them.

## 11. Magellanic penguin *Spheniscus magellanicus* chick with two cloacae and four legs

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With the exception of bill deformities, there are few reports of penguins with developmental abnormalities, either because these are uncommon or because affected individuals perish before they can be observed. On 27 January 2023 a Magellanic penguin (*Spheniscus magellanicus*) chick with two cloacae and four legs was found at Martillo Island (54°54'26"S 67°23'06"W), Tierra del Fuego, Argentina. The chick was at the last stage of parental care before emancipation, having almost completely lost its down plumage (estimated 75-90 days-old), and was in good body condition. However, it showed an extra set of legs and feet projecting caudally, connected to the body ventral to the tail. The anatomy of these supernumerary limbs resembled that of functional limbs (normal bone and soft tissues, fully formed digits and feet, etc.) but they were smaller and appeared non-functional, dragging behind the body as the chick walked. Upon closer inspection, it was also noticed that the bird had two cloacae, one on each side of the tail, both seemingly functional based on the presence of faeces on surrounding feathers. The nest was visited again four days later but the chick was not found. Survival of chicks is low during the first year and is expected to be even lower for individuals with mobility restrictions from deformities, representing a hindrance on their parents' breeding success. Future research would be valuable to determine the genetic and environmental factors triggering developmental abnormalities in penguin chicks and to quantify their impacts on breeding success at the population level.

## 12. Offspring sex, hatching order, and brood reduction: different strategies lead to different sex ratios?

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Southern rockhoppers penguins (RP, *Eudyptes chrysocome chrysocome*) are obligate brood reducers with large egg-size dimorphism within a clutch (20%) and a highly biased survival of first hatchlings (1<sup>st</sup>-H) belonging to the larger eggs. Magellanic penguins (MP, *Spheniscus magellanicus*) are facultative reducers with a more similar egg-size dimorphism. Taking into account the hatching order within a brood, we molecularly sexed MP hatchlings at Isla Quiroga (Sta. Cruz, Argentina) from 2010 to 2012 (n=117 broods) and RP hatchlings at Isla Pingüino (Sta. Cruz, Argentina) in 2015, 2018 and 2022 (n=68 broods). We found that 1.3% of the RP couples raised both nestlings, with 1<sup>st</sup>-H surviving the most; while in MP 46% of the couples raised both nestlings and 1<sup>st</sup>-H survived 10% more than second hatchlings. We found, for both species, an unbiased hatchling sex ratio (MP = 0.53, RP = 0.49). But, while in RP and MP the overall 1<sup>st</sup>-H' sex ratio was 0.48 ( $X^2=0.07$ ,  $p=0.79$ ) and 0.56 ( $X^2=1.75$ ,  $p=0.18$ ), second hatchlings' sex ratio was 0.36 ( $X^2=2.95$ ,  $p=0.08$ ) and 0.67 ( $X^2=12.9$ ,  $p<0.001$ ), respectively. So, the sex ratio of 1<sup>st</sup>-H was unbiased in both species, while second hatchlings were biased towards females in RH and towards males in MP. Probably this result is related to the different brood reduction strategies and survival probabilities of second hatchlings between species. In RP, these, together with a possible lower cost of raising a smaller female, might lead to a bias in second-hatchlings towards females that permits raising two nestlings if conditions are optimal.

### 13. Studying phenology and reproductive biology of southern rockhopper penguins using time-lapse cameras combined with individual marking

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Collecting temporal extensive data on phenology and reproductive success can be logistically challenging in remote colonies. Autonomous time-lapse camera systems offer an opportunity to provide such coverage. During two breeding seasons we studied phenology, parental care behavior and reproductive success of the southern rockhopper penguin (*Eudyptes chrysocome chrysocome*) population of Isla Pingüino, Argentina, through the use of autonomous time-lapse cameras combined with individual marking. We found that their breeding cycle was in agreement with existing data from other colonies of the SW Atlantic. Regarding nest and chick attendance we observed, on average, that females invested 14.6% more time than males incubating the eggs, while males dedicated 53.8% more time chick guarding than females. Feeding events during guard stage were more frequently held by females (approximately 83% of the recorded events); males' contributions increased then during the crèche period. The mean hatching success was similar in both seasons ( $\sim 0.84 \pm 0.5$ ), while breeding success varied between years ( $0.23 \pm 0.39$  and  $0.45 \pm 0.44$ ). The effect of heat stress on chick survival was assessed, since uncommonly high air temperatures were reached during the study, causing unprecedented chick mass mortality. The most extreme cases of chick's mortality were recorded during days with maximum temperatures higher than 36°C. The combined methodology of time-lapse cameras with individual marking turned out to be useful to study phenology, parental investment and the variables that affect reproductive success (for instance, weather conditions), all aspects that were poorly known for this particular colony.

## **14. Bayesian additive regression trees (BART) applied to global-scale species distribution models (SDMs): present and future projections of penguin species**

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Within the current scenario of ongoing global environmental change, Marine Ecosystem Models (MEMs) are developed to analyse the past and future dynamics of life in oceans. One of such efforts is EcoOcean, a complex, mechanistic and spatiotemporal explicit MEM of the global oceans based on a trophodynamic core. To predict species distributions and dynamics under contrasting scenarios of climate change and human pressures, EcoOcean requires as inputs the species native ranges, species-specific functional responses for key environmental variables, and time-varying maps of environmental variables delivered by Earth System Models (ESMs). The different sources of uncertainty in these inputs may influence the validity and precision of EcoOcean results. In this study, we evaluate the use of global Bayesian additive regression trees (BART) as a promising new alternative to traditional Species Distribution Models (SDMs) based on classification tree methods to generate these inputs to EcoOcean. To test BART's capability as an SDM on a global scale, we performed a suitability study with the worlds' 18 penguins as a widely distributed community in the Southern Hemisphere. Our results show that BART is a powerful approach to predict the potential distribution of penguin species, as well as their relationship with key environmental variables, on a global scale. Besides their usefulness as inputs for informing existing MEMs such as EcoOcean, our assessments provide insights on the past, present and future distribution of the whole penguin community. This information is essential for evaluating penguin responses to global change, and for guiding viable management approaches with global conservation policy objectives.



## 15. Ecological niche modelling to elucidate the history and fate of penguins

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Penguin species are considered bio-sentinels of the state of ocean and coastal ecosystems. Their life is shaped by the conditions of the sea for feeding and land for breeding. Their attachment to specific environmental and habitat conditions can be used with Ecological Niche Modelling to comprehend their biogeographical patterns and evolutionary processes. The paleohistory of the group recreated from an ancestral niche reconstruction with ENM show their progressive expansion to both cooler and warmer conditions, an adaptation that could be offset by increased prey availability. Furthermore, niche differentiation can be indicative of more recent regional speciation processes as seen in the case of the gentoo penguins. All these conditionings that reveal the drivers of penguin distributions offer the possibility to elucidate future changes and affections related to global change. Future-projected ENM show the general increase of more favorable niche conditions and an associated predicted range expansion at the southern range of distributions. In turn the conditions of the northermost populations will become more unsuitable and can lead to local population collapses. However, these patterns can fluctuate between areas and so sites with future increased prey availability can potentially compensate the increased physiological stress from warming. Lastly the external pressures from the fishing industry influence the picture, requiring additional study and mapping for inclusion in more refined modelling.

## 16. The geographic patterns of penguin's evolution

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Penguins are seabirds found only in the southern hemisphere and have existed since the late Cretaceous. The current distribution of living penguins closely resembles where fossils are found. However, there is an expansion in the type of climate shared between ancestors and living. The biogeographic history makes clear that fossils lived in essentially temperate climates, while today, we have the most diverse representation of climates ever occupied by penguins. The modern penguins inhabit different places since the polar cold, pass through temperate climates and reach the Equator. In addition, these birds have great dispersion capacity, which raises the question of why penguins do not occur in the northern hemisphere. Even though penguin species occur in places with different characteristics, all species share a common place, the marine environment with cold waters. This is possible due to adaptations that allow marine life in low temperatures. These characteristics that allow survival in cold places are atypical and unite all the species of the group, leading us to the hypothesis of phylogenetic niche conservation. Therefore, it may be that habitat limitation prevents penguins to reach the northern hemisphere. To answer these questions, we built a database with presence and absence data and temperature and marine productivity variables for analysis. We use modeling and phylogenetic niche conservation test. We found the highest suitability frequency in the southern hemisphere, although there are suitable locations in the northern hemisphere. The phylogenetic conservation rate was intermediate and may be related to the northern hemisphere absence of penguins.

## 17. 15 years of *Spheniscus* rehabilitation in Chile

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The Buin Marino Rehabilitation Center began operations in 2007, located within the facilities of the Buin Zoo in Santiago, Chile. Since 2014 Mundomar Foundation is the institution responsible for the rehabilitation of marine animals in these facilities.

Mundomar Foundation is a non-profit institution, its mission is to inspire the conservation of marine wildlife. It has a collaboration agreement with SERNAPESCA, and its objective is the rehabilitation of marine fauna, education, research and conservation.

Penguins of *Spheniscus* genus admitted to rehabilitation between 2007 and 2022: 174

Species of *Spheniscus* genus admitted: Humboldt and Magellanic.

Causes for which penguins were admitted: physical Injuries, dehydration and emaciation, blindness, covered in oil and others.

59% of the penguins died from natural causes or were euthanized. By performing necropsies and taking samples, the specimens become a source of knowledge of the genus *Spheniscus* from the scientific and the conservation point of view.

*Spheniscus humboldti* population trend is decreasing, they are listed as Vulnerable for the IUCN Red List. 43 Humboldt penguins that entered rehabilitation showed bilateral blindness, consequently, they were rehabilitated, but could not be reinserted into their natural environment. They remain at Mundomar Foundation for research and outreach purposes.

The reinsertion of the specimens is the successful culmination of any rehabilitation. The percentage of reinsertions correspond to the 15%.

The people who collaborate and works at Mundomar Foundation have shown interest in continuing the work done and are developing further projects for the conservation of the marine ecosystem and the rescue and rehabilitation of the Chilean marine fauna.

## **18. Artificial incubation of African penguin eggs rescued from breeding colonies to bolster the wild population**

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The African penguin population is estimated at 14 000 breeding pairs, approximately 1.4% of the population at the start of the 20th century, and the species is currently classified as Endangered. Since 2012, SANCCOB has employed techniques to incubate African penguin eggs that were abandoned by their parents due to severe weather events, onset of moult, low food availability, or nests being in unsafe locations. Artificial incubation and hand-rearing has become a valuable conservation tool to reduce the mortality of eggs and chicks in the wild and to bolster the wild population. From 2012 to 2021, a total of 2134 eggs (and 9311 chicks) were received by SANCCOB's Chick Rearing Unit. It was estimated that up to 50% of the eggs were not viable for incubation while over 70% of the viable eggs hatched after being artificially incubated and an overall release rate of 84% for hand-reared African penguins was achieved. These rates are comparable to or even higher than currently observed breeding success rates in the wild. Initial resighting data confirms that African penguins originating from artificially incubated eggs survive in the wild and successfully commence breeding. Artificial incubation and hand-rearing could be a valuable conservation measure to be used for other population recovery plans, seeing the increase in disastrous population impacts due to disease outbreaks, extreme weather events, oil spills, and other catastrophes.

## 19. Grapiprant as a treatment for early onset osteoarthritis in a gentoo penguin (*Pygoscelis papua*)

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Osteoarthritis is a common cause of lameness in penguins in human care. Long-term treatment of osteoarthritis often requires weighing up the cost of potential side effects of medications against overall improvement to quality of life. Grapiprant, a non-COX inhibiting NSAID, is currently approved as an analgesic for osteoarthritis-associated pain in dogs. Grapiprant works by antagonising the EP4 receptor, which plays a role in promoting pain and inflammation. Traditional NSAIDs such as meloxicam act by restricting the production of prostaglandins all together and may have significant adverse effects on the gastrointestinal, hepatic, and renal systems, especially when used chronically. Whilst currently approved for usage in dogs, studies involving grapiprant have shown promising results when employed as an analgesic in other species including cats, rabbits, and horses. To date, the sole documented use of grapiprant in an avian species reflects a number of pharmacokinetic studies involving red-tailed hawks (*Buteo jamaicensis*). Since December 2022, we have been trialling grapiprant in one of our Gentoo penguins (*Pygoscelis papua*) "Jeffrey", as an analgesic to manage his congenital hip dysplasia induced early onset osteoarthritis with promising results. So far, we have seen significant alleviation of lameness in the absence of appreciable renal and hepatic compromise on multiple blood tests throughout the trial. To our knowledge the current study is the first clinical case report documenting the use of grapiprant in an avian species.

## 20. Recovery attempt of the captive population by using artificial insemination technique of Southern rockhopper penguin (*Eudyptes chrysocome*)

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The declining population of the zoo and aquarium-managed southern rockhopper penguins in Japan has been an area of concern due to the low reproductive rate in managed environments. This study aims to provide the technical establishment of artificial captive breeding to improve the fertility rate of this endangered species.

The optimal timing for artificial insemination and day of egg laying were estimated by the three methods below;

1. Monitoring of fluctuation in calcium, triglyceride, and inorganic phosphorus concentrations in blood samples during breeding season.
2. Observation of mating behavior from nesting to incubation period through video recording.
3. Examination of follicle development using ultrasonography.

