

MESTRADO EM CIÊNCIAS DO MAR – RECURSOS MARINHOS
ESPECIALIZAÇÃO EM BIOLOGIA E ECOLOGIA MARINHAS

Environmental Enrichment for the Captive Humboldt Penguins (*Spheniscus humboldti*) at SEA LIFE Porto, Portugal

Ana Jacira Madureira Carvalho

M

2024

Environmental Enrichment for the Captive Humboldt Penguins (*Spheniscus humboldti*) at
SEA LIFE Porto, Portugal

Ana Jacira Madureira Carvalho

Instituto de Ciências Biomédicas Abel Salazar

ANA JACIRA MADUREIRA CARVALHO

**Environmental Enrichment for the Captive Humboldt Penguins
(*Spheniscus humboldti*) at SEA LIFE Porto, Portugal**

Dissertação de Candidatura ao grau de Mestre
em Ciências do Mar – Recursos Marinhos,
submetida ao Instituto de Ciências Biomédicas
de Abel Salazar da Universidade do Porto.

Orientadora – Ana Isabel Ferreira Magalhães
Gamito Carrilho

Categoria – Professora Auxiliar

Afiliação – Instituto de Ciências Biomédicas de
Abel Salazar da Universidade do Porto

Co-orientadora – Ana Sofia Poças da Costa
Ferreira

Categoria – Curadora de Exposições

Afiliação – SEA LIFE Porto

Agradecimentos

A concretização desta Dissertação não seria possível sem o contributo de várias pessoas, às quais dedico estes agradecimentos com o mais sincero apreço.

À professora Ana Magalhães, o meu profundo obrigado pela excelente orientação, ajuda, carinho e amizade. O seu apoio incondicional e a sua paixão pela área foram uma fonte constante de inspiração.

À minha coorientadora, Ana Ferreira, muito obrigada pela disponibilidade e incentivo constantes. Obrigada por assegurares todas as ferramentas necessárias à realização deste trabalho e por teres a paciência de me apoiar, sempre com um sorriso no rosto.

Ao Professor Doutor Pedro Oliveira, agradeço imenso o apoio valioso fornecido na análise estatística desta Dissertação. Um agradecimento adicional à Doutora Ana Valentim por toda a compreensão ao longo deste processo, e à Doutora Tania Monreal-Pawlowsky, cujo *feedback* e rigor estabelecido me motivaram diariamente a querer superar as minhas próprias capacidades.

À equipa do SEA LIFE Porto, um enorme obrigado por esta oportunidade e por me mostrarem que diversão e profissionalismo podem andar lado a lado. Ao longo deste ano, não houve um único dia em que tenha acordado desanimada por ir trabalhar convosco. Colocaram a fasquia muito alta.

Um agradecimento especial à equipa de Biologia, por todos os conselhos, apoio e companheirismo. Obrigada pela dedicação aos vossos animais, e por fazerem tudo ao vosso alcance para garantir o seu bem-estar. Foi um prazer imenso aprender convosco.

À minha família, o meu muito obrigado. Ao meu irmão, o meu colega de casa preferido (e também o único), obrigada por segurares as rédeas da casa enquanto eu desesperava na divisão ao lado. Aos que me criaram, os meus pais e avós, devo-vos tudo. São quem mais amo neste mundo e no próximo.

Aos meus amigos, agradeço por me lembrarem que a vida é para ser vivida. Não consigo imaginar o que seria de mim sem vocês.

Por fim, resta-me agradecer ao grupo de pinguins que tive o privilégio de acompanhar ao longo deste ano. Pelas bicadas (que prefiro acreditar serem gestos de carinho), pelo mau humor, pelas gargalhadas e até pelas frustrações que me provocaram, acompanhar-vos foi, sem dúvida, uma experiência única e inesquecível.

Ana Jacira Madureira Carvalho

Declaração de Honra

Declaro que o presente trabalho é de minha autoria e não foi utilizado previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referenciação. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

25 de novembro de 2023

O estudante

Ana Carvalho

Ana Jacira Madureira Carvalho

Abstract

Animal welfare became an academic field in 1965, with the Brambell Report. Since then, multiple definitions of 'welfare' have evolved, and more comprehensive frameworks have emerged. Animal welfare is an internal and subjective state inferred from observable indicators, including physical, physiological, and behavioural. Since no single indicator is definitive, a comprehensive evaluation combining all indicators is essential for understanding an animal's level of welfare.

Environmental enrichment (EE) enhances animal welfare by reintroducing complexity to captive environments. It aims to promote species-specific behaviours, improve reproductive success, and enhance overall fitness and health while reducing abnormal behaviours. Understanding the unique needs of each species is essential for effective EE.

Despite some debate, EE can include animal training which is supported in the literature as a possible positive and beneficial stimulus. Animals subjected to veterinary care or routine husbandry tasks may experience compromised well-being if they are unaccustomed to or lack training for such interactions. Therefore, training animals to participate voluntarily can be essential for maintaining their overall welfare. Even though the concepts of animal training and EE address different motivations and behaviours, these approaches are complementary to each other and represent a key component to ensuring animal well-being.

Humboldt penguins (*Spheniscus humboldti*) are native to Chile and Peru and are classified as "vulnerable" on the International Union for Conservation of Nature Red List, as their current population trend is decreasing. As an endangered species, keeping animals under human care ensures they have access to food and medical care, helping to extend their lifespan; provides a space for scientific research to occur; and, among others, educates and sensitises the public.

This study was conducted at SEA LIFE Porto and was divided into two simultaneous parts. The first part of this study aimed to evaluate the activity budget of Humboldt penguins and investigate how land and aquatic EE influenced their walking, exploratory, and swimming behaviours, along with enclosure use. The second part of this study focused on training the penguins to gradually approach keepers, allow their touch, and eventually reach the desired final position of remaining in the keeper's arms for a few seconds.

For the first part of the research, *ad libitum* observations were conducted to develop the species' ethogram. Systematic observations occurred over approximately seven months, from December 2023 to June 2024. EE items were selected based on comparisons between captive and wild penguin behaviours to ensure their ethological needs were met. Both the land and water-based enrichment items were presented up to five times a week for 30 minutes to an hour.

Recordings were analysed using The Observer XT 11.5 software by Noldus. To address the difficulty in identifying individual penguins, an instantaneous scan sample of the group was conducted once every minute. The Electivity index, total percentages of visible penguins and of each behavioural category were calculated. Differences between situations with and without enrichment were assessed using a paired samples t-test, with a normal distribution of differences.

The activity budget analysis revealed a decrease in the number of visible penguins as the reproductive and nesting season progressed (February through July), with most of the penguins' time spent on stationary behaviours. Social and swimming behaviours were the least frequently observed. The effectiveness of EE devices varied by month and type of EE. Additionally, the penguins showed little interest in the display areas near visitors, preferring to remain closer to their nests.

The second part of this research focused on training the penguins, as keeping animals in captivity requires careful measures to ensure their health and well-being. Animals unaccustomed to handling often experience fear and stress. However, positive reinforcement training (PRT) can help reduce fear and anxiety, enhance welfare, and promote positive emotions, giving animals more control over their environment.

In this research, PRT was used to train the penguins to lie on the keeper's arms while extending their legs and feet, to facilitate and reduce the discomfort associated with certain veterinary and handling procedures. The training methodology was developed to fit the keepers' routines, allowing for daily training sessions without the need to expand the team. Nine steps were defined and included the penguins being gradually closer to the keepers, applying increased pressure during touch, and eventually having the penguins remain on the keeper's lap.

Training occurred from January to May 2024 in the indoor area of the penguins' enclosure,

allowing the penguins to decide how long they desired to participate. A checkered blue and green board was used as a visual cue in each session, aiming for the penguins to associate it with being picked up and placed in a keeper's arms. Statistical analysis was performed to assess significant differences in training progress between sexes. A Mann-Whitney U test was utilised to compare the highest training steps achieved by males and females.

Step 7 was the highest step achieved in the training process, and it was achieved by three out of the eight penguins. The reproductive cycle notably impacted training, as some penguins hesitated to leave their nests during this time, impeding progress. Males consistently reached higher training steps than females, who remained more wary throughout the process. Nonetheless, the group exhibited improved comfort with physical touch and proximity to keepers during and after training sessions.

Keywords: *Spheniscus humboldti*, animal welfare, environmental enrichment, positive reinforcement training, penguin behaviour

Resumo

O bem-estar animal tornou-se um campo acadêmico em 1965, com o Relatório de Brambell. Desde então, surgiram várias definições de 'bem-estar', bem como *frameworks* mais abrangentes. O bem-estar animal é um estado interno e subjetivo inferido a partir de indicadores observáveis, incluindo indicadores físicos, fisiológicos e comportamentais. Uma vez que nenhum indicador isolado é definitivo, uma avaliação abrangente que combine todos os indicadores é essencial para compreender o nível de bem-estar de um animal.

O enriquecimento ambiental (EA) melhora o bem-estar animal ao reintroduzir complexidade nos ambientes cativos. O objetivo é promover comportamentos específicos da espécie, melhorar o sucesso reprodutivo e aumentar a condição física e a saúde em geral, e ao mesmo tempo reduzir comportamentos anormais. A compreensão das necessidades únicas de cada espécie é essencial para um EA ser eficaz.

Apesar de algum debate, o EA pode incluir o treino de animais, que é sustentado pela literatura como um possível estímulo positivo e benéfico. Animais submetidos a cuidados veterinários ou tarefas rotineiras de manejo podem ter o seu bem-estar comprometido caso não estejam habituados ou não tenham sido treinados para essas interações. Assim, o treino para que os animais participem voluntariamente pode ser essencial para a manutenção do seu bem-estar geral. Embora os conceitos de treino animal e de EA abordem motivações e comportamentos diferentes, estas abordagens são complementares entre si e representam uma componente fundamental para garantir o bem-estar dos animais.

Os pinguins de Humboldt (*Spheniscus humboldti*) são nativos do Chile e do Peru e estão classificados como “vulneráveis” na Lista Vermelha da União Internacional para a Conservação da Natureza (IUCN), dado que a sua população está atualmente em declínio. Sendo uma espécie ameaçada, manter estes animais sob cuidados humanos garante-lhes acesso a alimento e cuidados médicos, ajudando a prolongar a sua longevidade; proporciona um espaço para a realização de investigação científica; e, entre outros benefícios, educa e sensibiliza o público.

Este estudo foi realizado no SEA LIFE Porto e foi dividido em duas partes simultâneas. A primeira parte teve como objetivo avaliar o *budget* de atividade dos pinguins de Humboldt e investigar como o EA colocado em terra e em água influenciou os seus comportamentos de locomoção, exploração e natação, bem como o uso do recinto onde habitam. A segunda

parte do estudo concentrou-se em treinar os pinguins para se aproximarem gradualmente dos tratadores, permitirem o toque e, eventualmente, alcançarem a posição final desejada de permanecerem nos braços do tratador por alguns segundos.