Under physical restraint, semen samples were collected via digital expression at the genital papillae. The samples were diluted with equal amounts of Beltsville solution (Sexton, 1977) and extender before storage at 4 °C for 15 minutes. Secondary dilution was performed by mixing diluted semen and Beltsville extender containing 7.5% N-methyl acetamide as a cryoprotective agent at a 1:1 ratio. The samples were placed in plastic straws and frozen by exposing to liquid nitrogen vapor for 30 minutes. Straws containing frozen semen were then plunged into liquid nitrogen. Thawed semen was administered to female penguins under physical restraint at 5-10 days before egg laying. Blood samples were collected for DNA testing to confirm the identity of the male penguin. Successful a case of a newborn chick was achieved in 2022. Further advancement of this technology will contribute to improved fertility rate and management of the captive population.

## 21. Rehabilitation of Humboldt penguins (*Spheniscus humboldti*) after an oil spill in Lima – Peru

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The Humboldt penguin (*Spheniscus humboldti*) is a native species in Peru and Chile, categorized as vulnerable (VU) by the IUCN. Worldwide, pollution by oil spills affects wildlife such as seabirds. In January 2022, in Lima - Perú, an oil spill affected over 60 km of shoreline and islands. This poster describes the Humboldt penguin rehabilitation process implemented after this oil spill and the gaps related to the species' health knowledge in the wild. The rehabilitation process was undertaken at temporary facilities implemented at Parque de las Leyendas Zoo, in Lima. Between January 2022 and March 2023, more than 500 live seabirds were admitted to the rescue facilities, from which 14 individuals were juvenile Humboldt penguins, of which 79% (11/14) were oiled. During the rehabilitation process, three individuals died, 2 of them were oiled. Rehabilitation included body temperature stabilization, hydration, nutrition, and frequent physical and clinical evaluations for treatment accordingly. Oiled penguins with PCV above 30% and fair body condition were selected to be cleaned following the procedures detailed in IPIECA (2017). Although the rehabilitation process was successful, during recovery, some penguins presented cutaneous lesions compatible with poxvirus disease, reason why the federal authority in charge decided not to release the exposed penguins due to the risk of introducing a potential new disease, not previously reported in Humboldt penguins in Peru. It is necessary to study wild Humboldt penguins to evaluate their health status, including the presence or absence of Poxvirus to determine the natural source of the infection.

## 22. The survey of the prevalence of osteoarthritis in captive Humboldt penguins (*Spheniscus humboldti*)

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Osteoarthritis (OA) is a pathological condition that involves joint degeneration, inflammation, osteophyte formation, and infectious arthritis. There are only a few reports of OA in penguins, so we investigated its prevalence in captive Humboldt penguins in Japan.

We used X-ray imaging from three directions (dorsal, left lateral and right lateral and measured the body weight of 220 individuals (86 males and 134 females, aged 2.04 to 44.12 years) housed at nine zoos and aquariums. OA was diagnosed using X-ray images, and the severity of OA in the shoulder, elbow, hip, knee, and tarsal joints was evaluated using a three-grade scale (0: none, 1: mild, and 2: severe) with correlation analysis with age. The body weight of males was significantly heavier (mean 4402 g) than that of females (mean 3835 g). The prevalence of OA (grade 1 and 2) in the shoulder, elbow, hip, knee, and tarsal joints was 1.1%, 16.6%, 18.2%, 19.8%, and 4.3%, respectively. The severity of OA in the hip and knee joints increased significantly with age. Additionally, we observed severe lameness in four animals with patella luxation. Calcification of the gastrocnemius tendon was observed in 97.5% of penguins.

This study is the first report to statistically analyze OA in penguins. The results showed that OA was present in approximately 20% of the hip and knee joints and indicated that aging was a risk factor. Further analysis is necessary to understand its impact on penguins.



## 23. Adaptation capabilities to global warming in an endothermic marine predator, the king penguin: Consequences of body size on diving performance

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Penguins are major consumers in Southern Ocean trophic food webs. During the breeding season, king penguins (*Aptenodytes patagonicus*) breeding in the Crozet Archipelago mainly target myctophid fish. These fish are concentrated at shallower depths within the southern Polar Frontal zone than in areas closer to the penguins' breeding sites. Thus, penguins rely on the predictable spatial distribution of mesopelagic fish found close to the Polar Front, where they forage intensively. However, recent climate change models predict a gradual southward shift of the Polar Front that would double the required travel distance by 2100. Such drastic hydrological change would potentially impact the breeding success and ultimately, the Crozet population. To reduce foraging trip duration, penguins could target myctophids closer to the colony, rather than traveling to the Polar Front, but this implies having the diving capacity to access greater depth. Consequently, determining the factors that account for individual differences in diving and foraging ability is critical to understand the potential for species adaptation to warmer oceanographic conditions. In general, taller animals can dive longer and deeper, leading to the hypothesis that taller penguins may be "selected" in this context of less favorable oceanographic conditions. To test this, the effects of body size on diving performance are currently investigated using long-term biologging and physiological datasets over a period of 5 to 30 years. First results are presented and discussed here.

## 24. Penguins on the move: habitat availability and climate connectivity among present and future climate analogues

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As many other marine organisms, penguins are expected to track climate-driven changes in the availability and distribution of their optimal environmental niches. However, the capacity of individual species to reach future optimal areas (the so-called environmental analogues) may depend on the environmental constraints they face in the transition between current and future optimal patches. By combining the modelled habitat suitability and environmental responses for three penguin species (Magellanic penguin *Spheniscus magellanicus*, African penguin *Spheniscus demersus*, and Little penguin *Eudyptula minor*) with long-term climate projections for two contrasting 'Shared Socioeconomic Pathways' (SSP1-2.6; low climate forcing and SSP5-8.5; high climate forcing), we evaluate trends in penguins' optimal habitat availabilities along the 1850-2100 period. Furthermore, we use a circuit-theory framework to assess the connectivity between species' current habitat suitability distribution and their future environmental analogues. Our assessments predict an overall decrease in optimal habitat availabilities for the three species, with the likely exception of African penguins in the SSP1-2.6 scenario. The loss of optimal habitat will be enhanced in the SSP5-8.5 scenario. Likewise, our connectivity analyses reveal that penguins will be more environmentally constrained to reach their future environmental analogues in the SSP5-8.5 scenario, particularly in the case of the Magellanic penguin. Our approach can be extended to other marine organisms such as any other of the world's 18 penguin species and can be useful for tracking penguins on the move, while informing future and adaptive management and conservation policies.

## 25. The hotter, the worst: little penguin population responses to increasing ocean temperatures in New Zealand

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Ocean warming is unevenly distributed around the globe. Species from regions warming at higher rates face unprecedented environmental changes, potentially affecting population dynamics. This might be the case for little penguins inhabiting New Zealand, where ocean warming exceeded the global average. Evaluating the impact of ocean warming on these penguin populations is a significant challenge to understand and predict how they will adapt/respond to changing environmental conditions. This is what we have done in this work; we combined the most comprehensive dataset on little penguin population dynamics across New Zealand with the most spatially explicit assessments on climate velocity (i.e., trends in ocean temperature) based on satellite information. We revealed that ocean temperature is increasing all over New Zealand. However, certain regions in the western and eastern coasts of the North and the South Islands are warming at the highest rates. For little penguin populations, we show a non-linear, general pattern showing increased trends when facing relatively minor warming, remaining stable at intermediate climate velocity levels, and decreasing in areas facing higher warming rates. Only a few colonies facing 'low' warming rates show declining trends in individual numbers, thus pointing to other stressors affecting little penguin populations. These results are crucial for conserving penguins and their associated ecosystems. It could promote reducing or mitigating local stressors to enhance resilience to climate change in colonies facing higher ocean warming rates.

## 26. Are penguins “what they drink”? Relationships between eggshell carbonate and dietary water oxygen stable isotope values

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Eggshells have been used to examine avian physiology, diets, and to reconstruct past environmental conditions. This is because birds are thought to be “what they drink”. Specifically, the oxygen in eggshell carbonates is assumed to be derived from water consumed during eggshell formation. However, no study has tested this prediction in penguins. We compared the oxygen stable isotope ( $\delta^{18}\text{O}$ ) values of African penguin (*Spheniscus demersus*) eggshells and their corresponding water sources at 25 zoological institutions. Our study takes advantage of the fact that rain and tap water  $\delta^{18}\text{O}$  values vary dramatically, but predictably, across North America leading to a large range of water  $\delta^{18}\text{O}$  values across the zoological institutions examined (-22.2 to -1.6 ‰). Preliminary results applying the relationships between eggshell and water  $\delta^{18}\text{O}$  values observed in captivity to wild penguins predicts higher use of snow/melt water (vs. ocean/prey water) in fasting Adélie penguins (*Pygoscelis adeliae*) vs. foraging Gentoo penguins (*Pygoscelis papua*) during eggshell formation. The results provide support for the use of eggshell  $\delta^{18}\text{O}$  as a non-invasive method to track water sources in captive and wild penguins.

## 27. Developing refined foraging performance metrics that reflect energy expenditure in an endangered diving seabird, the African penguin

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Resource competition with industrial fisheries is one of the key threats to many seabird species, especially during the breeding season when seabirds can only forage in areas close to their breeding colonies. To assess competition between fisheries and seabirds, researchers typically use various metrics and proxies for seabird foraging performance. However, conventional metrics, such as trip duration and path length, vary in their response to prey supplies and do not accurately reflect energy budgets. Here, we use data from animal-borne video cameras, accelerometers and depth recorders to develop foraging metrics for the African Penguin that take into account both energy intake and expenditure. We assess the accuracy of these new metrics in terms of reflecting actual prey consumption and three-dimensional movements and compare these estimates to corresponding conventional path- and dive-derived metrics. These new foraging performance metrics have the potential to provide improved insights into functional relationships between penguins and prey, thus providing a useful means to assess the impacts of resource competition and other factors limiting prey supplies.

## 28. Dietary plasticity of endangered Northern rockhopper penguins in the South Atlantic

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The endangered Northern rockhopper penguin *Eudyptes moseleyi* is the northernmost crested penguin species breeding north or at proximity of the sub-tropical front in the South Atlantic and southern Indian Oceans. The Tristan da Cunha archipelago and Gough Island, in the South Atlantic, are home to ~ 90% of the global breeding population. A recent tracking study showed that populations from both island groups segregate in either time and/or space at sea. Combining the analysis of stomach contents with stable isotope analysis of several penguin tissues, we investigated whether these spatial and temporal foraging patterns translated into differing diet at several stages of the species' annual cycle. Stomach contents indicated that crustaceans (mostly Euphausiidae) dominated the diet at Nightingale (Tristan da Cunha) and Gough islands during breeding (37-89% by mass). The biggest difference was found in the proportions of cephalopods and fish, with more remains of both categories recovered in stomach contents from birds breeding on Nightingale than in birds breeding on Gough Island (cephalopods: 14% versus 2% by mass; fish: 25% versus 10% by mass). Stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of eggshells, blood and feathers segregated most penguins from both island groups confirming their differing foraging areas and trophic levels during pre-laying, incubation, chick-rearing and pre-moult. Their foraging and trophic ecologies were also influenced by year and sex. Ultimately, this study provides insights into the dietary plasticity of Northern Rockhopper Penguins in the South Atlantic and its resilience to future environmental changes.

## 29. Effects of rivers on seabird foraging ecology

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Rivers play a significant role in coastal trophic communities and seabird productivity worldwide. River-associated changes in ocean foraging conditions can impact the spatial distribution, feeding behaviour, breeding performance, phenology, health and survival of seabirds. However, research focused on the topic is limited, and most studies only provide circumstantial descriptions of seabird-river interactions. We present a literature review of quantitative and qualitative studies of the effects of river flow on seabird ecology. The most commonly investigated river effect was on seabird foraging habitat selection through spatial distribution studies, which describe environmental variables associating high bird densities with riverine areas, namely combinations of sea surface temperature, salinity, primary productivity, and water clarity in tropical and temperate seascapes. Long-term time series showed that the influence of rivers on seabird demographics varied with river magnitude: seabirds in regions influenced by very large rivers ( $>1000\text{m}^3/\text{s}$ ) were negatively affected by increased freshwater flows, while colonies influenced by medium-large rivers ( $<1000\text{m}^3/\text{s}$ ) showed a positive relationship with runoff. Diet studies revealed higher diversity of seabird prey in estuarine waters compared with marine regions yet provided no clear consensus on prey quality due to contamination. Our findings suggest that for highly mobile sea predators, freshwater-influenced areas may act as a buffer against unpredictable marine resources. Little penguins (*Eudyptula minor*) were among the most frequently studied species, exceeded only by common murrelets (*Uria aalge*). This review emphasizes the need to intensify research efforts to further our understanding of the interplay between rivers and seabirds and effectively inform coastal management globally.

### 30. The fish component of Adélie, gentoo and chinstrap penguin diets breeding on two Islands in the South Shetland Archipelago

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The purpose of this study was to compare the fish component of the diets of sympatrically breeding Pygoscelid penguins breeding on two islands in the South Shetland Archipelago, north of the Antarctic Peninsula. We collected diet samples from penguins returning to feed their chicks using the water offloading technique. We removed otoliths from those samples and identified the species of fish consumed. We compared the diets of Adélie penguin (*Pygoscelis adeliae*), Chinstrap (*P. antarctica*) and Gentoo (*P. papua*) penguins that breed sympatrically on King George Island, at the Copacabana field site from 1987-2013 as well as the Chinstrap and Gentoo penguins breeding on Livingston Island at the Cape Shirreff field site from 1997 onwards during the years when data were collected at both sites. We compared interspecific differences in the fish species consumed within breeding colonies and inter-colony intraspecific differences in fish types. These data can serve as a baseline to compare to future penguin diet studies and may inform some of the divergent responses of these penguin species/populations to changing ice conditions.



### 31. Estimating foraging times of the African Penguin, *Spheniscus demersus*, using transponder and ground reader data

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The population of the endangered African Penguin is in serious decline. It is widely believed that this decline can be mainly attributed to declines in their main food source, sardine and anchovy. Foraging trip duration is a potential indicator of the local availability of prey. If prey are abundant and close to the colonies, we generally see shorter foraging trips than if prey are scarce and more distant. Here we examined the possibility of using data from penguins fitted with Passive Integrated Transponders (PITs), automatically recorded by ground readers at four (Robben Island, Simon's Town, Stony Point and Dyer Island) penguin colonies, to estimate the duration of foraging trips. We only used data from birds that crossed the readers twice on the same day to estimate foraging trip duration. Foraging trip durations varied seasonally and annually at all colonies. Penguins at Robben Island had the longest foraging trip durations, perhaps indicating that prey around the Island is less abundant or comparatively further away. Penguins at Simon's Town had the shortest foraging trips, perhaps indicating the presence of more prey, locally abundant. Female penguins generally had longer foraging trip durations than males, across all colonies. We believe the current approach could in future provide useful information on the local availability of prey around penguin colonies, and thus has the potential to be used to advise management on a dynamic ecosystem-based approach to fisheries management.

## 32. Genetic characteristics of a captive population of little penguin (*Eudyptula minor*) in Japan

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As of 2019, a total of 52 individuals of little penguins (*Eudyptula minor*) are kept at five facilities in Japan. The Japanese Association of Zoos and Aquariums (JAZA) manages the studbook to avoid inbreeding and to maintain genetic diversity. However, more than 80% of all individuals are kept in one aquarium, and most offspring come from specific breeding pairs. The purpose of this study was to determine the genetic characteristics of this captive population. The genetic diversity and structure based on the mitochondrial DNA and microsatellite polymorphisms were analyzed using 49 samples. Analysis of 357 bp of the mitochondrial control region sequence revealed five haplotypes with a total of seven nucleotide substitution sites. Phylogenetic tree analysis showed that all 49 samples were classified into the same genetic category as the entire wild population of the Australian Little Penguin, consistent with the studbook records. Moreover, microsatellite analysis revealed the expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity were 0.00–0.40 and 0.00–0.43, respectively. Although the genetic diversity indices of the captive populations closely resembled those of wild populations, half of the markers used in this study did not show polymorphism, suggesting the need for additional analysis using other markers. Notably, STRUCTURE analysis revealed that this population could be divided into three genetically differentiated groups. Together with information from the studbook, these results can contribute to the maintenance and conservation of this population of Little Penguin in Japan.

### 33. MHC-DRB gene diversity in species survival plan and native *Spheniscus demersus* penguins

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The *Spheniscus demersus* AZA Species Survival Plan<sup>®</sup> (SSP) aims to maintain a genetically diverse and sustainable population of African penguins within AZA-accredited facilities. MHC genetic diversity comprises a major component of ecologically relevant genetic variation by coding for cell-surface proteins involved in immunological resistance to pathogens. The goal of this study is to compare the MHC allele diversity of SSP penguins with that of penguins sampled in their native habitats using polymerase chain reaction, denaturing gradient gel electrophoresis, cloning and DNA sequencing. Our results revealed seven allelic variants of the *DRB1*-like gene. These include the previously described *cape001*, *002*, *003* and *004* alleles and three previously unreported alleles that we have provisionally designated *cape003b*, *004b* and *005*. A notable feature of the samples examined is the predominance of the *cape001* allele; observed in 84% of the SSP penguins (19% homozygous & 81% heterozygous) and 91% of the wild penguins (20% homozygous & 80% heterozygous). *Cape 003*, *003b*, *004* and *004b* alleles were detected in both populations. *Cape002* was detected only in the SSP samples and *cape005* only in the wild samples. Overall, these results indicate that the *Spheniscus demersus* SSP MHC allele pool reflects that of its wild counterpart with considerable accuracy. The high level of *cape001* heterozygosity may reflect recent and/or ongoing positive selection. Further sampling of both populations is recommended to more accurately ascertain the status of the *cape002* and *005* alleles and to ensure against rare allele (e.g. *004* and *004b*) drift from the SSP population.

## 34. Neutral and adaptive evolution in the speciation continuum of the rockhopper penguins (*Eudyptes*)

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Studies along the speciation continuum provide great opportunities to assess the contribution of neutral and adaptive evolution on the evolutionary history of species. When populations are geographically distant, the lower probability of random mating between populations would increase genetic divergence, by means of lower genetic connectivity and higher genetic drift. However, the increase of population divergence could also be related to adaptation to specific environmental conditions, and the contribution of each denotes the main drivers of speciation. Rockhopper penguins (genus *Eudyptes*) are crested penguins conformed by three main lineages, characterized by high vagility and low genetic divergence. Recently, it was found with a restricted set of genomic markers obtained from ddRAD sequencing, that these three major lineages represent three different species with low genomic variability. However, the main evolutionary processes that disentangle the drivers of speciation, such as introgression, incomplete lineage sorting and natural selection, have not been assessed with a robust and representative dataset. Here, we sequenced whole genomes of the Northern (n=12; *E. moseleyi*), Southern (n=21; *E. chrysocome*) and Eastern (n=26; *E. filholi*) rockhopper. Genetic structure analysis shows a clear lineage divergence between the three species, each with a characteristic demographic history trajectory, but with shared ancestry

about 1 mya, giving support to the hypothesis of three defined species. Also, the three species show trends of population expansion during the last glacial period, followed by a decrease in  $N_e$ . Population gene flow analyses show great contribution of introgression along the evolution of the species, with migration vectors between the three species and their ancestral populations. Selection analyses are expected to show adaptations to the different historical processes, such as adaptation of the southern rockhopper to cold environment due to potential glacial refugium, or adaptations to warmer environments for the northern rockhopper which inhabits along the subtropical area.

## 35. Unraveling the secrets of sex: Exploring the role of sexual chromosomes in banded penguin speciation

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Sexual chromosomes and sexual selection have an important evolutionary role during speciation events. Although autosomal genes have an important role in ecological speciation, rapid evolution of sex-linked genes can also play a critical role in the evolution of sexual isolation by encoding traits with sex-specific fitness effects such as primary and secondary sexual characteristics. We analyzed sexual scaffolds of 113 genomes wide of the four banded penguin species, we assembled against the little blue penguin genome and identified 7 sexual scaffolds by heterozygosity and depth coverage method. Previous studies have detected that the major difference in whole genomes traits were due to sexual traits. Loci related with male reproduction traits and that could be involved with epigenetic and polygenetic regulation during speciation events were detected as well. Our main question is how important the sex chromosomes are in the formation of novel *Spheniscus* penguin species. In several taxa, as amphibian, the female heterogametic sex is the first to appear and then differences begin to accumulate in the sexual chromosomes. This is as a result of their rapid capacity for evolutionary rate, chromosomal rearrangement and small effective population size, which later differentiate into homogametic males. We found lower differences between females and males of most recent divergent species and higher accumulated differences in those species that have an older divergence through allopatry.

## 36. Developments in the management of hoiho in a changing and unpredictable environment

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Shortly before the last International Penguin Conference (IPC10) in August 2019, the Minister of Conservation in New Zealand launched a draft strategy and action plan to support the ecological and cultural health of the endemic hoiho / yellow-eyed penguin. This plan was developed in a partnership between Te Rūnanga o Ngāi Tahu, Department of Conservation, yellow-eyed penguin Trust and Fisheries New Zealand. Almost four years later the Northern population of hoiho is surviving at very low levels and numbers fewer than 200 breeding pairs. Little is known about the Southern population. Hoiho are subjected to a range of cumulative marine and terrestrial threats, which continue to cause mortalities and dramatically reduce breeding success. Implementation of the strategy and action plan is in part focused on intensive management interventions to support hoiho during key phases of their annual cycle. Conservation management however needs to be all encompassing, dynamic and flexible to cope with the range and changing nature of the threats that hoiho are facing. The threat of extinction looms ever closer and the hoiho is an unfortunate indicator of the perilous state of our coastal environment. However, dealing with the threat of climate change, fisheries (bycatch, extraction, and benthic disturbance), sedimentation and the myriad of other impacts is altogether a slow and political process. These are threats that need to be addressed with all haste before hoiho run out of time.

### 37. Empowering a conservation culture through the Global Penguin Society Education Program

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Fostering a conservation culture that values the protection of penguins and their habitats is crucial to engage and empower local and international communities. The Global Penguin Society (GPS) Education Program promotes conservation values of penguins and their habitats through several activities: a) School Trips and talks: GPS offers talks and trips for children to visit penguin colonies, benefiting over 7,000 children so far. Additionally, GPS streams online classes from remote penguin colonies via satellite antenna to thousands of students globally. b) Educational Material: Develops and publishes educational booklets and learning and activity books in Spanish and English. So far 10,000 educational books have been distributed for free in 5 Spanish speaking countries. c) Penguin colony clean-up: GPS annually takes hundreds of adolescents to collect garbage from beach and nesting areas of penguin colonies in Patagonia, Argentina. The materials are processed at a recycling center, where 74% of the collected garbage is plastic, mainly single-use items, and 10% is metal. Paper and paperboard, glass, organic waste, and other materials represent 4% each. d) GPS conducts an annual summer awareness campaign to reduce single-use plastics consumption in beach resorts in Patagonia. Thanks to the campaign, 32,000 straws and 25,000 single-use plastic cups were prevented from being used. Our actions were instrumental in promoting a law that bans plastic straws in a major city in Patagonia, which is a significant accomplishment for the organization.



### **38. High adult mortality at mainland African penguin (*Spheniscus demersus*) colonies and how the rehabilitation and release of penguins may be helping to bolster these colonies**

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Population numbers of African penguins *Spheniscus demersus* have decreased by more than 95% since the start of the 20th century. The species is currently listed as Endangered, with a global population of 14 000 breeding pairs. Since the 1980s there have been a number of attempts by African penguins to establish new colonies on the mainland of South Africa. As a result, African penguins started colonizing Stony Point and Simon's Town in the early 1980's and these colonies have thrived while most island colonies have declined. However, mortality events linked to mainland breeding can result in the death of hundreds of seabirds. Mortality events such as road kills and predation have become more frequent in mainland colonies. Between 2015 - 2022 a total of 773 adult penguin deaths were recorded by The Southern African Foundation for Conservation of Coastal Birds at these two colonies at an average of 96 adult birds per year from a range of non-health related reasons including dog attacks, road kills, bee envenomation as well as terrestrial predation. We predicted that with the high mortality rate of adult penguins recorded during this period there should be a greater decline in breeding at these colonies. Although these colonies suffer from these mortality events, they still perform better compared to island colonies. Here we present different mortality events affecting mainland colonies and the impact they have on their populations. We also discuss how the rehabilitation and release of penguins can help bolster African penguin population in mainland colonies.

### 39. Impacts of terrestrial and marine influences on little penguins, sentinels of coastal ecosystem health

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Seabirds are one of the most threatened groups of animals. Occupying both marine and terrestrial environments, threatening processes are multifactorial and complex. Many ecological studies investigating seabird health do not consider spatial variability, but doing so may help reveal key processes shaping health. Here we investigated variability in body condition (a primary measure of health) in a resident, coastal seabird, the little penguin *Eudyptula minor*, across 21 populations around Tasmania, Australia. Our sites represented a spectrum of marine and terrestrial conditions, providing an unparalleled opportunity to assess the impact of multiple processes shaping the health of this species. We show significant regional differences in body condition of breeding penguins, with east coast populations consistently in better condition. Using Bayesian multilevel models we found local marine productivity to be the strongest influencing factor ( $\beta_1 = 0.72$ , 95% CI [0.24, 1.23]). Breeding habitat loss and ectoparasite load also negatively influenced body condition, with parasite load having a greater negative impact on females ( $\beta_1 = -0.51$ , 95% CI [-0.88, -0.15]). Findings demonstrate that body condition (as a measure of health) varies spatially and is related to natural and anthropogenic stressors. The cross-sectional approach highlights the need for a deeper understanding of the specific factors underlying the observed variability in body condition in key populations. Little penguins are an ideal sentinel for coastal ecosystem health and by assessing condition across an array of sites we revealed potentially important health processes that would otherwise be difficult to discern.