Na primeira parte da investigação, foram efectuadas observações *ad libitum* para desenvolver o etograma da espécie. Seguiram-se observações sistemáticas que ocorreram ao longo de aproximadamente sete meses, de dezembro de 2023 a junho de 2024. Os itens de EA foram selecionados com base em comparações entre os comportamentos de pinguins em cativeiro e na natureza, para garantir que as suas necessidades etológicas eram satisfeitas. Tanto os itens de enriquecimento colocados em terra como os colocados em água foram apresentados até cinco vezes por semana, durante 30 minutos a uma hora.

As gravações foram analisadas usando o *software* The Observer XT 11.5 da Noldus. Para abordar a dificuldade em identificar pinguins individualmente, foi realizado um *scan* instantâneo do grupo a cada minuto. O índice de eletividade, as percentagens totais de pinguins visíveis e de cada categoria comportamental foram calculados. As diferenças entre situações com e sem enriquecimento foram avaliadas utilizando um teste t de amostras emparelhadas, com distribuição normal das diferenças.

A análise do *budget* de atividade revelou uma diminuição no número de pinguins visíveis à medida que a época de reprodução e de nidificação progrediu (fevereiro a julho), sendo a maior parte do tempo dedicado a comportamentos estacionários. Os comportamentos sociais ou de natação foram aqueles que ocorreram menos vezes. A eficácia dos dispositivos de EA variou consoante o mês e o tipo de EA. Adicionalmente, os pinguins demonstraram pouco interesse nas áreas de exibição próximas dos visitantes, preferindo permanecer mais perto dos seus ninhos.

A segunda parte desta investigação focou-se no treino dos pinguins, já que manter animais em cativeiro requer a implementação de medidas para garantir a sua saúde e bem-estar. Animais que não estejam habituados ao *handling* muitas vezes experienciam medo e stress. No entanto, o treino com reforço positivo (TRP) pode ajudar a reduzir o medo e a ansiedade, melhorar o bem-estar e promover emoções positivas, fornecendo aos animais mais controlo sobre o seu ambiente.

Neste estudo, o TRP foi utilizado para treinar os pinguins a deitarem-se nos braços do tratador, enquanto estendiam as pernas e as patas, de modo a facilitar e reduzir o

desconforto associado a certos procedimentos veterinários e de manejo. A metodologia de treino foi desenvolvida para se integrar nas rotinas dos tratadores, permitindo sessões de treino diárias sem a necessidade de expandir a equipa. Foram definidos nove passos, que incluíam os pinguins aproximarem-se gradualmente dos tratadores, aplicar maior pressão durante o toque e, eventualmente, fazer com que os pinguins permanecessem no colo do tratador.

O treino decorreu de janeiro a maio de 2024 na área interior do habitat dos pinguins, permitindo que os mesmos decidissem quanto tempo desejavam participar nas sessões de treino. Um tabuleiro quadriculado azul e verde foi utilizado como sinal visual em cada sessão, visando que os pinguins o associassem a serem colocados nos braços de um tratador. Foi realizada uma análise estatística para avaliar diferenças significativas no progresso do treino entre os sexos, sendo o teste de Mann-Whitney U utilizado para comparar as etapas de treino mais elevadas alcançadas por machos e fêmeas

O Passo 7 foi o mais elevado alcançado no processo de treino, atingido por três dos oito pinguins. O ciclo reprodutivo impactou o treino, uma vez que alguns pinguins hesitaram sair dos seus ninhos durante este período, o que impediu o progresso. Os machos atingiram consistentemente níveis de treino mais altos do que as fêmeas, que se mostraram mais cautelosas ao longo do processo. No entanto, no geral, o grupo demonstrou um maior conforto com o toque físico e a proximidade dos tratadores durante e após as sessões de treino.

Palavras-chave: *Spheniscus humboldti*, bem-estar animal, enriquecimento ambiental, treino com reforço positivo, comportamento dos pinguins

Table of Contents

Agradecimentos	II
Declaração de Honra	III
Abstract	IV
Resumo	VII
List of Acronyms, Abbreviations and Symbols.....	XII
List of Figures	XIII
List of Tables	XV
Conference Presentations	XVI
Introduction.....	1
Animal Welfare	1
Environmental Enrichment.....	5
Animal Training	6
Humboldt Penguins (<i>Spheniscus humboldti</i>).....	9
<i>Species Distribution and Biological Traits</i>	9
<i>Behavioural Patterns in the Wild</i>	12
<i>Behavioural Patterns in Captivity</i>	13
<i>Population Status and Threats</i>	14
Keeping Animals Under Human Care	15
Objectives	17
Study Location and Subjects.....	17
Study Site	17
Study Area.....	18
The Penguins of SEA LIFE Porto.....	19
Chapter 1: Assessing the Impact of Environmental Enrichment	23
1. Introduction	23
2. Materials and Methods	25
2.2. Systematic Observations	26
2.3. Chosen Environmental Enrichment Devices	29

2.4.	Behavioural Analysis	32
2.5.	Statistical Analysis.....	33
3.	Results.....	34
3.1.	Activity Budget.....	34
3.2.	Effects of Environmental Enrichment.....	38
3.3.	Enclosure Use	41
4.	Discussion.....	42
4.1.	Activity budget	42
4.2.	Effects of Environmental Enrichment.....	44
4.3.	Enclosure Use	46
5.	Conclusion	48
	Chapter 2: Behavioural Training of Humboldt Penguins.....	49
1.	Introduction	49
2.	Materials and Methods	51
2.1.	Training Procedure	51
2.2.	Training steps: Description, Goals, and Challenges.....	55
2.3.	Statistical Analysis.....	60
3.	Results.....	60
4.	Discussion.....	64
5.	Conclusion	67
	Final Remarks.....	69
	Bibliographical References	70
	Appendices.....	78
	Appendix A	78
	Appendix B	85
	Appendix C	93
	Appendix D	96
	Appendix E	97

List of Acronyms, Abbreviations and Symbols

AM	Ante Meridiem
cm	Centimetres
EAZA	European Association of Zoos and Aquaria
EE	Environmental Enrichment
ENSO	El Niño Southern Oscillation
FAWC	Farm Animal Welfare Committee
GB	Gigabyte
HCS	Humboldt Current System
IUCN	International Union for Conservation of Nature
Kg	Kilogram
m	Metres
m²	Square metres
m³	Cubic metres
PM	Post Meridiem
PRT	Positive Reinforcement Training
SPSS	Statistical Package for the Social Sciences
WAZA	World Association of Zoos and Aquariums

List of Figures

Figure 1. The Five Domains model for comprehending and evaluating various states of animal welfare.	3
Figure 2. Humboldt penguin (<i>Spheniscus humboldti</i>) distribution map (area highlighted in orange).	9
Figure 3. Adult Humboldt penguins (<i>Spheniscus humboldti</i>) at SEA LIFE Porto (A) and juvenile Humboldt penguin (B)	10
Figure 4. SEA LIFE Porto penguins' exhibition.	18
Figure 5. Tommy (male, 9 years old) and Hickory (female, 5 years old) at SEA LIFE Porto.	20
Figure 6. Beaker (male, 8 years old) and Luna (female, 7 years old) at SEA LIFE Porto.	21
Figure 7. Pumpkin (male, 8 years old) and Pip (female, 7 years old) at SEA LIFE Porto.	21
Figure 8. Bramble (male, 6 years old) and Baobab (female, 7 years old) from SEA LIFE Porto.	22
Figure 9. Division of the penguins' enclosure into different zones, taking into account their use by the penguins.	29
Figure 10. Percentage of visible penguins from December 2023 to April 2024.	34
Figure 11. Percentage of visible penguins engaging in maintenance behaviours from December 2023 to April 2024.	35
Figure 12. Percentage of visible penguins engaging in stationary behaviours from December 2023 to April 2024.	35
Figure 13. Percentage of visible penguins engaging in locomotion on land behaviours from December 2023 to April 2024.	36
Figure 14. Percentage of visible penguins engaging in swimming behaviours from December 2023 to April 2024.	37
Figure 15. Percentage of visible penguins engaging in social behaviours from December 2023 to April 2024.	37
Figure 16. Percentage of visible penguins engaging in environmental interaction behaviours from December 2023 to April 2024.	38

Figure 17. Effect of environmental enrichment: Comparison of significant differences in the mean percentage of behavioural categories (A-H) based on the absence (represented in blue) or presence (represented in green) of environmental enrichment (EE).	40
Figure 18. Variation in the Electivity index according to the different display zones (A-E), as described in Figure 9, and time of the year (December 2023 to January 2024, February to March 2024 and April to May 2024).	41
Figure 19. Illustrative representation of the intended penguin behaviour (created by the author).	50
Figure 20. The checkered green and blue board used as a visual cue in every training session.	55
Figure 21. Humboldt penguins' training steps.	57
Figure 22. Penguins' training progress: highest step achieved in each session for the different penguin pairs.	62
Figure 23. Independent-samples Mann Whitney U test.	63
Figure 24. Sprinkler.	93
Figure 25. Aquatic aerator.	93
Figure 26. Artificial algae, with approximately one meter in length.	93
Figure 27. Artificial algae and a net with fish, suspended from the bridge (not represented in the picture).	94
Figure 28. Artificial algae and leaves.	94
Figure 29. Artificial plant.	94
Figure 30. 3D printing of the Peruvian booby bird (<i>Sula variegata</i>).	95
Figure 31. Two of the feeding balls used with artificial algae (on the left) and fish (on the right).....	95
Figure 32. Training steps flowchart.	96

List of Tables

Table 1. Categories and descriptions of behaviours displayed by the Humboldt penguins during observations.....	25
Table 2. Overview of the selected environmental enrichment devices, including their categories, functions, purposes, and placement within the exhibition.	30
Table 3. Criteria used to classify each penguin's training sessions as “Terrible”, “Bad”, “Good”, “Excellent”, or “Did not enter the indoor area”.....	52
Table 4. SEA LIFE Porto Humboldt penguins’ ethogram.	85
Table 5. Session classification for each Humboldt penguin at SEA LIFE Porto. The columns contain the penguins’ names, while the rows represent the training session number. The colours are attributed based on Table 3, portraying the criteria for session classification. Green = “Excellent”; Yellow = “Good”; Orange = “Bad”; Grey = “Did not enter the training area”.....	97

Conference Presentations

The results obtained between December 2023 and April 2024 were presented at 3 conferences.

In poster format:

- Carvalho, J., Ferreira, A., & Magalhães, A. (2024, May 10). *Activity Budget of Humboldt Penguins (Spheniscus humboldti) Under Human Care at SEA LIFE Porto and How Environmental Enrichment May Influence Activity Levels* (Presentation in Poster format). Investigação Jovem da Universidade do Porto 17^a edição. Porto, Portugal. <https://www.up.pt/ijup/ijup-2024/>
- Carvalho, J., Ferreira, A., & Magalhães, A. (2024, July 10). *Activity Budget of Humboldt Penguins (Spheniscus humboldti) at SEA LIFE Porto and How Environmental Enrichment Influences Activity Levels* (Presentation in Poster format). Universities Federation for Animal Welfare – International Animal Welfare Conference 2024. Porto, Portugal. <https://www.ufaw.org.uk/ufaw-events/ufaw-international-animal-welfare-conference-2024-porto>
- Carvalho, J., Ferreira, A., & Magalhães, A. (2024, November 5). *Behavioral Patterns of Humboldt Penguins (Spheniscus Humboldtii) in Captivity: the Impact of Environmental Enrichment on Activity Levels* (Presentation in video format). Animal Behaviour Live 2024, annual online conference. <https://animalbehaviour.live/conference>.