## 40. Natural and anthropogenic impacts on Humboldt penguins (*Spheniscus humboldti*) on the northern coast of Lima, Perú

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The Humboldt penguin is an endemic species of the Humboldt Current (IUCN status: Vulnerable). In its habitat, it presents with natural and anthropogenic threats, which tend to cause decreases in its total population. Knowing the main causes of its mortality is critical to implement adequate conservation strategies. At the same time, evaluating environmentally critical areas, taking as an indicator the total number of live and dead penguins present, allows for the calculation of the incidence and severity of the impacts that could be affecting this species. The objective of the present work is to evaluate and compare two current threats: an oil spill and the avian influenza outbreak on the Humboldt penguin population along the northern coast of Lima, between January 2022 and April 2023. A higher mortality of penguins was observed during the avian influenza outbreak, especially at the beginning of their breeding season (March 2023), showing although anthropogenic impacts are important for biodiversity conservation and should be avoided, natural impacts can be also harmful and should be studied, prevented and when it happens, should be properly managed. The impact of both the avian influenza outbreak and the oil spill on the Humboldt penguin population in the study area needs to be clarified to subsidize the necessary measures for population recovery and protection of the species.

## **41. The activity report of Penguin Fund**

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This poster reports on the activity of “Penguin Fund” (PF) in Japan. PF, founded in 1986, is a volunteer group of penguin lovers in Japan with the motto: “Sharing our future with Penguins”. Aims of PF are as follows: (1) To open and sustain contacts with individuals and groups engaged in penguin research and protection all over the world, and provide support in any way possible, (2) to coordinate the sending of volunteers to penguin habitats and reserves, (3) to publish books, brochures and other informative materials on penguins, and (4) to collect literature on penguins and penguin goods. To accomplish these aims we have meetings every two months to hear talks of people such as penguin researchers, keepers of zoological gardens, artists and penguin lovers. We also do fundraising activities by auctioning penguin-related goods every two months. Through these activities and donations, we have contributed to conservation and research of wild penguin all over the world, including Phillip Island Nature Park, Charles Darwin Research Station, Yellow-eyed Penguin Trust, and SANCCOB. Donation also went to many individual penguin researchers as well. During the COVID-19 pandemic it has been difficult for us to gather in person, so we held online lectures and auctions to continue our activities. And we donated to penguin conservation groups around the world as usual. We were convinced that our feelings for penguins were connected even though we were far apart. We are looking for lecturers who can give lectures about penguin research and new penguin information.

## 42. A case report of intracoelomic hemorrhage due to ovarian torsion in a captive Humboldt penguin (*Spheniscus humboldti*)

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Ovarian or oviduct torsion have been reported in some avian species, however, no case has been reported from *Spheniscidae*. Here, we share a case report of ovarian torsion in a captive penguin.

A 21-year-old, captive female Humboldt penguin (*Spheniscus humboldti*) was considered in good health condition until the day before its death. The animal suddenly showed symptoms of critical illness including vomiting and anorexia, and then died in shortly after. The hematocrit value just before death fell into 19 %, indicating severe anemia. Radiographs showed increased opacity of the coelomic cavity suspicious for ascites, and ultrasonography also showed images suspicious for sanguineous ascites.

At autopsy, a hematoma measuring 10.0 x 4.5 x 4.0 cm was found in the left abdominal air sac, and a large amount of dark red fluid was accumulated in the body cavity, with a total volume of 280 ml, including the hematoma. A 4 cm in diameter, dark red, spherical mass was found in the ovary, and the mass was filled with blood clots. A 2.5 x 2.0 x 2.0 cm degenerated egg, which appeared to be an internal ovulation, was also observed. Based on the findings described above, it was diagnosed that the animal died of hypovolemic shock due to massive intracoelomic hemorrhage caused by vascular collapse resulting from ovarian torsion.

This is the first report of ovarian torsion in penguins. Further investigation is needed to understand its pathophysiology and frequency in penguins.

### **43. Adenovirus detection on *Aptenodytes patagonicus* at Reserva Natural Pingüino Rey, Bahía Inútil, Tierra del Fuego between 2019 and 2020**

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The Penguin Siadenovirus A is a non-enveloped double-stranded DNA adenovirus, which has been detected since 2016 in *Pygoscelis antarcticus*, *Pygoscelis adeliae* and *Pygoscelis papua* in Antarctica. In addition, during the same period, it has been isolated in specimens of *Spheniscus humboldti* in a colony located in Peru. The aim of this thesis was to expose the development and results of an investigation focused on the detection of adenovirus in fecal samples of King Penguin from a colony located in the King Penguin Natural Reserve, Bahía Inutil, Tierra del Fuego, Chile. The fecal samples were collected during 2019 and 2020, and they were analyzed through the technique of conventional PCR with degenerate primers. Taking a total of 90 fecal samples into account, solely one pool was initially labeled as suspicious, which represented the 5,5% of samples analyzed (5/90). When that pool was opened and each sample was examined, the presence of adenovirus was not detected. Consequently, it is possible to conclude that there is no presence of adenovirus in the fecal samples. Nevertheless, considering the scope of the sample and its time limitation, the results do not allow us to pose a permanent absence of adenovirus within this colony. This research is an approach to promote future studies on virology and other infectious agents in this colony, contributing to the conservation of the King Penguin.

#### 44. Avian pox virus outbreak on Magellanic penguin (*Spheniscus magellanicus*) from Magdalena Island; Magellan Region, Chile

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Avian Pox Virus can affect more than 330 wild and domestic/captive bird species, including seabirds and it is described in at least four species of penguins. This virus is highly contagious and can cause skin lesions (cutaneous form) and/or in organs (diphtheritic form). From 2019 on Magdalena island, Monumento Natural Los Pingüinos, Región de Magallanes, Chile. We did surveillance of disease of Magellanic penguins, and from January to March 2023, we found live and dead chicks penguins with suspected skin lesions of Avian Pox Virus. We requested a special permit to SERNAPESCA for manipulating the penguins and collect samples for laboratory analysis. We found the chicks in three areas of the island and we recorded the macroscopic lesions from 15 penguins, 9 dead and 6 sick chicks. The macroscopic lesions were observed mainly in the face area (around the eyes and beak), the wings, the feet and the abdominal area. We obtained skin samples from 7 dead animals, preserved in alcohol and subsequently histopathological and molecular analyses were performed. We compared the weather conditions, rainfall, and temperature between October and March from 2010 to 2023. We compare the molecular results with those from individuals from previous years to figure out if it was the same strain. The increase in temperature and decrease in rainfall can predispose the vectorial transmission and spread of Avipoxvirus and can be the principal cause of this outbreak. This disease can be relevant for the breeding success in this declining population of the Magellanic penguin colony.

## 45. Fungal contamination in the environment of penguin communities in the French Southern Territories

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Data from captive populations in temperate countries indicate that penguins are highly susceptible to airborne fungal infections, particularly aspergillosis caused by the saprophyte mold so called *Aspergillus fumigatus*. *A. fumigatus* is commonly isolated in soils and vegetation worldwide at temperatures ranging up to 57°C. However, little is known about the representation of this species and other ones in the natural habitats and about the risks of fungal infection for penguins. Sixty-four soil surfaces were swabbed twice in Nov-Dec 2021, around eight penguin communities of the French Southern Territories in sub-Antarctic Crozet archipelago (king (x5), gentoo and rockhopper penguins) and subtropical Amsterdam Island (Moseley's penguins). Samples were inoculated onto malt extract agar and incubated at 35°C for ≤7 days. In case of any fungal growth, colonies were counted, expressed in colony-forming units (CFU), and identified by phenotypic and genotypic investigations. In total, 14% spots in Crozet and 100% in Amsterdam were contaminated with ≥1 CFU, but *A. fumigatus* was isolated only once (2 CFU in one spot of Crozet). Whatever the species consideration, the total fungal load was measured at 11 CFU±17 per spot. Several species belonging to the Mucorales order -with pathogenic potential- were found highly prevalent among the local fungal flora, representing notably 216 UFC/415 around the king penguin communities in Crozet, primarily in the reproduction and grassy areas therein. Repeating the surface sampling procedure sounds necessary, as well as exploring the clinical impact of these findings in terms of fungal infection occurring in penguins living in these ecosystems.



## 46. Mosquitoes at penguin colonies in Argentinean Patagonia: previously underestimated or an emerging threat due to climate change?

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Penguins are highly susceptible to avian malaria, a disease caused by mosquito-borne *Plasmodium* protozoa. Avian malaria can cause rapid outbreaks in penguins in captivity, with up to 50–80% mortality, and has also been implicated in the mortality of wild penguins in New Zealand following the climate change-potentiated spread of mosquitoes. In previous surveys in 2012 and 2014, we failed to detect avian malarial parasites at breeding colonies of Magellanic penguins (*Spheniscus magellanicus*) in the Patagonian coast of Argentina, and speculated that this was attributable to the arid climate of this region being unsuitable for mosquito vectors. During fieldwork in October 2022, however, we found a small number of female *Culex* sp. mosquitoes host-seeking at the Magellanic penguin colony in Cabo Dos Bahías (44°55'S 65°33'W). A survey of the area revealed that the rocky shores near the penguin colony had small brackish water ponds, several of which contained mosquito larvae. These brackish water ponds had a range of salinity levels (3.8 to 79.4‰), suggesting they resulted from a combination of rainwater, sea spray and evaporative processes, with mosquito larvae being most prevalent in ponds with intermediate salinity levels (6.4 to 34‰). The presence of mosquitoes at penguin colonies is acutely concerning because it implies favourable epidemiological conditions for the transmission of malarial parasites. Further studies are warranted to explore the ecology of mosquitoes in this region, how it may be affected by climate change, and the implications for the transmission of mosquito-borne parasites to penguins.

## 47. Nasal mites in wild Magellanic penguins (*Spheniscus magellanicus*) in Chubut, Argentina

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Rhinonyssids (Mesostigmata: Rhinonyssidae) are haematophagous mites that parasitize the upper respiratory tract of vertebrates. These minuscule spider-like parasites (approx. 1 mm) can cause significant discomfort, itching, sinusitis and may contribute to secondary respiratory infections. Two species of nasal mites are known from penguins: *Rhinonyssus sphenisci* parasitizes Humboldt (*Spheniscus humboldti*) and Magellanic penguins (*Spheniscus magellanicus*) and *Rhinonyssus schelli* parasitizes Adélie (*Pygoscelis adeliae*) and Gentoo penguins (*Pygoscelis papua*). Additionally, *Rhinonyssus sphenisci* sensu lato has been reported parasitizing African penguins (*Spheniscus demersus*). We examined the upper respiratory tract of 30 juvenile-plumaged and 18 adult-plumaged Magellanic penguin carcasses collected from April to November 2022 at Chubut province, Argentina. Nasal mites were found in the nasal cavity and paranasal sinuses of 8 individuals (16.7%), comprising 4 juveniles (13.3%) and 4 adults (22.2%). Average abundance was  $1.6 \pm 5.8$  parasites/examined host, and average intensity was  $9.4 \pm 12.1$  parasites/infected host (median = 4, range = 1–34). No significant differences were detected in the prevalence of these parasites among categories of age, sex, carcass collection context (beach-cast, colony survey), carcass preservation (fresh, moderate decomposition, advanced decomposition, severe decomposition), or cause of death (paralytic shellfish poisoning, predation, starvation, other). The prevalence in this study was comparable to that reported in previous surveys on Magellanic penguins beach-cast in southern Brazil (juveniles 17.6%, adults 12.5%) and in African penguins in South Africa (juveniles 29.4%, adults 26.7%). Morphological and genetic characterization of these parasites is under way in order to clarify the taxonomy and evolutionary relationships of the nasal mites from different penguin species.

## 48. Protocols to protect king penguin (*Aptenodytes patagonicus*) from an avian influenza AH5N1 outbreak

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In 2023 highly pathogenic avian influenza (HPAI) H5N1 caused the death of a large number of wild mammals and birds on the Chilean coastline. Spread by migratory birds, such as Anseriformes and Charadriiformes, this virus raises concern all over the world due to its potential to trigger a zoonotic disease.

The only king penguin colony in Chile is located in Tierra del Fuego, Chile, a place where a great number of migratory birds have been described. In order to prevent contagion, we developed protocols to avoid infection during penguin research, to handle birds in case of infection and to report massive deaths to governmental agencies.

This study was observational and we were able to establish site specific protocols which will allow the "Reserva Natural Pingüino Rey" staff to act appropriately in case of an outbreak.

Further research should focus on long term surveillance, as other authors have suggested. Also, the interaction between king penguins and other birds that act as natural hosts, should be studied thoroughly in order to identify disseminating species in the area.

## 49. Stranding and mass mortality of penguins in continental Chile related to HPAIV-H5N1

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Currently, HPAIV-H5N1 is causing a panzootic that affects wildlife and domestic animals globally. The virus has led to the deaths of millions of animals. To date, positive cases of HPAIV H5 Clade 2.3.4.4b in wildlife have only been reported in African Penguins. In Chile, the virus was confirmed in December 2022 affecting several animals including penguins. This work aims to summarize the events of strandings and mass mortality of penguins and to provide evidence of HPAIV-H5N1 related to these cases. The National Fisheries and Aquaculture Service (SERNAPESCA) of Chile collected information about the stranding and mortality events. Samples were conveniently collected for influenza detection, which were processed at the Animal Virology Lab of the Agriculture and Livestock Services (SAG) in Lo Aguirre, Chile. Between January and March 2023, 320 penguin stranding events were recorded, with a total of 875 individuals, of which 786 were dead, mostly Humboldt penguins (97%). Most of the cases (88%) were observed between Arica and the Coquimbo Region. The data was compared with strandings from previous years and a significant increase in cases was determined. 124 cases were sent for diagnosis of HPAIV. Most of these (84%) belonged to Humboldt penguins. From the total cases, only four cases were positive for influenza detection, all of them HPAIV-H5N1. The results confirm that massive mortality can be related to HPAIV outbreaks. The low percentage of HPIAV detection can be explained by the quality of samples and the pathogeny of the virus in penguins which should be further investigated.

## **50. Successful rehabilitation of African penguin chicks after high pathogenicity avian influenza (H5N1) infection**

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High pathogenicity avian influenza (HPAI) has affected seabirds in Southern Africa since 2018. The highest mortalities were seen in Greater-crested terns and Cape cormorants in 2018 and 2021, respectively. However, several hundred African penguins died of HPAI in Namibia in 2019 and more than 300 in South Africa in 2021 and 2022. Affected penguins in the wild showed severe neurological symptoms and either died soon after they were found or were euthanised. SANCCOB, a seabird rehabilitation centre in South Africa, assisted with the management of these disease outbreaks in the wild; euthanising affected birds and testing symptomatic or dead birds found in the wild. Seabirds requiring rehabilitation for other reasons were admitted after a clinical examination and, in some cases, an HPAI test. SANCCOB managed to prevent HPAI outbreaks in its rehabilitation facilities until late 2022 when positive cases were detected in African penguins undergoing hand rearing and rehabilitation. Clinical signs were mostly limited to cloudy eyes and only a few cases showing more severe neurological symptoms died or were euthanised. After several weeks, during which SANCCOB was under quarantine, affected African penguins recovered and tested negative and could be released back into the wild. Most affected birds were abandoned chicks that had been hand-reared at SANCCOB. We will present on clinical signs seen, testing protocols, biosecurity and management of cases in African penguins, which could guide other rehabilitation centres dealing with HPAI in penguins and other birds.

## 51. Surveillance of avian influenza virus in penguins from different areas of Chile (2019 - 2023)

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Avian influenza virus (IAV) is an important concern for animal and human health. The virus has been detected in several penguin species from both different continents, including Antarctica. In December 2022, high mortality rates were recorded in wild birds on coast of Chile due to highly pathogenic influenza A. We performed active surveillance for IAV detection in several locations in continental Chile between 2019-2022 and in Antarctica in 2023. To assess the occurrence of IAV in penguins from different areas of Chile, 335 samples were collected for IAV testing by RT-PCR from five different species. Of the total amount, environmental and cloacal samples from Magellanic penguins at Magdalena Island (n=194), Humboldt penguins at Valparaíso Region (n=49), and chinstrap (n=5), gentoo (n= 71), and Adélie (n=1) were taken. Additionally, 13 sera samples from Antarctic penguins were taken. We found IAV evidence, including positive cases of Antarctic penguins, Magellanic, and Humboldt penguins. By real-time RT-PCR 8 samples were classified as suspects, and 1 out of 13 samples was positive for IAV ELISA. The study highlights the occurrence of IAV in penguins from different regions of Chile, indicating the potential threat of the virus to animal health. We were not able to detect high concentrations of IAV, but we still were constantly detected in these populations. Notably, the detection by ELISA seems to be a good alternative for sampling. Continued surveillance and research are essential to better understand the virus's ecology and its potential impact on penguins and human populations.

## 52. Bird-borne video cameras record unseen feeding strategies of breeding Humboldt penguins

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Small video-cameras attached to seabirds have revealed aspects of foraging behavior not previously detected with other dataloggers. We used TechnoSmart video-cameras in tandem with TDRs/GPS loggers to record underwater behavior and prey capture rates on 12 chick-rearing Humboldt penguins at Punta San Juan, Perú in July-August 2022. Penguins were selective, capturing only fish and ignoring red squat lobster schools (*Pleuroncodes monodon*) available during their underwater excursions. Overall, 64% of fish consumed (N = 124 prey items) identified in the videos was anchoveta (*Engraulis ringens*), captured at a rate of 27 fish/h. In 95% (N=184) of the catches, fish were attacked from below, captured mostly during ascend. All hunting took place between 0 and 43 m deep (mean = 14.2 m), and began after penguins reached >1 m depth. The rate of prey capture increased from 1 catch/5.4min to 1 catch/15s on average when penguins circled the school and moved it close to the surface. Prey was captured solitarily, with their congeners (1-7 individuals) or in mixed groups with Peruvian boobies (*Sula variegata*) and Inca terns (*Larosterna inca*) when the fish were at shallow depths. Improved night vision video-cameras could be useful in revealing capture behaviors during nocturnal dives.

### 53. Bycatch and mortality of Humboldt penguin (*Spheniscus humboldti*) inshore Peruvian southern waters

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Of all seabirds, penguins are one of the most endangered groups according to the IUCN Red List. Humboldt penguins are considered vulnerable and interaction with fishing gear is among the threats they face at sea. In Peru, gillnets have been considered the main artisanal fishery causing mortality to Humboldt penguins. Here we presented preliminary results of an ongoing observer program in artisanal gillnet fishery inshore Peruvian southern waters coming from Tambo de Mora (13° 30' S, 76°11' W) and San Andrés (13° 43' S, 76°13' W) inlets. On-board observations were registered in surface gillnets and bottom-set gillnets. The target species were Eagle ray (*Myliobatis peruvianus*), guitarfish (*Rhinobatos planiceps*), and whiptail stingray (*Dasyatis brevipes*), among others. Vessels operated from 0.5 nm to 5 nm and the characteristics of nets were 145m – 182m in length, 9-11 m in height, 12"- 14" of mesh size with 10-15 nets, totalizing 1.85 to 2.22 km of the net. From February 2017 to April 2019, we observed 42 fishing trips mainly during the austral months (December-April), and estimated a BPUE of 0.95 animal/trip<sup>-1</sup> for Humboldt penguin in the study area. In addition, as part of a mitigation strategy to reduce sea turtle mortality, 56 experimental trips testing LED lights were observed from December 2021 to November 2022 with a BPUE of 0.88 animal/trip-1 with a greater capture of individuals in the nets using lights. The analysis is ongoing and has noted higher captures near a penguin colony that aggregates in a breakwater, an artificial man-made structure.



## 54. Case Report: A unique king penguin (*Aptenodytes patagonicus*) colony in Tierra del Fuego, Chile

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In 2010 the first breeding attempt of king penguin (*Aptenodytes patagonicus*) at Bahía Inutil, Tierra del Fuego, Chile was recorded. This was an unprecedented circumstance because all king penguin colonies are located in the open sea. Given the settlement of this colony in Tierra del Fuego, these penguins have to deal with native and introduced mammals, such as Guanaco (*Lama guanicoe*), Grey Fox (*Lycalopex griseus*), and mink (*Neovison vison*). The last two were introduced in the 50' and now are predating on king penguin chicks, which is unprecedented and poses a threat to breeding success. Furthermore, this colony is the only one where the main foraging areas are the same year-round and are not located in the High Seas. Another quality of this colony is that they don't eat myctophids and eat in the same feeding areas all year.

Lastly, due to the occupation area and the threats they face, this species in Chile is categorized as endangered, while the IUCN classified it as Least Concern

Further research should focus on describing the phenology and population dynamics of this colony, their interactions with native species, and determining invasive species' impacts on breeding success.

## 55. Consistency among plot-based and plotless methods for Magellanic penguin density estimations in Tierra del Fuego

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Density estimations are not independent from the methodology used. Factors than can influence density estimation in seabirds include spatial arrangement, colony dynamics and history over time. As burrow nesting seabirds, *Spheniscus* penguins' numbers are not easily assessed with methods such as aerial surveys. There are at least five methods being used for estimating density and abundance of Magellanic penguins (*Spheniscus magellanicus*), with no current consensus on which is more reliable. Moreover, to date there is no comparison among methods for a single study area. During the breeding season of 2021, we compared the level of agreement between two methods for density estimation of Magellanic penguins in Isla Martillo (Argentina), a 44.8 ha island in the Beagle Channel. We compared a plot-based method (i.e., total counts in 20 m radius plots) and a plotless method (i.e., the point-centered quarter method) and assessed density and abundance estimations in three previously established strata and for the entire area. Methods were consistent among each other (i.e., confidence intervals of density estimations widely overlapped). Current stratification has no effect on the total abundance estimation. Point-centered quarter was a less time-consuming method, but density accuracy was sacrificed in the process. Truncation of the data up to 20 m in this method also had no effect on density estimation. In future studies and in order to improve the quality of information for species breeding in very different environments throughout their range, we recommend assessing the consistency between at least two methodologies, with their associated field efforts.

## 56. Exploring the success of a new penguin colony in Patagonia: Growth, occupation, and breeding patterns

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Studies on the formation and growth patterns of penguin colonies since the very first nest settlement are almost nonexistent. In the Chubut province, Argentina, the population trends of Magellanic penguin (*Spheniscus magellanicus*) colonies vary between regions. Abundances in the southern and central areas of Chubut have substantially declined, while colonies in the northern part of the province are on the rise. In this context, the El Pedral colony emerged in 2008 and we studied its population growth trend, occupation pattern and breeding performance. We conducted 15 annual censuses by direct count of active nests and estimated the breeding success during seven years. A remarkable exponential growth was observed: from 6 (2008) to over 4,000 (2022) breeding pairs ( $\lambda = 1.6$ ). To assess the magnitude of the growth fueled by immigration versus local recruitment, we developed an ecological matrix model. We contrasted the growth rate observed with the growth calculated using the maximum parameters of fecundity and survival of Magellanic penguins in Patagonia. Results indicate that colony growth was mainly due to immigration of individuals from other colonies rather than local recruitment, as expected for a recently established colony. This study represents the first detailed characterization of the demographic processes of a Magellanic penguin colony during its first 15 years of existence.

## 57. Heat-related death of gentoo penguin *Pygoscelis papua* chicks at Martillo Island, Argentina

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Heat waves have detrimental effects on most species, either through direct or indirect effects. Nowhere is the potential for direct impact greater than the polar and sub-polar regions, where adaptations against heat loss leave animals vulnerable to hyperthermia. Gentoo penguins *Pygoscelis papua* belong to Antarctic and subantarctic regions. We placed a Reconyx timelapse camera overlooking a colony has recently established colony at Martillo Island within the Beagle Channel, Argentina. This camera has recorded images between 2013 and the present (4 images per day to every 15 minutes during the breeding season). On the 21<sup>st</sup> of January 2015, five of the twenty-five chicks died between 5:45pm and 6pm. A sixth chick died later that same day, reducing the total number of chicks by 24%. Temperature registered by the camera showed increased maximum temperatures that day (24°C) coinciding with the time the chicks died. All the chicks died at their nest. On the 20<sup>th</sup> and 21<sup>st</sup> of January 2021 another event of high temperature was registered (maximum temperatures of 25°C and 24°C), yet on this occasion no dead chicks were registered in the photographs. As opposed to previous seasons, in recent years most of the chicks that enter crèche (beginning of January) use nearby bushes to cool down in their shade. Behavioural changes may help poorly adapted species withstand moderate heat events where they have scope to move, yet these behavioural adaptations may not suffice in future global warming scenarios or when chicks are in the brood guard phase.

## 58. Individual identification using black spots pattern on Humboldt penguins' (*Spheniscus humboldti*) chest

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A pattern of black spots on the Humboldt penguins' chest is considered useful for non-invasive individual identification. In order to confirm the universality, uniqueness, permanence, and versatility of patterns, we analyzed them using image processing and visual identification accuracy. The analyzed dataset was composed of 91 images of 30 Humboldt penguins including 8 subadults housed at Enoshima Aquarium. Each image was taken from the front after annual molting and the dataset includes 1-6 years' worth of images for each penguin.

To evaluate similarity of patterns, images were trimmed as they include only the upper chest, normalized with homography transformation, and binarized. We newly introduced the maximum overlapping ratio (MOR) as a suitable evaluation function for black spots and checked whether images of the same penguin mark high MOR score. Additionally, 84 aquarium staffs took part in a visual identification test.

Results indicated that all 30 penguins had black spots on their chest. Moreover, 90 of 91 images had other images of the same individual in the dataset, and 80 of 90 images marked the highest MOR for another image of the same individual, while 87 of 90 images marked in the top three MOR scores. This result suggests that individual patterns are consistent over time and different between individuals. The visual identification test resulted in a high rate as 96.7%.

According to the results, the black spots on the chest of Humboldt penguins are a common and distinctive feature that is useful as a non-invasive biological marker for individual identification.