Appendix A contains the abstracts submitted for each conference and the posters and PowerPoint presented.

As oral communication:

- Carvalho, J., Ferreira, A., & Magalhães, A. (2024, July 12). *Optimizing Veterinary Care: Practical Training of Humboldt Penguins (Spheniscus humboldti) to Facilitate Medical Care at SEA LIFE Porto* (Oral communication). 2nd Regional meeting of ISAE - International Society for Applied Ethology. Porto, Portugal. <https://www.i3s.up.pt/event?v=308>

Publications:

An article titled “Optimizing Animal Care: Training of Humboldt Penguins (*Spheniscus humboldti*) to Facilitate Veterinary and Husbandry Procedures” was submitted to the Journal of Zoo and Aquarium Research and is awaiting review. <https://www.izar.org/izar>.

In addition, I collaborated in the production of a video as part of the “Animal Welfare in Aquaculture and Public Aquariums” module during the advanced training in Aquaculture that took place in Porto between February 19 and March 1, 2024, as part of the ERASMUS+ international project (2023-2025), called AFISHE - Development of Aquaculture and Fisheries Education for Green Deal in Armenia and Ukraine: from Education to Ecology, coordinated by ANAU - Armenian National Agrarian University and with partners in Armenia (NAS RA - National Academy of Sciences of the Republic of Armenia), Ukraine (SNAU - Sumy National Agrarian University, in Sumy, and NUWEE - National University of Water and Environmental Engineering, in Rivne) and Portuguese partners (U. Porto), Croatian (UNIDU - University of Dubrovnik) and Slovakian (SUA - Slovak University of Agriculture, in Nitra) partners, co-financed by the European Union (<https://erasmusplus.am/course/afishe/>).

The video featured a visit to SEA LIFE Porto, where the scope of the dissertation project was outlined. It covered the environmental enrichment strategies implemented, detailing the selection process, rationale, and their significance. The video emphasised the critical role of environmental enrichment and the study of animal behaviour in promoting the welfare of the animals in captivity.

Introduction

Animal Welfare

The formalisation of animal welfare as an academic field began in 1965 with the release of the Brambell Report, commissioned by the British government, which focused on the welfare of animals kept under intensive livestock husbandry systems (Brambell Report, 1965; Carenzi & Verga, 2009). Since then, a considerable amount of scientific research has gone into this topic, but even so, the concept of 'welfare' has different definitions, reflecting varying perspectives throughout the literature (Carenzi & Verga, 2009).

Indeed, over time, the human-animal relationship has suffered a cultural evolution of its societal perception, therefore resulting in the suggestion of several welfare definitions (Carenzi & Verga, 2009). Dockès and Kling-Eveillard (2006) revised that animal welfare should be based on four major concerns:

- (i) Biological and technical definitions – Focused on the animals' basic requirements, specifically their Five Freedoms (further elaboration on this topic will be provided later in this document), the opportunity for animals to adapt to environmental challenges (Dantzer & Mormède, 1983; Capdeville & Veissier, 2001), as well as the effect of man-animal interactions on technical tasks (Hemsworth & Coleman, 1998, as cited in Dockès & Kling-Eveillard, 2006).
- (ii) Regulation approaches – Acknowledging the animal as a sentient being, whose owner must provide conditions that satisfy the species' biological needs. Such perspectives have led to the creation of several regulations and laws.
- (iii) Philosophic approaches – Exploring ideas regarding the status of animals, whether they are considered sentient beings or mere machines, and whether they possess freedom or are merely tools.
- (iv) Communication between man and animal – Taking into consideration that the farmer's welfare is directly related to the animal's welfare, and how breeding techniques might influence this interaction (Porcher, 1997; Porcher, 2001, as cited in Dockès & Kling-Eveillard, 2006).

While initially directed toward farm animals, one might argue that these issues offer a comprehensive framework that addresses fundamental aspects of animal well-being and that might be applicable across all species, therefore being a holistic approach to the meaning of animal welfare.

The establishment of the Five Freedoms was a turning point in the history of animal welfare. Over the years, this framework has been refined (FAWC, 1993, 2007, 2009), and the Five Freedoms originally defined by the Farm Animal Welfare Committee (FAWC) - currently known as the Animal Welfare Committee – have evolved to be, as quoted verbatim from FAWC (2009, p.2):

- (i) **Freedom from hunger and thirst**, by ready access to water and a diet to maintain health and vigour.
- (ii) **Freedom from discomfort**, by providing an appropriate environment.
- (iii) **Freedom from pain, injury and disease**, by prevention or rapid diagnosis and treatment.
- (iv) **Freedom to express normal behaviour**, by providing sufficient space, proper facilities and the appropriate company of the animal's own kind.
- (v) **Freedom from fear and distress**, by ensuring conditions and treatment, which avoid mental suffering.

Even though the Five Freedoms have managed to form a concept of something as multifaceted as animal welfare into a set of practical guiding principles, they focus on the absence of negative affective states and do not necessarily mean that an animal is experiencing positive emotions, and vice-versa, therefore not being sufficient to guarantee a satisfactory state of welfare (Yeates & Main, 2008; Beausoleil et al., 2023).

In order to evaluate animal welfare with more pragmatism, the World Zoo and Aquarium Animal Welfare Strategy, issued by the World Association of Zoos and Aquariums (WAZA), recommended that zoos and aquariums employ the 'Five Domains' model, a simple framework designed to "understand and assess different animal welfare states" (Mellor et al., 2015, p.18; Beausoleil et al., 2023). This approach considers four physical/functional domains: Nutrition, Physical Environment, Health, and Behavioural Interactions, alongside a fifth domain focused on the animal's Mental State (Mellor et al., 2015; Mellor et al., 2020). The first three physical/functional domains focus on "internal survival-related factors", i.e. genetically determined impulses that urge animals to have certain behaviours which ultimately contribute to their survival (Mellor, 2017, p.4). The fourth physical/functional domain ("Behavioural Interactions") is correlated to "external situation-related factors" (Mellor, 2017, p.4; Mellor et al., 2020, p. 3). It concerns the animal's perception of the surrounding environment and its interactions with other animals and human beings (Mellor et al., 2020). These physical/functional domains encompass subjective positive and negative components which in turn result in subjective positive or negative emotional experiences, ascribed to the mental domain (*Figure 1*; Mellor, 2017). The animal's welfare

status is determined by all the affective experiences and, therefore, the overall mental state (Figure 1; Mellor, 2017).

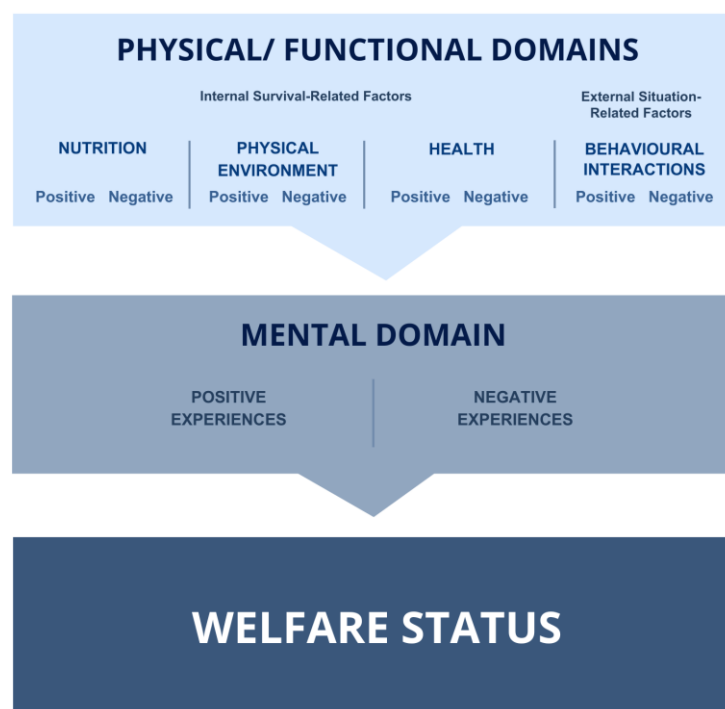


Figure 1. The Five Domains model for comprehending and evaluating various states of animal welfare. Internal and external factors lead to positive or negative subjective experiences for each of the physical/functional and mental domains. The entire affective experience determines the welfare status. Adapted from Mellor et al. (2015), with additional information from Mellor (2017) and Mellor et al., (2020).

This model underlines the idea that addressing an animal's emotional needs is equally as crucial as tending to its physical demands since for each physical alteration there can be a corresponding emotional response. It also emphasises that minimising or avoiding negative experiences is insufficient to achieve good or acceptable animal welfare, pleasant experiences are required as well (Mellor et al., 2020). The capacity of animals to experience positive emotions is a key characteristic of the positive animal welfare concept (Lawrence et al., 2019). An animal that is free from pain and suffering, but lacks access to positive welfare opportunities can be characterised as experiencing a neutral state of welfare (Muhammad et al., 2022).

Positive animal welfare indicators can include, for instance, behaviours that demonstrate comfort or engagement with both the physical and social surroundings (such as resting, interacting with the environment, being active, or interacting with others of the same species), measures considered 'species-typical', or those that indicate positive

affective states (Ward et al., 2024). Recently, behavioural diversity has been recognised as a possible positive welfare indicator, although further research and validation are needed (Miller et al., 2021).

Since animal welfare relates to an animal's "internal and subjective state", it can only be deduced from observable characteristics that reflect the animal's affective state (Beaulieu, 2024, p. 2). Its assessment should be based on a diverse range of measurements including, physical, physiological, and behavioural indicators (Broom, 1988). These indicators, help infer how animals feel and, hopefully, determine their level of welfare.

Physical health is the most widely accepted and visible indicator of animal welfare (Dawkins, 2003). It is commonly accepted that when animals get hurt, sick, or have physical problems, they suffer and have poor welfare (Dawkins, 2003). While some believe that as long as animals are not sick or injured, they are in good welfare, others argue that it entails much more than simply avoiding circumstances that put the animals' health at risk or expose them to situations where death is likely (Dawkins, 2003). This is one of the reasons why additional forms of welfare measurement have been searched (Dawkins, 2003).