## 59. King penguin (*Aptenodytes patagonicus*) sightings and breeding attempts at Martillo Island, Tierra del Fuego, Argentina

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King penguins (*Aptenodytes patagonicus*) have a circumpolar distribution and inhabit subantarctic islands between 45°S and 55°S. Historical and archaeological records show this species used to inhabit the Fuegian region before their eradication by humans for commercial use in the 19<sup>th</sup> century. In recent years this species began to reappear in several areas of previous occupation. In 2013 a camera trap was placed facing a Gentoo penguin colony at Martillo Island, Tierra del Fuego and has been taking photos ever since. King penguins were registered, between 1 and 4 individuals at a time. Individuals were seen moulting, resting and even breeding. Breeding attempts such as displays, mating and incubation of rocks were observed the first years. In 2019-2020, a pair successfully produced a chick, for the first time, that lived 19 days. In 2020-2021 the chick lived 19 and in 2021-2022 only 9 days. In 2022-2023 adults stopped the reproductive event during incubation, abandoning the egg. Necropsies of the dead chicks did not reveal an apparent cause of death. Even if the early death of chicks produced by the breeding pair is overcome, breeding success will not occur until a larger amount of breeding pairs is recruited as chicks need to crèche during the winter months. The reoccurrence of species in previously vacated places may be due primarily to the lifting of anthropogenic impacts and may also indicate the environmental conditions are favourable for resettlement in this area.

## 60. King penguin chick mortality related to predator presence in Tierra del Fuego, Chile

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During the breeding cycle, which occurs on land, several species have been reported to kill chicks and adults, and also eat abandoned eggs and dead penguins. King penguin (*Aptenodytes patagonicus*) has the longest breeding cycle among penguins, this situation makes them susceptible to terrestrial threats. Since 2010 this species was reported breeding in Tierra del Fuego, Chile. On this island, two predators were introduced grey fox (*Lycalopex griseus*) and mink (*Neovison vison*) in 1940-1950, this has faced King penguins with terrestrial predators, which is the main cause of chick deaths during winter.

## 61. King penguin mortality related to heat wave events in 2019 and 2020 at Bahía Inútil, Tierra del Fuego

**Maite Arriagada<sup>1</sup>**

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Climate change has increased extreme weather events all over the planet. Species faced with these events can either adapt or suffer the consequences.

On Tierra del Fuego, high-temperature peaks of 30°C and 24 °C were recorded in 2019 and 2020, respectively. We reported the deaths of 7 adults and 4 chicks in 2019 and 2 adults and 9 chicks in 2020, most of them had dehydration signs and stomachs with food content.

Only the birds that died in 2020 were analyzed. All of them had stomach content. The adults had a thick mucous secretion on the beak and eyes.

We attributed the deaths to dehydration, mainly because the chicks had full stomachs and this made them unable to thermoregulate properly.

Heat mortality events have been reported on Magellanic Penguin (*Spheniscus magellanicus*) in Punta Tombo, Argentina on January 2019 (Holt & Boersma, 2022). In addition, several authors have discussed the challenges penguin species are facing with climate change (Tworkowski *et al.*, 2019; Le Bohec *et al.*, 2008)

At King Penguin Natural Reserve we established a protocol, that when cloud cover and wind are poor, added to high temperatures (more than 20°C), the first refuge will be closed, due to its proximity to the colony and the stress tourists might generate on breeding king penguins.

Predictive model results show that extreme weather events will be more frequent and severe (Murali *et al.*, 2023; Jones *et al.*, 2015), therefore, research should focus on ways to protect penguins.



## 62. Lessons from a Magellanic penguins long-term monitoring in Southern Patagonia: unified methodology, scale-dependent density, and stable population trends

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The main touristic attraction of Monte León National Park (MLNP) in Patagonia (Argentina) is the colony of Magellanic penguins (*Spheniscus magellanicus*), which suffers predation from pumas (*Puma concolor*). For this reason, the colony is monitored since 2005 to assess population abundance, but using different methodologies during 10 years. Since 2015, a methodology was developed to unify the colony monitoring. Field methodology involves the counting of all nests on 44 fixed 20 m radius circular areas spaced systematically every 100 m in the entire colony, identifying number of occupied nests every concentric radii of 1 m. Total abundance is calculated using the total area of the colony from circles density. Density and abundance estimations of MLNP colony from 2015 to 2021, calculated with radii of 10, 15 and 20 m, are reported. Two analytical methods, quadrat-count and distance sampling with uniform distribution without adjustments, in R language were used. The colony showed no trend over the time, despite puma predation, with 43,764 nests in average (95% CI 31,076 - 56,452). Both methods did not show densities significant differences for each radii, with distance sampling being more time-consuming for both fieldwork and analysis. Larger radii showed significantly lower values, suggesting a tendency towards regular distribution at 20 m. Results emphasize the scale-dependent nature of density, and highlights the importance of using standardized, comparable and cost-effective methods for long-term wildlife populations monitoring.

### **63. Long-term monitoring of breeding and molting colonies of Humboldt penguins (*Spheniscus humboldti*) at the Humboldt Penguin National Reserve, Coquimbo, Chile**

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Monitoring the conservation objectives is crucial for any conservation program. This turns out to be especially true for the Humboldt penguin, an emblem, and vulnerable species of the Reserva Nacional Pingüino de Humboldt (RNPH). The purpose of this paper is to report the results of the long-term monitoring carried out by CONAF at the Choros and Damas Island colonies of Humboldt penguins at the RNPH. The annual reproductive monitoring carried out between 2015 and 2021, indicates an average abundance of 737 reproductive individuals at Choros Island (min-max range: 388-1240) for the period 2015-2021 with non-significant variations, suggesting population stability although a low population size. Monitoring during the molting period on the Reserve shows that the average abundance for the period 2020-2023 is 664 individuals. For the year 2020, a strong decrease of 71% of the population was detected in relation to the oldest reference value for the year 2013 (1498 individuals). The results obtained in recent years showed a tendency towards the recovery of the molting population. The stability of the reproductive population and the gradual recovery of the molting population in recent years are very encouraging, however, mortality due to Highly Pathogenic Avian Influenza (HPAI) during 2023 could be causing a significant decrease in the population in this region. Monitoring of reproductive and molting individuals will be essential to determine the impact of this disease on the population dynamics and the actions and measures that we can carry out to mitigate its impact on the viability of the species.

## 64. Penguin Monitoring 2.0: How transponders and weighbridges revolutionised the way we study penguins

**Andre Chiaradia<sup>1</sup>, Ross Holmberg<sup>1</sup>, Kean Maizels<sup>2</sup>**

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Individual identification is essential to study the life cycle of penguins. From last-century techniques such as metal flipper banding to the use of transponders coupled with weighbridges, penguin research arrived in the 21st Century. Weighbridges can collect massive amounts of data on attendance patterns and body mass without manual handling, allowing continuous monitoring of individual penguins and population-wide ecological studies. At Phillip Island, Australia, little penguins (*Eudyptula minor*) marked with transponders have crossed weighbridges for over 25 years. We have been upgrading the system as technology has improved over the years. The current system can store every single record for post-analysis, providing the transponder ID and weight of all birds crossing a weighing platform, even when multiple birds are present. The system records over 20 million points per year. By modelling the dynamics of each penguin as a continuous series of simple movements, we mathematically verified that the best way to calculate a penguin's mass is by using the erratic measurements recorded from the weighing platform with a relatively high sample frequency. The system addresses the challenges of animal automated weighing, such as errors due to unpredictability in the penguins' behaviour during weighing and multiple birds on the weighing platform simultaneously. Over the past 25 years, transponders and weighbridges have revealed new frontiers in the foraging ecology of little penguins, presenting an exciting opportunity for future research on penguins in general.

## 65. Population parameters of a king penguin colony (*Aptenodytes patagonicus*) in Bahía Inútil, Tierra del Fuego, Chile

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Until 2010, the king penguin (*Aptenodytes patagonicus*) was considered an occasional visitor in Isla Grande of Tierra del Fuego, Chile. In 2011, a small group of penguins was established in Bahía Inútil, which promoted conservation initiatives at King Penguin Nature Reserve. Since then, its particular characteristics and various events that have influenced its development have been researched. Due to its confinement and distance from the open ocean, this colony differs from other penguin settlements and thus, this study aimed to analyze its population parameters. Observations were recorded from 2015 to 2021, to describe population parameters, evaluate the interannual viability of the chicks and analyze the influence of environmental variables on the absolute and specific mortalities of chicks. Results showed a survival rate higher than one in the first five years, an increase in the annual number of offspring, and greater mortality during the winter months, though no significant time trend in the number of fledged chicks. A strong association between the hatching period and the chick's fledging was observed, where those born between January and February had greater chances of fledging. Regarding environmental effects, mean, maximum, and minimum monthly temperature showed a significant association with mortality, while no significant association was found with wind speed. These analyses allow to approach this colony's particular features and give guidelines for its management.

## 66. Oceanographic and habitat traits affecting colony size in Humboldt penguins (*Spheniscus humboldti*) in Chile

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Understanding the factors that model colony size in seabird populations is essential to understand their ecology and develop adequate conservation strategies. In this study we investigate how island surface area, habitat diversity, distance to the mainland, latitude, the presence of invasive species, the protection status of the islands and food availability affect population size at ten Humboldt penguin (*Spheniscus humboldti*) colonies in north-central Chile during the 2021 and 2022 breeding seasons. We used generalized linear models (GLM) to test whether these variables predict colony size in Humboldt penguins. Our preliminary results indicate that the protection status (e. g. national park, nature reserve) and latitude have no significant effects on colony size. However, the presence of invasive vertebrate species, including rats (*Rattus* sp.) and European Rabbit (*Oryctolagus cuniculus*) are the most important factors affecting the size of colonies, thus when these two invasive species are present on the islands, the number of penguin pairs decreases significantly. We discuss the importance of these preliminary findings to the development of sound management plans for the Humboldt penguin. We dedicate this presentation in loving memory of Gabriel Knauf.

## 67. Methodological constraints for estimating the Humboldt penguin population in Chile

**Paulina Arce<sup>1</sup>, Maximiliano Daigre<sup>1</sup>, Florencia Vial<sup>1</sup>, Alejandro Simeone<sup>1</sup>**

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In the current scenario of declining biodiversity, it is crucial to provide quality information on population sizes and trends of threatened species to allow their classification within red list categories. This requires accurate, species-focused, and standardized methodologies to obtain and compare data in time. The Humboldt penguin (*Spheniscus humboldti*) occurs along the Pacific coast of Peru and Chile where researchers have used different methods to determine population sizes, including the direct count of molting birds, active nests, and indirect estimates through statistical methods (e.g. distance sampling, monitoring quadrats). The use of different methods makes difficult to obtain comparable data, both within and between colonies and countries. In this presentation we analyze how population size of Humboldt Penguins has been determined at their main colonies in Chile (e.g. Pan de Azúcar, Chañaral, Choros, Tilgo, Cachagua) and how the different methods employed have produced contrasting results: 6 to 8 times higher numbers of breeding pairs are obtained using statistical approaches compared to traditional terrestrial direct counts of active nests. We discuss how these different results derived from different methodologies preclude comparison and difficult adequate management strategies for the species. We recommend the use of a standardized method based primarily on the direct count of active nests (=breeding pairs= mature individuals) following IUCN criteria to derive the total population size for the species and supplement, when possible, with direct counts of molting birds. We dedicate this presentation in loving memory of Gabriel Knauf.

## 68. Pre-molting trips: Detrimental effect of GPS on body weight gain, returning date and blood isotopic values?

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Biologgers are often used to study distribution of penguins at sea, but their effects on the studied birds are rarely assessed. Many penguin species spend several weeks at sea foraging to gain weight before the molt; an energetically demanding process. Within the framework of a study on pre-molting trips of rockhopper (*Eudyptes chrysocome*) and Magellanic penguins (*Spheniscus magellanicus*) at Isla Pingüino, Argentina, we analyzed the effect of GPS on weight gain, returning dates and blood isotopic values, by comparing equipped with non-equipped (controls) marked birds. Equipped Rockhoppers gained 2.6 times less weight than controls (mean gain:  $285 \pm 384$  vs  $753 \pm 249$  gr.; N:17 and 17;  $p < 0.001$ ). Their trips were 52% longer; they returned 15 days later (median: 44 vs 29 days, N:13 and 10,  $p = 0.005$ ). Equipped Magellanics gained 1.4 times less weight than controls (mean gain:  $1042 \pm 486$  vs  $1482 \pm 384$  gr., N:13 and 14,  $p = 0.045$ ). Their median returning date was 18 days later than controls ( $p = 0.002$ ). Within both species, the equipped and control birds had similar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values ( $p > 0.1$ ). Overall, we found possible detrimental effects of GPS on returning dates and weight gain in both species. This gain is of vital importance during pre-molting period since poor conditions can compromise the survival during the molt. Our results highlight the need to include the assessment of biollogger effects with controls when using GPS on penguins. Furthermore, our results also call into question the interpretation of published studies on penguins based on several weeks' long GPS deployments.

## 69. Progress in understanding drivers of *Pygoscelis* penguin demography and population dynamics near Palmer Station, Antarctica

**Megan Cimino<sup>1</sup>**

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Since 1990, *Pygoscelis* penguins have been routinely studied near Palmer Station as part of the Palmer Long-term Ecological Research program. The long-term objective of this penguin research is to identify and understand the mechanisms regulating contrasting penguin population trends against a backdrop of rapid regional warming. Climate projection studies suggests the West Antarctic Peninsula (WAP) may no longer contain suitable habitat for ice-obligate species, like the Adélie penguin, by the end of the 21<sup>st</sup> century so understanding the threats to these species face is a pressing issue. While WAP-wide studies suggest a decrease and poleward shift in Antarctic krill (the primary prey species), there is no directional change in the krill population near Palmer Station despite strong interannual variability driven by the dominant cohort. On a large-scale, winter/spring sea ice appears to function as primary driver of penguin demography while on a smaller-scale, summer precipitation and local weather, including interactions with landscape features, appears to be a secondary driver. Spring preconditioning, specifically the timing of sea ice retreat and its extent, is a dominant physical force governing biological processes – including penguin breeding phenology – and shifts in the timing of sea ice retreat could ultimately lead to mismatches that influence chick survival. The complexities and interrelated aspects of nesting habitat conditions, oceanographic foraging environments and prey distributions will be discussed in relation to influencing penguin population dynamics.