Physiological measurements offer an objective assessment of an animal's health and condition (Cooke et al., 2014). When the physiological states of animals deviate from their typical baseline, it signals a potential disturbance that could lead to negative consequences (Jerez-Cepa & Ruiz-Jarabo, 2021). These physiological reactions, however, vary across taxonomic groups and can be unique to individual species (Jerez-Cepa & Ruiz-Jarabo, 2021; Browning, 2023). Additionally, many physiological signs used to assess welfare are, in reality, autonomic reactions related to activity or arousal that are not necessarily linked to poor welfare (Dawkins, 2003). Moreover, these indicators naturally fluctuate with factors such as breeding conditions, time of the day, and temperature, besides having some invasive or intrusive measuring methods that inevitably disturb the animal (Dawkins, 2003) and might compromise results.

Behavioural cues are frequently employed as the primary indicators for evaluating animal welfare (Binding et al., 2020). Behavioural assessments have included measuring differences between animals in captivity versus those in the wild or free-ranging environments, as well as examining uncharacteristic or harmful behaviours like stereotypes – repetitive actions lacking clear purpose or function (Mason, 1991; Dawkins, 2003). Although many behavioural and physiological signs can signal stress, none are specific to a particular stressor (Morgan & Tromborg, 2007). Some stressors can result in identical or

highly similar effects on animals, while others lead to varying effects depending on factors such as age, sex, or prior experiences (Morgan & Tromborg, 2007).

The various welfare indicators complement each other, highlighting the importance of comprehensive welfare evaluations. Each of these indicators is an individual piece of a puzzle providing useful information which, when combined, creates a complete picture of an animal's welfare. Welfare assessments are complex and multidisciplinary, requiring careful analysis and the integration of diverse factors to ensure that all aspects of an animal's well-being are covered.

Environmental Enrichment

Environmental enrichment (EE) can be succinctly described as the introduction of complexity to a habitat that has a positive overall effect on an animal (Kresnye et al., 2022), hence improving its quality of life and overall well-being. It serves as a vital tool, particularly in captive settings where the de-complexification of wild environments may be more apparent, allowing for reintroducing some of the lost environmental complexity (Kresnye et al., 2022).

Ideally, EE should involve biologically relevant improvements to the environment that positively impact animals in captivity, such as, for instance, increased reproductive success, fitness, or overall health, rather than merely concentrating on altering the environment or increasing its complexity (Newberry, 1995). The ultimate goals of EE should include fostering species-specific behaviours, enhancing the capacity to manage challenges, broadening the range of exhibited behaviours, encouraging positive interactions with the environment, and potentially minimising or eradicating abnormal behavioural patterns (Wells, 2009).

EE comprises a range of categories including social, occupational, physical, sensorial and dietary (Arechavala-Lopez et al., 2021). These categories often intersect and are not entirely distinct from one another (Kresnye et al., 2022). They serve as the building blocks for developing comprehensive enrichment plans, where integrating all categories is crucial to ensure the animals are provided with a more dynamic and evolving environment (Maple & Perdue, 2013). EE programs go beyond simply offering appealing items to animals; they are a systematic and process-oriented method to consistently deliver varied forms of stimulation to every animal (Barber, 2009). A successful enrichment plan should consider

the animals' natural history, alongside the populations' activity budgets in the wild while aiming to encourage behaviours specific to each species (Mellen & Sevenich MacPhee, 2001). An effective enrichment program should be included in a dynamic and constantly evolving framework that includes setting objectives, planning, executing the plan, documentation, and making the necessary adjustments (Mellen & Sevenich MacPhee, 2001). Therefore, one might argue that a continuous evaluation of the enrichment program is necessary to ensure it remains appropriate amidst the evolving needs and changing behaviours of ageing animals.

Welfare assessments often concentrate on identifying pain and evaluating the frequency and severity of illness or injury, focusing mainly on the current health status rather than addressing other aspects of welfare (Wolfensohn et al., 2018). This issue is particularly pertinent for animals in captivity as, occasionally, there is not enough attention given to the broader welfare aspects, such as the appropriateness of their environment and the opportunities to engage in diverse normal behaviours (Wolfensohn et al., 2018). These issues can be addressed, for instance, through the implementation of EE. As suggested by Ward et al. (2024), EE significantly predicts positive welfare indicators such as activity and interaction with the environment, therefore highlighting the crucial role of enrichment in assuring positive welfare is put into practice.

Animal welfare and EE are, therefore, intertwined notions within animal care, with enrichment strategies serving as fundamental tools to ensure that animals can engage in natural behaviours, cope with challenges, and experience positive emotions, ultimately leading to improved health and well-being.

Animal Training

As Bain (2019) explains, animal training is essentially the process of influencing and shaping behaviour. When this process leads to a change in the animal's actions, then learning has taken place (Bain, 2019). The concept of training has a wide range of applications in animal management, from veterinary care and breeding efforts to educational and entertainment purposes; from ensuring proper nutrition and modifying behaviours to optimising enclosure use and promoting human-animal interactions (Melfi & Ward, 2019), training proves to be a valuable and versatile tool.

According to the European Association of Zoos and Aquaria (EAZA) Animal Training Guidelines, animal training should result in an overall welfare benefit, not disrupting, hindering or compromising typical social behaviours of the species (Heidenreich et al., 2023). It should be designed to enhance animal well-being, ensuring that involvement in the training program maximises benefits and minimises potential harm to the animals (Heidenreich et al., 2023). A universal approach or perfect method for training animals does not exist (Heidenreich et al., 2023). Frequently, it requires a deeper, more individualised look into how behavioural change can be effectively achieved for each animal (Heidenreich et al., 2023).

Animal training implies having the ethical responsibility of ensuring that the welfare of each animal is a priority (Heidenreich et al., 2023). Regular risk/ benefit analyses are recommended to determine whether to proceed with training, to guarantee the training program achieves the best outcomes efficiently and effectively, and to evaluate if the chosen training procedures are suitable (Heidenreich et al., 2023). One important aspect is ensuring that the animal agrees with what is happening, rather than merely accepting it (assent vs. consent) (Heidenreich et al., 2023). Evaluating assent involves observing the animal's body language and offering it the choice to move away or not participate (Heidenreich et al., 2023). Additionally, it is important that the animal has several ways to achieve what it desires; restricting options to just one, particularly for essential needs like food, can be perceived as coercive (Heidenreich et al., 2023).

The emphasis on offering animals choice and control within their environments can be linked to the ongoing debate about whether training can serve as a form of enrichment, thus being enriching. The reasoning is that if enrichment aims to enhance the welfare of animals in captivity, and training is thought to contribute to improved welfare, it can be inferred that training is enriching (Fernandez, 2022).

Based on published data, Melfi (2013) analysed several hypotheses related to the enriching effects of training on animals, including its ability to promote learning, enhance human-animal interactions, introduce change to daily routines, and support traditional environmental enrichment methods. The author argued that once a behaviour is learned, it stops being enriching, as it no longer provides a learning experience and its associated benefits (Melfi, 2013). While training can lead to the same results as EE, the fact that the methods through which they bring about behavioural changes differ qualitatively, along with the likelihood that the outcomes themselves may not always feel enriching for the animals, further challenges the idea that training is inherently enriching (Melfi, 2013). The author

additionally noted that while training introduces changes to an animal's life, this alone may not be enriching, as change is not necessarily beneficial (Melfi, 2013). However, training sessions often involve a learning component that may enhance communication between humans and animals, potentially fostering positive interactions and relationships if done correctly (Hosey & Melfi, 2012, as cited in Melfi, 2013; Melfi, 2013). Moreover, evidence suggests that training can facilitate the provision of EE (Melfi, 2013). Although this field needs more research, the author emphasises that the enriching effects of training are just one of the factors to consider, and a cost-benefit analysis should determine whether training should be used, regardless of its enriching potential (Melfi, 2013).

Westlund (2014) offered an alternative perspective on several topics, specifically focusing on four hypotheses regarding the effects of formal training – defined as “the trainer using operant conditioning in direct interaction with the animal” – as a form of enrichment (Westlund, 2014, p.4). The hypotheses explored related to formal training enhancing the animal's control over its environment, increasing behavioural choices, encouraging species-specific behaviours, and empowering the animal to effectively manage challenges (Westlund, 2014). The author concluded that because animals learn from the outcomes of their actions, which are influenced by their behaviour, then formal training is linked to the animal's control over its environment, therefore being considered a form of enrichment (Westlund, 2014). Furthermore, as participation in training sessions is voluntary and animals can decide to engage or not, and because formal training can alleviate fears and therefore broaden behavioural opportunities, it can be considered a form of enrichment (Westlund, 2014). The author stated that, although at first glance formal training might not seem to encourage species-specific behaviours, its common use of food reinforcers stimulates the animal's natural foraging instincts, prompting species-specific covert behaviours (Westlund, 2014). As such, formal training can serve as enrichment by this standard (Westlund, 2014). Lastly, Westlund (2014) argues that formal training may help animals manage sudden stressful situations, such as relocation, transport or medical procedures. While conventional EE provides benefits, it may not fully prepare animals to better cope with novel or aversive stimuli (Westlund, 2014). In contrast, formal training equips animals with the necessary skills to effectively handle these challenges, thus qualifying as a form of enrichment (Westlund, 2014). Although training and conventional EE target different motivations and behaviours, both approaches complement each other and are essential for maximising animal welfare (Westlund, 2014).

Humboldt Penguins (*Spheniscus humboldti*)

Species Distribution and Biological Traits

Humboldt penguins (*Spheniscus humboldti*) are natural to the coasts and offshore islands spanning from Isla Foca (5° 12' S) in Peru to Isla Guafo (43° 32' S) in Chile (Figure 2) (Hays, 1986; BirdLife International, 2020). These regions are bathed by the Humboldt Current System (HCS), an upwelling system characterized by its cold, extremely productive and nutrient-rich waters, allowing for high primary productivity (Hays, 1986; Montecino & Lange, 2009). The food supply that results sustains various schools of fish on which seabird populations rely (Hays, 1986).

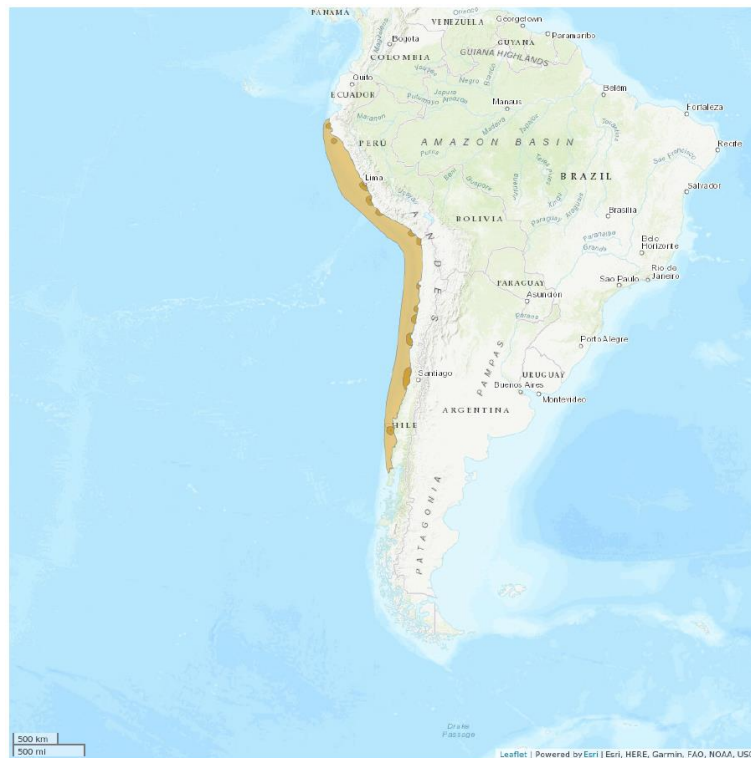


Figure 2. Humboldt penguin (*Spheniscus humboldti*) distribution map (area highlighted in orange). Adapted from BirdLife International, 2020.

The plumage of Humboldt penguins is similar in both males and females, making it difficult to discern between the sexes based solely on plumage characteristics (Zavalaga & Paredes, 1997). These penguins have black flippers, bills, feet and back, a white belly with black spots and a black band across the chest (Figure 3A). A white stripe runs across their heads, and a patch of bare skin on their face begins at the base of the beak, extending around and circling each eye (Figure 3A). In turn, juveniles' are differentiated from adults

by their brownish head feathers, greyer cheeks, and the absence of the distinctive white stripe on the head and the black band across the chest (Figure 3B; De la Puente et al., 2013).



Figure 3. Adult Humboldt penguins (*Spheniscus humboldti*) at SEA LIFE Porto **(A)** and juvenile Humboldt penguin **(B)**. Left and centre images by the author; right image by Grefor Mima via [Pixabay](https://pixabay.com/photos/humboldt-penguin-penguin-bird-7711121/), accessible at <https://pixabay.com/photos/humboldt-penguin-penguin-bird-7711121/>.

Spheniscus humboldti is a medium-sized penguin, normally weighing around 5 kg (Hays, 1986; Smith et al., 2008) with a height of 67 to 72 cm (Coker, 1919). Humboldt penguins are approximately emmetropic both in air and water, enabling them to see well in both environments (Sivak et al., 1987), and can particularly discern colours with wavelengths in the blue-green region of the spectrum (Bowmaker & Martin, 1985). In their natural habitat, Humboldt penguins typically live for 15 to 20 years (Schneider et al., 2014, as cited in Romero et al., 2023), however, in captivity, they can live as long as 25 to 30 years (Wallace, 2014). At around 3 to 4 years old, they reach sexual maturity (Araya et al., 2000, as cited in De la Puente et al., 2013).

Even though males tend to be bigger and heavier than females (Wallace et al., 2008), the differentiation between the sexes based on this fact alone is flawed as there are exceptions and variations; it is possible that the opposite occurs, and females have larger bodies than certain males. While sexing based only on physical aspects is difficult, there is a trait that allows individuals to be distinguished: the pattern of black spots on each animal's chest, which is unique to each penguin as no two penguins have the same pattern (De la Puente et al., 2013), much like a fingerprint on people.

Each year, Humboldt penguins go through a crucial process known as moulting, during which, to keep their feathers in optimal condition, they lose and regrow new ones, ensuring proper insulation against environmental conditions both in the sea and on land (Ancel et al., 2013). In their natural habitat, Humboldt penguins undergo moulting once a year (Merritt & King, 1987), between January and March (Paredes et al., 2002; Wallace & Araya, 2015). During this time, which lasts around 3 weeks (Paredes et al., 2003), moulting individuals remain on land rather than venturing into the sea to search for food (Luna-Jorquera et al., 2000), thereby undergoing a fasting period (Croxall & Davis, 1999). At SEA LIFE Porto, moulting season happens between July and August, with each penguin usually completing the process within 2 to 3 weeks.

After moulting, Humboldt penguins in their native regions typically breed from March to December (Paredes et al., 2002), with breeding peaks occurring twice a year, in the spring (September-November) and autumn (March-May) (Simeone et al., 2002a). Following successful mating 1 to 2 eggs are laid with approximately 3 to 4 days between each laying (Schwartz et al., 1999; Taylor et al., 2001). In these regions, Humboldt penguins also experience two distinct peaks of egg-laying – usually in April and August/September, or in May and October, depending on the location (Paredes et al., 2002). As a result, they can lay up to 4 eggs per year. For SEA LIFE Porto's group of penguins, the breeding and nesting season begins around February and can extend through July, with each pair typically experiencing two egg-laying peaks during this period.

The eggs are incubated for roughly 40 to 42 days and they hatch asynchronously, typically within 2 days of each other (Paredes & Zavalaga, 2001; Quispe et al., 2020). Chicks turn into fledglings at around 10 to 12 weeks old (Quispe et al., 2020). Even though *Spheniscus humboldti* develops pair-bonds, it does not exhibit a rigorous monogamous mating behaviour, as it has been documented engaging in extrapair copulations (Schwartz et al., 1999). In most penguin species, incubation is a shared responsibility, with each parent taking turns incubating the egg (Ancel et al., 2013). Since the chicks are semi-altricial and remain in the nest, once the egg hatches, one parent looks after the offspring while the other forages for food (Culik et al., 2000); both parents participate in feeding by regurgitating food for their young (Ancel et al., 2013). Once the chicks reach a certain stage in their development, depending on factors such as breeding location, climate, and oceanographic conditions, they are left alone at the nest, which allows both parents to forage for food at the same time and thus satisfy the growing needs of the chicks (Culik et al., 2000).

Breeding Humboldt penguins have remarkable adaptability when selecting nesting sites, doing so in a diverse range of locations including cliffs, shorelines and rock crevices (Simeone & Bernal, 2000; Paredes & Zavalaga, 2001). Moreover, they can also nest on “dug-out bowls under rocks or vegetation”, on caves, and on burrow nests excavated within guano deposits, i.e. dried seabird excrements (Schwartz et al., 1999; Taylor et al., 2004; Ellenberg et al., 2006, p.97; Zavalaga & Alfaro-Shigueto, 2015).

Spheniscus humboldti's diet primarily consists of pelagic species, with a particular focus on those that form large groups (Herling et al., 2005). That includes, for instance, Peruvian anchovy (*Engraulis ringens*), Araucanian herring (*Strangomera bentincki*), Inca scad (*Trachurus murphyi*), Garfish (*Scomberesox saurus*), Silverside (*Odontesthes regia*), Common hake (*Merluccius gayi*), and South American pilchard (*Sardinops sagax*) (Herling et al., 2005). Cephalopod beaks and crustacean carapaces have also been collected from Humboldt penguins' stomach samples, although in smaller quantities (Herling et al., 2005). Each species' role in the penguins' diet varies depending on the location and time of year (Herling et al., 2005); however, fish generally make up its primary component.

Behavioural Patterns in the Wild

To date, there is limited information in the literature regarding the activity budget of this species, specifically the amount of time these animals spend on each activity in their repertoire.

The only information available regarding the species' behavioural patterns in the wild pertains to the time spent foraging and, consequently, swimming. According to Culik (2001), during the breeding season, the foraging behaviour of Humboldt penguins, such as range and duration, is influenced by the demands of their offspring and the availability and closeness of their main food sources. Therefore, these behaviours can vary widely within the same species (Culik, 2001). Nonetheless, it is possible to differentiate between two types of foraging trips: day and overnight trips, i.e. outings that last less or more than one calendar day, respectively (Taylor et al., 2002; Hennicke & Culik, 2005).

Although the penguins' varying foraging patterns might be attributed to other factors, the difference in foraging trips may reflect the compromise breeding birds make between meeting their own nutritional needs and provisioning for their young, as the types of trips could be the method used to sustain their fat reserves while ensuring their chicks are fed

(Taylor et al., 2002). Adults normally undertake shorter trips to ensure their chicks' provisioning rate is met (Taylor et al., 2002). In contrast, longer overnight foraging might be linked to the penguins' own survival, since these allow for a more extensive search of food (Taylor et al., 2002). Humboldt penguins may prioritize self-feeding during the initial part of their overnight foraging trips, followed by a period dedicated to gathering food for their young (Taylor et al., 2002). This flexible foraging strategy was likely adopted to ensure that they could balance both the demands of parental care and individual survival.

Additionally, no sex differences in mean forage depth, duration, and proportion of overnight and day trips were observed (Taylor et al., 2002), suggesting that, at least concerning looking for food, both sexes share this responsibility equally. A difference was noted, however, between failed breeders and their counterparts, with the former making lengthier foraging trips and less frequent but longer and deeper dives than the latter (Taylor et al., 2004).

Even though further research is needed, the available information indicates that foraging and swimming constitute a major portion of the Humboldt penguins' daily routine.

Behavioural Patterns in Captivity

Keeping animals under human care offers the opportunity to conduct in-depth studies on various species, enabling researchers to obtain valuable in-sights on certain topics that may be difficult or impossible to get in the wild.

Regarding *Spheniscus humboldti*, as previously discussed, information on how they allocate their time in the wild is scarce and mostly related to behaviours performed in water. Studies made in captivity allow to bridge this gap, providing more information on their time allocation to other behavioural categories. Although the environments and external factors potentially influencing behaviour differ, all the information gathered remains valuable.

In captivity, Humboldt penguins predominantly engage in stationary, locomotion, maintenance and swimming behaviours (Simeone et al., 2002b, Marshall et al., 2016; Zhang et al., 2021; Goodenough et al., 2023). Their activity fluctuates throughout the day, with rest being more common during the afternoon, and an increase in activity both before and after feeding (Zhang et al., 2021; Goodenough et al., 2023).

Spheniscus humboldti's behavioural patterns also vary with season. According to Goodenough et al (2023), behaviours performed in water were more frequent in autumn, with terrestrial inactive behaviours, such as standing and resting, being higher in spring. Indeed, weather conditions seem to be another factor influencing the penguins' behaviour. Although Goodenough et al (2023) reported that aquatic behaviours (swimming, aquatic auto-preening and aquatic feeding) were more frequent in warmer and sunny conditions, Zhang et al (2021) noticed the opposite, since swimming activity was higher on rainy days. Even though these studies were conducted in the United Kingdom and the Republic of Ireland, respectively, one might consider the geographic proximity of these countries to suggest similar climatic conditions, raising the possibility that the observed differences in behaviour might have been group-specific.

Visitor presence has additionally been mentioned as a potential factor influencing behaviour. Studies have shown that penguins' locomotion, preening, feeding and engagement with visitors were positively associated with visitor numbers (Zhang et al., 2021), and that penguin activity tended to decrease on days when visitors were absent (Goodenough et al., 2023).

Biological factors, such as the stages of the breeding phase, further shape behavioural patterns. Marshall et al (2016) found that Humboldt penguins spent significantly different proportions of their time in water depending on the breeding stage, with the penguins spending more time in water during the pre-nesting phase, which decreased during the nesting and post-nesting phases.

Population Status and Threats

As of the year 2000, Humboldt penguins have been classified as "vulnerable" on the International Union for Conservation of Nature (IUCN) Red List, as the current population trend is in decline (BirdLife International, 2020).