## 70. Return rate of Magellanic penguins, *Spheniscus magellanicus*, from Martillo Island, Beagle Channel, Argentina, using two different recaptures methodologies

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Magellanic Penguins from Isla Martillo colony, Argentina, have been tagged with subcutaneous microchips since 2006 to identify fledglings (between 150 and 190 per season), adults and to replace the stainless-steel bands used between 2003-2005. Therefore, exact or minimum age were known for each individual. We calculated the return rate between 2018 and 2019 of 142 adults identified in 2017, and compared two recapture methods: manual scanning of the penguin's back and, since 2019, a passive reader which was rotated amongst main paths used by penguins throughout the season. From the 142 manually registered adults in 2017, 60% returned in 2018, 39% in 2019, 30 % in 2021, 25% in 2022, only 11% returned every study season and 27% were never recaptured. Reproductive skips were recorded, for example. 8% were not recorded in 2018 but were recaptured the following season. Comparing both methods, 14% and 22% of the marked birds were recorded by both systems, 72% and 58% were recorded by the passive reader only, and 15% and 20% by the manual method only in 2021 and 2022, respectively. The passive reader had a higher return rate (8% in 2021 and 3% in 2022) than the manual method. Manual readers allow to record philopatry at a smaller scale and to monitor individuals for reproductive, trophic or health studies, but with higher sampling effort. On the other hand, passive readers detect a greater number of records for survival studies by age and allow to infer their reproductive status.

## **71. Successful colonization of Humboldt penguins in breakwaters: The case of the PERU/LNG port terminal**

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The PERU LNG port terminal is a liquified natural gas infrastructure located 163 km south of Lima and that started operations in June 2010. The port terminal is protected by a breakwater of 800-m long, built parallel the coast and 2km off-shore. The breakwater was built with a combination of natural rock and concrete boulders, and it has become an artificial inshore island. There is a secondary 190-m breakwater connected to the Jetty. Both breakwaters offer ideal conditions for breeding and resting of Humboldt penguins and other seabirds. Likewise, the undisturbed breakwaters and maritime area -exclusive for port operations- around the terminal provide unique conditions for seabird colonization and settlement. Since May 2011, the Biodiversity Monitoring and Assessment Program of the Smithsonian Institution and PERU LNG have been monitoring the marine biodiversity and abundance around the terminal. Humboldt penguins were initially counted twice a year since 2014 by direct observations, but the use of drones allowed monthly counts since 2017 onwards. First penguins in the main breakwater were sighted in 2012, two years after its construction. Maximum annual number of penguins during the molt showed an exponential increase from 316 birds in 2014 to 2091 birds in 2021, becoming one of the major Humboldt penguin sites in Peru. However, a sharp decline of the colony was reported between 2022 (1759 birds) and 2023 (502 birds). We discuss the possible causes for the rapid colonization, population decline and the vision to implement a penguin conservation plan in this site.

## 72. The Fall and rise of the little penguin on Phillip Island, Australia

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While penguins face many threats in both the marine and terrestrial environments, the terrestrial threats are easier to mitigate and achieve positive outcomes for populations. Phillip Island was once home to ten little penguin colonies, but by the late 20th century, the island's penguins faced extinction attributed to predation from domestic and introduced animals, disturbance, and habitat loss. By removing or managing most of the introduced terrestrial threats, Phillip Island's only remaining colony on the Summerland Peninsula is now the species' largest, with 32 000 breeding penguins. An additional benefit of the island-wide conservation work has been the re-establishment of three previously extinct colonies. We visited each colony during peak breeding over five years to gain an understanding of penguin numbers and colony extent. At two sites, we recorded the number of burrows and breeding activity, and at one hard to access location, we counted the sets of penguin footprints and their direction either to or away from the colony. All three sites recorded an increase in the number of burrows or footprints, with peak growth ranging from 172% to 410%. The total population size for each site is estimated to be between 100 and 200 breeding penguins, with the extent of two colonies increasing by approximately 15%. In a time where most of the world's penguin species are threatened, this highlights the importance of managing terrestrial threats and ongoing monitoring for penguins as they face a rapidly changing and uncertain future.

### 73. The quest for long-term monitoring, research, and conservation of the little penguin/kororā

**Rachel Hickcox<sup>1</sup>, Averil Parthonnaud<sup>1</sup>, Kevin Parthonnaud<sup>1</sup>**

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Long-term research projects are vital to implementing effective conservation management and advancing knowledge about a species. In some instances, repeated data collection over many years results in post-recognition of the project as long-term. Alternatively, a project is started with the intention of becoming a long-term programme. While some actions are unique to managing a specific project, both ways follow a common process of (1) conceptualisation and defining objectives, (2) creating the initial project plan, (3) applying for permits and funding, (4) refining protocols and methodologies, (5) preparing for field work, (6) training, (7) annual data collection, (8) data analysis, and (9) sharing results and measuring conservation success. Here we present these steps specific to the establishment of the Pōhatu/Flea Bay little penguin (*Eudyptula minor*) long-term monitoring and research programme by the Helps Pōhatu Conservation Trust. On the quest to better understand the population dynamics of the largest mainland colony of little penguins in New Zealand and to develop effective conservation strategies, we monitor a subsection of over 200 nest boxes using a combination of field observations, transponder marking, and GPS tracking. We are also conducting research on penguin habitat use, foraging behaviour, and diet. Our collaboration with several organisations provides opportunities for training and research for students and early career researchers. We believe that this programme provides a model for the establishment of similar long-term projects for other penguin and marine species around the world.

## 74. The winter distribution of chinstrap penguins from Deception Island, Antarctica

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Chinstrap penguins are top marine predators that mainly forage on krill (*Euphausia superba*) in Antarctica, and have been designated a key monitoring species under the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR). This species departs from breeding colonies after chick fledging and spend up to 8 months over-wintering at sea. Despite the obvious importance of the non-breeding season to their survival, winter distributions and behaviors of Chinstrap penguins in the Antarctic Peninsula are mostly unknown.

Several studies have shown in different organisms how movements can be fitted to different patterns to optimize search for food resources. From GPS locations, we verified the fitting of movement patterns to the functions of Lévy and Brownian strategies. This analysis of food search patterns can be used as an indirect measure to track changes in food availability across a broad range of environmental conditions. We used satellite telemetry to track the movements of 13 fully molted adults from the Vapour Col penguin rookery in Deception Island, South Shetlands (63° 00'S 60° 40'W). The analysis of the satellite location allows us to obtain accurate information on movement patterns, specific geographic regions used by penguins during non-breeding months, and the spatial overlap between fishing and foraging grounds which may highlight factors influencing their survival. The analysis of Chinstrap penguins' wintering movements is important under the current context of global change which is expected to affect migratory species.

## 75. Unveiling the mystery underlying two consecutive catastrophic breeding seasons in a large king penguin colony

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Large-scale breeding failures, such as offspring die-offs, can disproportionately impact wildlife populations that are characterized by a few large colonies. Yet, identifying the cause of these breeding failures is challenging, especially when there is a delay between the triggering events and the die-offs itself. Such is the case in species with long breeding cycles, when carryover effects might only result in offspring mortality weeks, if not months after the adults experience poor conditions. We investigate two unresolved dramatic breeding failures that occurred in consecutive years (2009 and 2010) in a large king penguin *Aptenodytes patagonicus* colony, a long-lived species with a breeding cycle lasting over a year. Here we found that a single period, winter 2009, was likely responsible for the breeding failure of both breeding seasons, suggesting that adults experienced poor foraging conditions at sea at that time. Following that unfavorable winter, the 2009 breeders – who were entering the late stage of chick-rearing – immediately experienced high chick mortality. Meanwhile, the 2010 breeders delayed their arrival and egg laying, which would have otherwise started not long after the winter. This delay caused a domino-effect for the 2010 chick cohort, eventually leading to the death of all chicks in winter 2010, a whole year after the unfavorable events of winter 2009. This study highlights the importance of carryover effects from the winter period on phenology and reproduction success for wildlife that breed in few large colonies.

## 76. Winter migration and isotopic niche of Adélie penguins from Western Antarctic Peninsula: species ecological insights to contribute to marine spatial planning and management

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Adélie penguins (*Pygoscelis adeliae*) undertake long-distance migrations during winter. This period includes the molt phase and is crucial for juvenile and adult survival and subsequent breeding success. Adélie penguin population declines in one of the most rapidly warming places in the Southern Hemisphere have been linked to the reduction of total biomass and size of available Antarctic krill. Since 2021, we are studying winter migration and isotopic niche of individuals breeding in a declining colony in King George/25 de Mayo Island, Western Antarctic Peninsula. Every year, we tracked penguins' movement using geolocators (GLS) attached to the legs, and collected body feathers for Carbon and Nitrogen stable isotope analysis. Preliminary results revealed migration routes across the Bransfield Strait, north of the Antarctic Peninsula, and the Wedell and Scotia Seas, encompassing three different CCAMLR management subareas. Penguins depend on resources available in subarea 48.5 during the post-breeding and molting stage, and resources distributed across subareas 48.1, 48.2 and 48.5 during the winter. We identified differential use of areas between years and estimated distances covered by penguins.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values obtained in body feathers reflect enrichment in pre-molt locations, where penguins replenish fat reserves before undergoing a catastrophic molt process. Areas used immediately before and after molting represent key areas for individual survival, but are not currently considered within current MPAs proposals. Adélie penguins' dependence on resources from different subareas throughout the year emphasizes the need to incorporate the temporal dimension of resources utilization when designing conservation measures for this region.

## **77. A systematic review and meta-analysis of the pollutant exposure in penguins through the southern hemisphere**

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As top-predators that integrate pollutants along marine food webs, penguins are exposed to increasing pollutant levels in marine ecosystem. However, comprehensive and systematic efforts aimed at evaluating the exposure to pollutants by the penguin community is clearly lacking. Here, we filled this gap of knowledge by systematically gathering available information about pollutant levels for the different penguin species.

Our systematic review identified 98 papers where 3583 pollutant levels for different penguin species were reported. Available information contrasts among pollutants and penguin species. As a case study, we performed a meta-analysis of mercury (Hg) levels in blood and feathers of the different penguin species geographic area using hierarchical three-level mixed-effects models with moderators.

We show that there are differences in Hg concentrations between species and geographic areas, with higher levels observed in species inhabiting the Indian Ocean and Oceania, and those occupying higher trophic levels. We provide the most comprehensive compilation and assessment of pollution concentrations in penguins, while identifying information gaps, and further steps required in penguin pollutant research. We also reveal that penguins can be used as effecting sentinel species for monitoring environmental pollution throughout the Southern Hemisphere.



## 78. Magellanic and gentoo penguin mortality linked to a toxic dinoflagellate bloom at Beagle Channel, Argentina, during austral summer 2022

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During the austral summer 2022, an intense bloom of the toxic dinoflagellate *Alexandrium catenella*, one of the main producers of paralytic shellfish toxins (PST), occurred at Beagle Channel, Tierra del Fuego, Argentina. Some weeks later, a striking number of seabirds, including two penguin species, showed intoxication symptoms and died along the channel shoreline and inner islands. Five dead Magellanic penguins (*Spheniscus magellanicus*), including adults and chicks, and one adult Gentoo penguin (*Pygoscelis papua*) were processed to assess the presence of PST in the stomachal content and the liver, including non-digested squat lobster (*Munida gregaria*) collected from *P. papua* esophagus. Thirty-one more *S. magellanicus* were counted dead at the shoreline but were not tested for toxins due to the carcass' degradation or signs of starvation. Quantitative and qualitative measures of toxins were performed by high-performance liquid chromatography.

*S. magellanicus* livers had toxin concentrations between 28 and 54  $\mu\text{g STX eq kg}^{-1}$ , and *P. papua* had an intermediate liver toxin concentration. The stomach content of two *S. magellanicus* showed toxin concentrations  $>1400 \mu\text{g STX eq kg}^{-1}$ . Toxin concentration of preys found in *P. papua*'s esophagus was  $>3400 \mu\text{g STX eq kg}^{-1}$ .

Harmful algal blooms (HABs) are natural phenomena, but pollution and climate change could alter their frequency and magnitude. Although not every toxic bloom triggers massive organisms' die-offs, increasing seabird mortalities linked to HABs have been reported globally, hence becoming relevant events for conservation.

## 79. Per- and polyfluoroalkyl substances (PFAS) in nesting material and blood of little penguins along a gradient of urbanisation in Tasmania

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Persistent organic pollutants (POPs) are globally widespread and ubiquitous within marine environments. Higher order predators are especially vulnerable to deleterious impacts on individual health through bioaccumulation. Per- and polyfluoroalkyl substances (PFAS) are an increasing health concern for marine animals. This study investigates the exposure to 14 PFAS in the nesting materials ( $n=51$ ) and 49 PFAS in serum ( $n=40$ ) of little penguins, a peri-urban resident species in Tasmania.