Population decreases among Humboldt penguins can be attributed to a variety of factors. These include the impacts of fisheries bycatch, especially in gillnets (Crawford et al., 2017), as well as entanglement in fishing equipment and consequential drowning (Simeone et al., 1999; Taylor et al., 2002). Additionally, the competition with the fishing industry for their primary prey species presents a potential risk to their population, particularly if overfishing occurs (Herling et al., 2005). Unsupervised guano harvesting (for

use as fertiliser) can negatively impact the *Spheniscus humboldti* population, either by destroying possible nesting sites or by disturbing nesting birds during the breeding season (Wallace & Araya, 2015). Added to this is the growth of ecotourism and the consequential repeated human visitation that may lead to nest abandonment, potentially causing breeding failure (Ellenberg et al., 2006). Moreover, unattended eggs are vulnerable to predation by rats and Kelp Gulls (Simeone & Luna-Jorquera, 2012), and penguins may be susceptible to other predators, such as foxes and domestic dogs (Schwartz et al., 1999). Another contributing factor is the scarcity of food during El Niño Southern Oscillation (ENSO) episodes (Culik, 2001), specifically during El Niño events, a phenomenon during which sea surface temperatures rise, resulting in the interruption of upwelling nutrient-rich water in the south-eastern Pacific Ocean and ultimately leading to a decrease in primary productivity, impacting the whole food web (Hays, 1986; Culik, 2001). Other related factors involve keeping bycatch Humboldt penguins for human consumption or sale (Alfaro-Shigueto et al., 2010), as well as egg harvesting (Trathan et al., 2015).

Keeping Animals Under Human Care

The discussion of whether to keep animals in captivity or not is a long and hard one. On the one hand, there are negative aspects of life in captivity that should not be ignored. As emphasised by Morgan & Tromborg (2007), the sources of stress in captivity are numerous as these environments are rich in sensory inputs that may be unfamiliar or even distressing to an animal. To quote, these include sound; light conditions; odours; thermal and tactile experiences; substrate properties; restricted movement; absence of retreat space; forced proximity to humans; aversive routine husbandry; restricted feeding and foraging opportunities; and abnormal social groups (Morgan & Tromborg, 2007).

On the other hand, animals under human care usually have suitable access to food and water, as well as medical care and safeguarding both from predators and conflicts, which is why they tend to have better health, longer lifespans, and higher reproductive success compared to many species living in the wild (Mason, 2010). Additionally, zoos play an important role in supporting conservation efforts, either *in-situ* (in the natural habitat) or *ex-situ* (outside the natural habitat) (Tribe & Booth, 2003). These measures include, for instance, rescuing endangered species, protecting and restoring habitats, reintroducing animals, conducting research, managing genetic diversity, captive breeding of vulnerable species, etc. (Tribe & Booth, 2003).

Captive breeding, in particular, can act as a form of insurance for vulnerable populations, protecting them from threats until they can be safely returned to their natural habitats (Conde et al., 2011). It involves introducing rare or endangered animals into captivity to create stable populations for future release into the wild or act as a genetic reservoir, to later boost diminishing populations or diversify lineages (Cohn, 1998; Williams & Hoffman, 2009). As an *ex-situ* approach, it is employed to safeguard species by providing controlled and protected environments where they can thrive and reproduce, thereby ensuring their ongoing survival. While a single zoo may have relatively few individuals of a specific species, the combined efforts of multiple zoos can support substantial populations of various species, including those facing significant threats (Conde et al., 2011). Nonetheless, the ethical consequences of keeping animals in captivity must not be overlooked while always bearing in mind that ensuring animal well-being is crucial.

As stated by Hill and Broom (2009), captivity offers animals varying niches and opportunities, or lack thereof, compared to those available in the wild. As such, animals should be kept in an environment as similar as possible to their natural habitats, so they can express species-specific behaviour, an effort that helps inhibit stereotyped behaviour (Keulartz, 2015) by ideally satisfying their behavioural and physical needs. In this sense, any information regarding an animal's natural history is crucial for understanding its needs and determining the environmental conditions necessary for its care in captivity (Wolfensohn et al., 2018).

The transformation of zoos from animal collections to conservation-focused parks reflects their evolution as educational establishments (Tribe & Booth, 2003). Modern zoos aim to present animals within the context of their natural habitats, rather than just showcasing them as curiosities, employing them in diverse educational roles (Tribe & Booth, 2003). Indeed, conservation and education have become focal themes in zoos' mission statements, emphasising how these elements are significant in zoo culture (Patrick et al., 2007). However, as Tribe and Booth (2003, p. 66) wrote "Zoos are still seen by some as being superficial, expensive, ineffective, and therefore indefensible" and added, "others are simply skeptical of the conservation claims of zoos, believing them to be merely window dressing".

Reaching a consensus on whether animals should be kept in captivity is undeniably challenging, with pros and cons on both sides. Nevertheless, it remains an important conversation that must be pursued if we want to keep making real and positive changes.

Objectives

This dissertation's objectives were defined in collaboration with SEA LIFE's Porto team. SEA LIFE Porto is committed to improving the welfare of their animals, with research being a key factor for such a goal. As such, it was hypothesised that implementing EE would increase the penguins' walking, exploratory, and swimming-related behaviours, contributing to the penguin's welfare, not only by helping to meet their ethological requirements but also by increasing their exercise levels, thus contributing to their physical and mental health.

To test this hypothesis, four objectives were defined:

- (i) Evaluation of SEA LIFE Porto's Humboldt penguins' daily activity patterns before the introduction of EE, and develop an ethogram specific to this group of animals;
- (ii) Apply the EE items based on the evaluation of past activity and investigate the penguins' behaviour in the presence and absence of EE devices;
- (iii) Assess if EE introduction increased enclosure use;
- (iv) Develop a practical training method for penguins that can be integrated into the daily routine of the SEA LIFE Porto team, to facilitate husbandry and veterinary procedures.

Study Location and Subjects

Study Site

This research took place throughout the school year of 2023/2024, between October 2023 and June 2024, at SEA LIFE Porto (41°10'07.2"N, 8°41'14.9"W), one of the few aquariums in northern Portugal.

Open since 2009, SEA LIFE Porto is located in the city of Matosinhos, district of Porto, and it is one of the SEA LIFE Centres in Europe. Belonging to Merlin Entertainments, which owns at least 50 aquariums and 2 sanctuaries scattered around the world, SEA LIFE Centres are dedicated to promoting awareness and appreciation for the ocean and its inhabitants, in addition to spreading the message about the importance of their conservation [Merlin Entertainments (SEA LIFE), n.d.].

SEA LIFE Porto has more than 3000 marine creatures (Merlin Entertainments, 2024a), many of them face the threat of extinction, have been saved but are unable to be released back into their natural habitats, or were born and raised in other SEA LIFE Centres as part of the groups' conservation initiatives (Merlin Entertainments, 2024b). Some of such creatures are the *Spheniscus humboldti* penguins, a species regarded to be decreasing in the wild (BirdLife International, 2020; IUCN, 2023). As an endangered species, aquariums such as SEA LIFE play a vital role in teaching the public about the threats that these penguins face in their native habitat and what preventive measures can be taken.

Study Area

The penguins' exhibition features both indoor and outdoor sections. The outdoor space has an area of 66 m² and is furnished with some rocks, pebbles, sand, and a pool (Figure 4). The pool covers 39 m² and has a depth that gradually increases from 1 m to 1.80 m, holding a total volume of 60 m³ (Figure 4). The indoor section, also referred to as the technical area, has a quarantine space, nests, and a room where the penguins' food is prepared. Along the wall adjacent to the technical area are entrances to seven nests, each intended for a pair of penguins, although some pairs may occupy multiple nests (Figure 4).



Figure 4. SEA LIFE Porto penguins' exhibition.

Each nest is 80 cm in length, 60 cm in width, and 70 cm in height, resulting in an area of 0,48 m². The floor of the nest has a perforated carpet to allow their excrement to pass through, preventing buildup and helping to maintain a cleaner and more hygienic environment for the penguins.

The nests are always open, allowing the animals to go in and out, at any time of the day. Maintenance of the nests consists of deep cleaning, replacement of the rubber mats for clean ones, and daily disinfection with the product Safe4 (<https://safe4disinfectant.com/store/disinfectant-products/10l-disinfectant-concentrate>). The outside display and pool are washed and scrubbed daily, with a diver entering the pool once a week to remove more stubborn algae from the wall. Both the pool and the nests are kept at ambient temperature.

The front and sides of the display are made of transparent glass, allowing the public to view and interact with the penguins while preventing physical contact. There is also a net covering the entire exhibit to prevent predators from entering.

The Penguins of SEA LIFE Porto

SEA LIFE Porto has a group of 8 adult penguins: 4 males—Tommy, Beaker, Bramble, and Pumpkin—and 4 females—Hickory, Luna, Baobab, and Pip. They were all born in captivity in England, the Netherlands, and Belgium and brought to Porto's exhibition in 2019.

The penguins are fed twice daily: once in the morning around 9 AM, before the centre opens to the public, and again at 4:30 PM. During the morning feeding, the penguins have been trained to enter the technical area and step onto a scale. This routine ensures that when the monthly weighing occurs, the penguins willingly step onto the scale, eliminating the need for the keepers to chase them around the exhibit. The afternoon group feeding is held in the outdoor area, where the penguins engage in friendly competition for their food, i.e. without showing aggressive behaviours before, during and after feeding. In both feedings, the penguins are hand-fed capelin (*Mallotus villosus*) or sprat (*Sprattus sprattus*). During the weekdays they are visible to the public from 10 AM to 6 PM and on the weekends from 10 AM to 7 PM.

The biology team at SEA LIFE Porto regularly examines the penguins' plantar region for any signs of lesions, such as ulcerative pododermatitis (bumblefoot), and monitors their weight to detect any extreme fluctuations, ensuring that any variations fall within the normal range for the species and this specific group.

Like in the wild, penguins under human care live in pairs. At SEA LIFE Porto 4 couples have formed over the years. Tommy and Hickory are the most recent couple (*Figure 5*). Tommy, the oldest penguin of the group, is a male born in 2015 at SEA LIFE Scheveningen. He couples with Hickory, the youngest female of the cluster, born in 2019 at SEA LIFE Weymouth. One of Tommy's curious characteristics is that at every afternoon feeding he picks up one or two fishes that have fallen on the floor and takes them to his nest. As a particularly responsive penguin, Tommy frequently interacts with its keepers, displaying a strong curiosity to explore its surroundings and investigate new objects. Hickory, in turn, often interacts with visitors by following their shadows or movements on the glass but is more wary and less inclined to interact with other animals or its keepers.



Figure 5. Tommy (male, 9 years old) and Hickory (female, 5 years old) at SEA LIFE Porto.

Luna is a seven-year-old female born in 2017 at SEA LIFE Scheveningen. It mates with Beaker, a male from SEA LIFE Scarborough born in 2016 (*Figure 6*). Beaker prefers living a quieter life, only making a fuss when someone trespasses on their nest territory, where it becomes highly protective. In contrast, its partner Luna is one of the few penguins that actively interacts with visitors, chasing their shadows or reflections created by cell phone screens and watches. Maybe due to this curious behaviour, Luna is easily distracted, often losing focus on the task at hand (or, in this case, the task at flipper).



Figure 6. Beaker (male, 8 years old) and Luna (female, 7 years old) at SEA LIFE Porto.

Pumpkin and Pip are a couple both from SEA LIFE Blankenberge, born in 2016 and 2017, respectively (*Figure 7*). Pumpkin is generally a calm male during feedings but occasionally pecks staff members who enter the display area outside of feeding times. For example, when someone enters the exhibition for the morning lecture, Pumpkin often follows the lecturer around, pecking at their feet and legs. It is a very observant penguin, frequently using the behaviour and interactions of other penguins as a guide to adjust its own actions, appearing to learn through observation. Pip is a highly energetic penguin, particularly noticeable during feeding times, when it regularly pecks at the keepers' legs, seemingly trying to attract their attention so she may be fed faster.

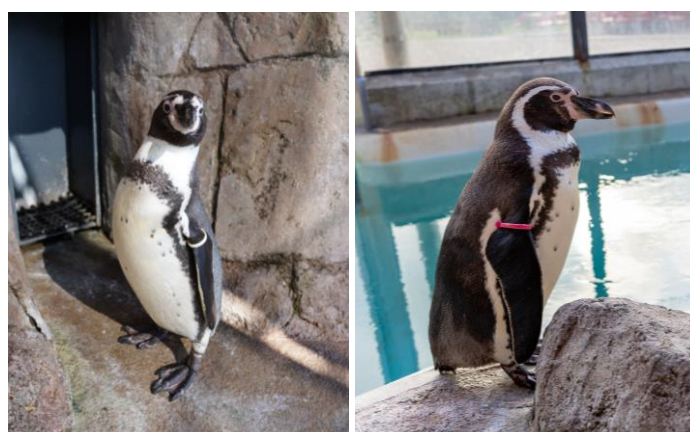


Figure 7. Pumpkin (male, 8 years old) and Pip (female, 7 years old) at SEA LIFE Porto.

Bramble and Baobab, both from SEA LIFE Weymouth, are the calmest couple in the centre (*Figure 8*). Baobab, a female born in 2017, and Bramble, a male born in 2018, are also the centre's couple most attached to each other, as usually where one goes, the other follows. Bramble is frequently seen entering other birds' nests to collect pebbles for its own nest, often during feeding times when other penguins are distracted trying to get food. Baobab, for its part, is a cautious penguin, typically waiting until the other birds have been

fed before approaching the keepers; or remaining in a quieter part of the exhibit, away from the group's commotion, waiting for the food to go to her.



Figure 8. Bramble (male, 6 years old) and Baobab (female, 7 years old) from SEA LIFE Porto.

Even though the Humboldt penguins are one of SEA LIFE Porto's most recent attractions, they are quite popular with visitors, who often go there on purpose to view them.

Chapter 1: Assessing the Impact of Environmental Enrichment

1. Introduction

In the wild, animals devote much of their time and energy to finding food and locating mates, while staying vigilant against predators (Anderson & Wood, 2001). In captivity, however, food and mates are generally easy to access, and the threat from predators is significantly reduced or eliminated, which can result in the animals lacking the activities that would typically occupy their time (Anderson & Wood, 2001). EE, formerly succinctly defined as the addition of complexity to a habitat that provides overall benefits to an animal (Kresnye et al., 2022), is a strategy designed to encourage behaviours, interactions and activities that mirror those that the animals would have in the wild while reducing potential stress and preventing abnormal or harmful behaviours. EE is arguably one of the most effective tools available for enhancing the welfare of an individual, and its use is gradually becoming more common as a proactive approach to fostering an enriching and engaging environment (Maple & Perdue, 2013).

While routine enrichment practices have become part of the standard care of animals in captivity (Kresnye et al., 2022), implementing EE presents challenges and limitations. Even though it requires careful consideration of species-specific behaviours and an animal's natural history, the reality is that detailed information about the behaviour and activity patterns of wild populations is frequently lacking (Mellen & Sevenich MacPhee, 2001) or is insufficiently documented. Furthermore, individual differences in personality among animals suggest that their responses to enrichment may vary substantially (Sroka et al., 2024). As a result, the same EE that may be enriching and beneficial for one animal could have the opposite effect on another. It is likewise important to recognise that EE does not always achieve its intended objectives (e.g., Carlstead et al., 1991; Swaisgood & Shepherdson, 2005; O'Brien & Cronin, 2023) and may carry inherent risks (Coleman & Novak, 2017; e.g., Marashi et al., 2003; Seier et al., 2005).

Over the years, a few studies have researched the impact of EE on Humboldt penguins and other penguin species under human care. Some of the positive outcomes reached, depending on the implemented enrichment methods, included increased overall enclosure use (measured in entropy), with a significant rise in the total variability of enclosure use in the presence of enrichment (Fernandez et al., 2021), as well as higher swimming and activity levels (Fernandez et al., 2021; Razal & Miller, 2021). As noted by Reisfeld et al. (2013), spending more time in the water can, for instance, aid in reducing pododermatitis

lesions, a positive outcome that enhances the health and well-being of animals. In Fiordland penguins (*Eudyptes pachyrhynchus*), a music concert series – with a stage located approximately 300 m from the penguin exhibit – led to changes in behaviour and habitat use (Fanning et al., 2020). Even though there was insufficient evidence to confirm if concerts might have caused behavioural responses related to stress, an overall increase in pool and nest use was noticed on concert days, alongside a decrease in preening and habitat interactions, with variations between individual penguins (Fanning et al., 2020). Visitor presence might have influenced results, although supplementary research is necessary to better comprehend its effect on the animals' welfare (Fanning et al., 2020). Nonetheless, according to the authors, the Fiordland penguins showed no notable signs of fear or avoidance related to visitors, as the penguins did not retreat to out-of-sight areas during visitor hours and were observed using the pool more frequently in the afternoons of concert days compared to the evenings, despite higher visitor presence (Fanning et al., 2020).

Providing favourable stimuli to animals in captivity, capable of evoking positive emotions, is of equal importance to the obligation of preventing or reducing distress (Arechavala-Lopez et al., 2021). Although EE is known for its significant impact on animal behaviour, it is crucial to ensure that these changes are beneficial and contribute to the animals' overall well-being. It is therefore especially important to have a deep understanding of the specific species being studied, in order to tailor enrichment strategies accordingly. Regarding penguins, particularly *Spheniscus humboldti*, our understanding of their captive behaviour, both with and without EE, remains limited. For example, more information is needed to explore how their seasonal cycle influences their responsiveness to EE, to determine which types of EE are most effective and when, to understand whether the species requires varying levels of stimulation throughout the day, to assess the long-term effects of enrichment programs, etc.

The research conducted at SEA LIFE Porto aimed to assess the daily activity patterns of Humboldt penguins in the absence of EE, and to investigate how the introduction of EE would impact their behaviour. Additionally, this study sought to determine whether EE devices would encourage walking, exploratory, and swimming behaviours while promoting greater use of the display area.

2. Materials and Methods

2.1. Preliminary Observations

Ad libitum observations were performed from October 4th to the 27th, in 2023. Excluding weekends and holidays, 43 observations with arbitrary duration were made for 17 days, totalling 67 hours and 46 minutes. The primary goals of these observations were to become familiar with the groups' behavioural patterns and to develop an ethogram specific to the animals under study, which was later used for further analysis.

A total of 63 behaviours were identified and are detailed in *Table 1*, which served as a basis for the systematic observations. It was refined throughout and until the end of all observations (*ad libitum* and systematic), incorporating not only my observations but also those of the keepers, and supplemented with valuable information obtained from literature research (Eggleton & Siegfried, 1979; Merritt & King, 1987; Razal & Miller, 2021; Zhang et al., 2021). *Appendix B* provides a more detailed description of each behaviour.

Table 1. Categories and descriptions of behaviours displayed by the Humboldt penguins during observations.

Category	Description	Behaviours
Maintenance	Behaviours related to self-care involving grooming, elimination of waste products from the body, and other physical and physiological necessities	Preening; Stretching; Flipper flapping; Shaking; Scratching; Sneezing; Excretion; Yawning; Coughing; Head rub
Stationary	Behaviours performed while the penguin remains stationary	Lying down; Standing up; Crouching; Slender posture; Evasive posture; Standing with the neck retracted
Locomotion on land	Behaviours that enable the penguin to move from one site to another on land	Walking; Running; Hopping; Slender walk; Retracted walk
Postural transitions	When the penguin changes its body posture	Motion of lying/looking down; Motion of standing/looking up
Swimming	Any behaviours that involve the penguins going into, remaining, and getting out of the pool	Diving; Surface swimming; Underwater swimming; Porpoising; Swimming on their side/back; Getting out of the pool

Table 1 (continued).

Category	Description	Behaviours
Social	Behaviours performed in conjunction with or directed to other individuals	Allopreening; Calling; Ecstatic display; Soundless ecstatic display; Flipper patting; Mounting; Lying down together; Rhythmical vocalisations; Bowing; Chasing; Neck intertwine
Agonistic	Aggressive or warning behaviours directed toward other individuals	Staring; Beaking; Boddy jabbing; Biting; Beak slapping; Threatening vocalisations
Environmental interaction	Behaviours involving interactions with the habitat	Observing; Touching; Lifting; Nudging; Tugging; Vocalising; Approaching; Exploratory walking
Human-animal engagement	Interactions that occur between humans and the penguins	Engaging with visitors; Engaging with keepers/aquarium staff
Feeding	Interactions that occur between penguins during feeding sessions, as well as physical and physiological responses	Individual; Group; Regurgitation
Nesting	Behaviours associated with nest building, as well as behaviours performed exclusively within the nest	Transporting materials into the nest; Nest scraping; Vibratory head shake
With no apparent motive or function	Behaviours with motivations or functions that are not yet fully understood	Nests' mat lifting and dropping
Imperceptible	The penguin is visible but its behaviours are not discernable	
Not visible	The penguin is out of sight, staying inside the nest or in the camera's blind spots	
Other	Any behaviour not listed in the previous categories	

2.2. Systematic Observations

Systematic observations occurred for about 7 months, from the 7th of December 2023 until the 27th of June 2024. Observations occurred without EE from the 7th of December

2023 to the 10th of January 2024, and randomly alternated with and without EE from the 22nd of January 2024 until the 27th of June 2024.

The penguin's behaviour was filmed with and without EE items using the camera Tapo C-310 version 1.0 with a 126 GB microSD card. The camera was placed at the exhibit's outer left corner at approximately 4 metres of height, to film as much of the display as possible from a single angle. To prevent any diurnal effects, recordings were made at the same time frame each day at 10:30-11:30 AM and 3-4 PM. The enrichment devices were randomly alternated at the authors' discretion, along with the time of day they were introduced (either morning or afternoon). This approach aimed to prevent habituation and maintain the novelty factor while minimising learning effects.