Six PFAS were found in the nesting materials in 15 out of 16 sites tested and seven PFAS were found in serum samples, across all populations tested ( $n=8$ ). Mean and observed ranges (ng/mL) were highest for Perfluoro-n-octanesulfonate (PFOS), Perfluoro-n-octanoic acid (PFOA) and Perfluoro-n-hexanesulfonate (PFHxS) in serum across all sites (PFOS = 0.715, <0.5 – 22.3; PFOA = 1.47, <1.0 – 2.7; PFHxS = 1.15, <0.5 – 5.6). Concentrations were moderately correlated with urbanisation (Spearman's Rank; PFOS = 0.49, PFOA = 0.55, PFHxS = 0.49), defined by total length of road network within 1km of the site, similar correlations were also evident in nesting materials. The serum of male birds had higher concentrations of PFAS on average than those of females, which is likely reflective of the excretory process of egg-laying in females.

This study is the first to document PFAS concentrations in little penguins and their breeding habitat and explores the associations between PFAS and individual health. These results highlight the utility of penguins as sentinels for ecosystem health.

## 80. Variation in mitochondrial metabolism during fasting in breeding king penguins

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In numerous avian species fasting is an inherent part of the breeding cycle. In king penguin chicks the mechanisms and consequences of the fasting process have been widely investigated because of their extremely long fasting period (4 to 5 months) during the growth. However, king penguin adults also experience relatively long fasting periods during their breeding cycle. For breeders, the success of fasting does not only influence their own condition and survival, but the chick's survival as well, and therefore their fitness. Unraveling the physiological mechanisms underlying adults fasting success can therefore provide information to understand variation in fitness. Previous studies focusing on king penguin adults showed that the whole-organism metabolic rate decreases across the different fasting stages. However, mitochondrial metabolism has been recently raised as a promising proxy of animal performance as it allows evaluating the balance between the energy production and its constitutive damaging sub-products (leading to oxidative stress). Here, the purpose of our study was to investigate changes in mitochondrial metabolism (in red blood cells) in breeding king penguins adults during a fasting period (i.e., egg incubation). For this purpose, we collected blood samples in males and females and measured mitochondrial respiration rates in the beginning (3 days on land) and at the end (10 days on land) of their natural fasting period. By studying changes in mitochondrial metabolism in regards to fasting we aim to better understand the cellular constraints shaping adult king penguin's performance.

## 81. Examination of microplastics in captive penguin fecal samples

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Microplastics have been found in all areas of the environment, including the air, oceans, soil, and within organisms themselves. Although the long-term effects of microplastics on aquatic organisms is still being explored, researchers have been able to quantify these plastics within various species. To our knowledge, no research has been published examining the presence of microplastics in captive penguin species. In addition, there is no standardized approach for extracting and identifying microplastic in biological samples. The goal of this project is to standardize the methods used for isolating and identifying microplastics, and to determine if captive penguins are exposed to microplastics. Samples were collected from Mystic Aquarium (n=107) and a second anonymous aquarium (n=28). All samples were digested in 30% H<sub>2</sub>O<sub>2</sub> to eliminate organic matter, then a density separation was performed with saturated NaCl. From there, the digested samples were filtered using a vacuum filtration system to isolate potential microplastic fibers. Fibers were quantified based on their color, size, and identification. A total of 148 fibers were found in the Mystic Aquarium samples, and 76 fibers were found from the second anonymous aquarium samples. The fibers found were most commonly black, blue or red, and they fell within the 0.5-5 mm range. The most common microplastic identifications include polyester (PET) and polypropylene. The source of these microplastics is still being examined and the results of this experiment will be compared to results obtained from wild penguins in Argentina.

## 82. Examination of the presence of microplastics in wild Magellanic penguins from Punta Tombo, Argentina via fecal analysis

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Microplastics, synthetic fibers less than 5 mm in length, have become a hot topic in the media recently as they have been discovered in human blood and placenta. This relatively new field of research has focused a lot on quantifying microplastics in all areas of the environment, especially in marine habitats. There are very few papers examining microplastics in wild penguins, and even fewer that examine microplastics in penguin chicks. The goal of this project is to expand the field of microplastic research to wild adult and chick penguins. Fibers were analyzed from 50 Magellanic penguin chick and 90 Magellanic penguin adult fecal samples collected in Punta Tombo, Argentina. Both groups followed a digestion, density separation, and filtration protocol, however, the digestion and density separate steps were flipped for the wild adult samples due to increased amounts of sand and rocks. From the 50 chick fecal samples analyzed, 222 fibers have been identified and described based on their color and size. Microplastics have been identified in numerous samples, including identifications such as polyester (PET), polyethylene, and polypropylene. Quantification and identification of fibers from the 90 adult fecal samples will be reported on in the near future. The results of this study will help us to understand the relationship between microplastics in adult and penguin chicks in the wild, and these methods can be used for dirty biological samples in future research investigations.

## 83. Microplastic ingestion of African penguins in South Africa

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Coastal birds act as bio indicators and can be used to draw inferences about the quality of the coastal environment. Currently, a significant knowledge gap exists on the occurrence and impact of microplastics on coastal birds such as the African penguin. The African penguin is classified as endangered, and its foraging behaviour being tactile and visual dependent makes it susceptible to the accidental ingestion of microplastics in the water as well as from its prey of choice. This study focused on quantifying microplastics ingested by the African penguin of different age classes by examining and digesting the gastrointestinal tracts using a KOH and Wet-Peroxide Oxidation method, isolating, and characterising the dominant types, sizes, and colour of microplastics. Preliminary analyses show that the number of microplastics found in the oesophagus, stomach and intestine range from 1409 to 1653 microplastics per penguin. The intestine has significantly more microplastics compared to the other components of the Gastro-Intestinal Tract. The majority of microplastics were white fibres in the size class of 125µm. Compared to other coastal birds, the African penguin holds the highest microplastic abundance due to greater gastrointestinal mass. The accidental consumption of macro and microplastics may cause bowel obstruction that may lead to starvation or release harmful chemicals into the organs leading to long term issues that may be passed to on to their offspring. Further research is necessary to assess the transport of microplastics in the coastal ecosystem and their impacts on key species such as the African Penguin.

## 84. Oil spill risks for African penguins and other seabirds in Namibia and South Africa

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South Africa and Namibia have an extensive history of oil spills, impacting large numbers of seabirds. Coastal developments and ship-to-ship bunkering activities have led to several oil spills in recent years. Compared to historic oil spills, such as the *Treasure* spill in 2000, these have been small in terms of quantity of oil spilled and number of birds affected, but still of concern to threatened seabirds. Here we present a meta-analysis of oil spills in the region and demonstrate how spilled volume is not necessarily an indicator of number of seabirds affected but how the proximity to seabird colonies does play a key role. Compared to international data, oil spills in southern Africa have a higher probability of affecting seabirds due to the overlap of human activities and important breeding and foraging areas. African penguins are most affected although flying seabirds may be underreported (due to mortality at sea). Analyses of rehabilitation data from over 1500 African penguins from eight recent spills (2001-2021) showed a high release rate after rehabilitation; penguins rescued in the earlier days after a spill had a higher body mass, better body condition and were less dehydrated. In light of ongoing economic development in the region, the prevention of oil spills and the preparedness to rescue and rehabilitate oiled seabirds is critical. Algoa Bay is of particular concern: since the expansion of Port of Ngqura and the implementation of ship-to-ship bunkering, vessel numbers and collision risk have increased as have the number of oil spills.

## 85. Plastic ingestion by Magellanic penguins (*Spheniscus magellanicus*) throughout their annual cycle

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The Magellanic penguin (*Spheniscus magellanicus*, MP) is the most abundant seabird and an emblematic species in the Patagonian Sea. We investigated the ingestion of plastics by MP along their breeding areas in central Argentina and wintering/migration areas in Argentina and Brazil, 2019-2022. In Argentina, the data was obtained from beach/colony surveys in Chubut province (n=68), and from beach surveys and rehabilitation centers in Buenos Aires province (n=29). In Brazil, data from beach surveys and rehabilitation centers was obtained from Beach Monitoring Program (n=785). Macro-mesoplastics (>5mm) were found in 37.6% of carcasses, with a higher frequency in individuals that died during rehabilitation (40.7% Brazil juveniles, 48.1% Argentina juveniles), and at migration areas (39.9% juveniles, 16.7% adults) versus breeding areas (6.5% juveniles, 9.1% adults). No plastic ingestion was recorded in carcasses collected at breeding colonies (nine adults), except one chick. In a subset of 21 carcasses of juvenile MP beach-cast in Buenos Aires coastline, we found that 100% showed microplastics (<5mm) in their stomach; a complete chemical identification of microplastics ingestion by MPs beach-cast at breeding areas (35 juveniles, 14 adults, one chick) is currently in process. These results corroborate that plastic ingestion is a common problem in MP and are thus useful indicators of marine plastic pollution in the Southwest Atlantic Ocean. These findings highlight the need to further assess subtler or indirect health effects of plastic ingestion, especially on reproductive birds.



## 86. Pollution Alert: Microplastics found in kidney and liver of Magellanic Penguins (*Spheniscus magellanicus*)

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Magellanic Penguin (*Spheniscus magellanicus*) is a seabird that is usually seen on the Brazilian coast between July and November. This penguin, as well as other seabirds, is impacted by the presence of plastic waste in the ocean. In addition to strangulation and ingestion of large plastic particles, there is also the problem of ingestion of microplastics (<5mm). Despite the impacts of these fragments on organisms still being studied, findings show that they can absorb toxic substances and release substances used in their manufacture, compromising the health, reproduction, and survival of the organism. Our objective was to investigate microplastic contamination in the kidneys and livers of *S. magellanicus*, for this purpose, 13 carcasses collected in 2019 were evaluated. The samples were digested with a 10% solution of Potassium Hydroxide in filtered ultrapure water, then kept in an oven at 40°C for 48 hours and vacuum filtered using a Millipore glass microfiber filter of 0.2µm to 0.6µm, subsequently analyzed using a stereomicroscope. Fifty-two particles were found, of which 75% were fibers. The most prevalent color was blue, totaling 51.92% of the items, followed by 21.15% transparent, 19.23% black, 5.77% pink, and 1.92% yellow. Particle metrics ranged from 3.263µm to 2982.707µm, with 25 particles found in the kidneys and 27 in the livers. We report unpublished records of microplastics in *S. magellanicus* livers and kidneys. In the next steps, chemical analysis will be carried out to identify the composition of polymers.

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- HOWELL, Lucy
- IRIGOIN-LOVERA, Cinthia
- ITO, Konomi
- JOLY, Nicolas
- JONGSOMJIT, Dennis
- JUAREZ, Ignacio
- KACZVINSKY, Chloe
- KARNOVSKY, Nina
- LAWRENCE, Simon
- LE MAHO, Yvon

- LEMONNIER, Camille
- LEÓN, Fabiola
- LESCROËL, Amélie
- LONDT, Logan
- LOPEZ, María Ignacia
- LUDYNIA, Katrin
- LYNCH, Heather
- MACHADO, Ana
- MARCHISIO, Nahuel
- MATTERN, Thomas
- MCGILL, Patricia
- MCINNES, Julie
- MCKELSON, Jessica
- MILLONES, Ana
- MNYEKEMFU, Mpumalanga
- MORAIS, Julia
- MORANDINI, Virginia
- MORGENTHALER, Annick
- MUÑOZ, Gabriela
- NEIRA, Victor
- NOLL, Daly
- OBERLIN, Maëlle
- OCHOA, Manuel
- OGATA, Akane
- OHARA, Ichiyu
- OKUBO, Michiko
- ORMEÑO, Milagros
- PALOMINO, Albert
- PERTIERRA, Luis R.
- PIZARRO, Eduardo
- PLANAS-BIELSA, Victor
- POLITO, Michael
- PÜTZ, Klemens
- RAMÍREZ, Francisco
- RAYA, Andrea
- REBSTOCK, Ginger
- REINHOLD, Sarah-Lena
- RITCHIE-PARKER, Heather
- ROBERTS, David
- RODRIGUEZ-PLANES, Lucia
- ROSSELL, Laia
- SANTOS, Amanda
- SARAUX, Claire
- SCHAEFER, Rebecca
- SCHMIDT, Annie
- SCIOSCIA, Gabriela
- SEGUEL, Mylene
- SIMEONE, Alejandro
- SHIRAKATA, Chika
- SNYMAN, Albert
- TISERA, Candela
- VANSTREELS, Ralph E. T.
- VARGAS, Renzo
- VIANNA, Juliana
- VIAL, Florencia
- VILLABRIGA, María Luján
- WAGNER, Eric
- WALKER, Brian
- WALLER, Lauren
- WASIAK, Paula
- WEBSTER, Trudi
- WEIDEMAN, Eleanor
- WELLS, Melanie
- WEINBERGER, Constanza
- WHITE, Jeff
- WIERENGA, Janelle
- WILLIAMS, Nicole
- WILSON, Rory P.
- WINQUIST, Suzanne
- ZAJKOVÁ, Zuzana
- ZALDÚA, Natalia
- ZAVALAGA, Carlos