Factors such as enclosure use, behavioural diversity, and the presence or absence of abnormal behaviours (atypical behaviours that deviated from the penguins' behavioural repertoire such as stereotypies and increased aggression) were assessed. In total, 180 recordings were made and 42 evaluated, each lasting 15 minutes. These specific periods were randomly selected from the two previously defined time windows, using *Microsoft Excel* software for randomisation.

The observation period was divided into three stages to encompass the different stages of the reproduction and nesting phase: before the onset, the first part, and the second part; however, due to logistical limitations, the introduction of EE was delayed and could only begin in late January 2024. For each phase and condition (without EE and with EE), one video was selected from the beginning, middle, and end of each month. This strategy was employed to account for potential temporal variations in the animals' responses to EE, thereby capturing possible behavioural changes over time.

1. From December 2023 to January 2024

A total of six videos were analysed, without enrichment: three from December and three from January (covering the beginning, middle, and end of each month).

2. From February to March 2024

Eighteen videos were analysed in total

- Without EE: three videos from February and three from March (covering the beginning, middle, and end of each month)

- With land-based EE: three videos from February and three from March (covering the beginning, middle, and end of each month)
- With aquatic-based EE: three videos from February and three from March (covering the beginning, middle, and end of each month)

3. From April to May 2024

A total of 18 videos were analysed

- Without EE: three videos from April and three from May (covering the beginning, middle, and end of each month)
- With land-based EE: three videos from April and three from May (covering the beginning, middle, and end of each month)
- With aquatic-based EE: three videos from April and three from May (covering the beginning, middle, and end of each month)

The enclosure was divided into distinct zones (*Figure 9*), each based on the usefulness of the space for the animals, to monitor their use of the different zones. Zone A (2.92 m²) was designated as the area immediately in front of the nests. Zone B (21.76 m²) was defined based on the location of the pebbles the penguins collected and took to their nests. Zone C (6.91 m²) was identified as the area on land where the penguins normally stood for the outside feeding. Zone D (28.23 m²) encompassed the area immediately in front of the high windows and any other spaces not occupied by the previous zones. Finally, the pool was classified as Zone E (38.88 m²), as it was the only water-filled area within the exhibit.

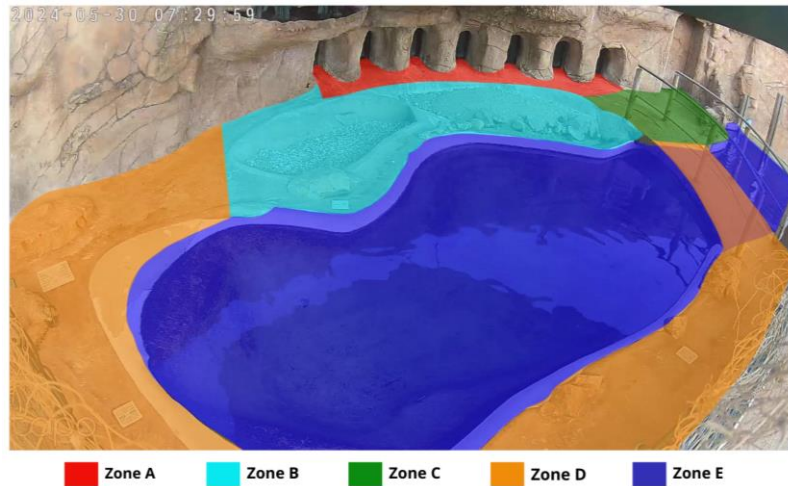


Figure 9. Division of the penguins' enclosure into different zones, taking into account their use by the penguins. **Zone A (2.92 m²)** – The area directly in front of the nests; **Zone B (21.76 m²)** – The sandbank and the area beneath and in front of the inactive waterfall, where the pebbles the penguins collected and took to their nests were located; **Zone C (6.91 m²)** – The space from the display entrance to the pool, including part of the bridge and the remaining area between Zones A and B; **Zone D (28.23 m²)** – The remaining land area of the exhibition; **Zone E (38.88 m²)** – The exhibition pool area.

2.3. Chosen Environmental Enrichment Devices

Over generations, as populations accommodate to captivity, their genetic structure may shift towards increased sedentarism and decreased wariness (Newberry, 1995). Providing proper EE can help extend the animal's behavioural repertoire and enhance overall activity. As such, the primary goals in implementing EE devices were A) to enhance the frequency of the penguins swimming in the pool, with an additional aim of extending their swimming duration, and B) to increase walking and exploratory behaviours, thereby expanding the area of the exhibit actively used by the penguins; with mental stimulation being a byproduct of this process.

Each device was chosen based on observations of the birds' behaviour in captivity compared to their behaviour in the wild. This ensured that the select devices were tailored to satisfy the ethological needs of the animals. This decision-making process was based on a thorough literature research that was later compared with insights shared by SEA LIFE Porto's keepers and information from various aquariums and zoos. Throughout the process, the penguins' varied sensory, cognitive, and physical capabilities were taken into account. *Table 2* details the chosen EE devices, highlighting the objectives and purposes behind

their selection, while specifying the zones in the exhibit where they were placed. Photos of the enrichment devices used can be found in *Appendix C*. The devices were presented a maximum of 5 times per week and were available on the display for 30 minutes to 1 hour. Exceptionally, the enrichment devices were occasionally left in the exhibit for more than an hour due to unforeseen circumstances, such as adverse environmental conditions or during educational lectures, to avoid disruption.

Table 2. Overview of the selected environmental enrichment devices, including their categories, functions, purposes, and placement within the exhibition.

Environmental Enrichment Device	Type of Environmental Enrichment	Function	Purpose	Exhibition Placement
Sprinkler	Physical Sensorial	Create the effect of rain on the pool's surface	Mimic natural aquatic conditions by creating a more dynamic and interactive environment, enhancing the animals' interest in the water, resulting in increased frequency and duration of swimming	Placed in Zone D, but most of the water droplets fell into Zone E.
Aquatic aerator	Physical Sensorial	Form bubbles or a water current in the pool, to make the water column more turbulent		Zone E
Artificial seaweed at the bottom of the pool	Physical Sensorial	Add areas with vegetation where penguins can swim through		Zone E
Artificial seaweed and a small net with fish, suspended from the bridge into the water	Dietary Physical Sensorial	Create an opportunity for the penguins to forage for food		Zone E

Table 2 (continued).

Environmental Enrichment Device	Type of Environmental Enrichment	Function	Purpose	Exhibition Placement
Other nesting materials, including artificial algae and leaves	Physical	Introduce different materials throughout the exhibition that can be used for nesting, in addition to the existing pebbles	Encourage nest-building behaviours while increasing exploration and exercise time	Zones A, B, C and D
Artificial plant	Physical Sensorial	Incorporate additional hiding spots within the display, allowing the animals to remain hidden without necessarily having to stay inside their nests	Provide the birds with greater control over their environment by adding extra hiding spots beyond their nests, allowing the penguins to better manage their exposure while in the exhibition	Zones B and D
Replica of the Peruvian booby bird (<i>Sula variegata</i>¹), combined with audio of its calls	Social Sensorial	Add replicas of birds found in their natural habitat, complementing them with a chirping sound	Recreate natural ecological interactions with other species, beyond human interaction	Zones A, B and D
Feeding balls with fish, pebbles, artificial algae and a rattle	Occupational	Add novel objects in the exhibition that increase the animals' problem-solving skills	Promote mental stimulation by requiring the animals to interact with the balls and understand how to use their physical abilities to access or be able to hear the objects inside	Zone A and B

¹ When selecting a species as a reference for the replicas, the focus was on choosing animals that do not pose a threat to Humboldt penguins in the wild, thereby minimising the risk of the replicas introduced into their habitat being a potential source

of discomfort. Ultimately, a species known for being one of the primary guano producers was selected (Weimerskirch et al., 2012). The species sounds were obtained via Xeno-Canto website, using recordings from Cristian Pinto Fernandez (XC727033, accessible at www.xeno-canto.org/727033; XC727034, accessible at www.xeno-canto.org/727034) and Ignacio Escobar (XC626094, accessible at www.xeno-canto.org/626094), both under the license Creative Commons Attribution-Non Commercial-Share Alike 4.0 License (<https://creativecommons.org/licenses/by-nc-sa/4.0/>). No changes were made to the recordings.

It is important to note that the enrichments “artificial algae, and a small net with fish suspended from the bridge into the water”, and “feeding balls containing fish, pebbles, artificial algae, and a rattle” were introduced only two times each, from June 2024 onwards. Consequently, these enrichments were not included in the analysed observations. Additionally, the sound of the Peruvian booby bird was introduced via a mobile speaker placed near the exhibit, out of the animals' sight. However, on rainy days the speaker could not be used, and only the replicas were utilised in those instances.

2.4. Behavioural Analysis

All 42 fifteen-minute recordings were analysed using *The Observer XT 11.5* software by Noldus. Since identifying the individual penguins in the recordings was impossible, an instantaneous scan sample of the penguin group was done once every minute. The identified behaviours were classified into 15 categories: maintenance; stationary activities; locomotion on land; postural transitions; behaviours associated with swimming; social interactions; agonistic behaviours; interactions with the environment; human-animal engagement; nest-building activities; feeding; behaviours with no apparent motive or function; imperceptible behaviours, when the specific behaviour could not be discerned; other behaviours; not visible, such as when the animals were inside their nests or in camera blind spots; and other behaviours.

The penguin exhibit was divided into zones based on their biological relevance (*Figure 9*). The areas of each zone were determined using the *Autodesk AutoCAD* software, and the Electivity Index (Vanderploeg & Scavia, 1979) was calculated to examine enclosure use. This index is particularly useful for assessing the significance of specific resources to animals (Brereton & Fernandez, 2022). It can be used in zones of different sizes, can detect whether resources or individual zones within an exhibit are being overused or underused (Brereton, 2020; Brereton & Fernandez, 2022), and should be applied, for example, in projects where enrichment is of interest (Brereton, 2020). This index produces a value that

ranges from -1, indicating underutilisation, to 1, representing overutilisation of each zone (Brereton, 2020; Brereton & Fernandez, 2022), while a value of 0 indicates that a zone is being used proportionally to its size (Brereton & Fernandez, 2022). It was calculated using the following formulas:

$$E = \frac{W_i - (1/n)}{W_i + (1/n)}$$

$$W_i = \frac{r_i/p_i}{\sum r_i/p_i}, \text{ where } p_i = \frac{\text{Area of a zone}}{\text{Total area of all zones}}$$

E = Electivity index

n = number of zones

r_i = observed use of a zone (number of sightings in each zone)

p_i = expected use of a zone, if all zones had been utilised evenly, considering their varying sizes

2.5. Statistical Analysis

As it was impossible to distinguish individual penguins, the number of visible penguins and their behavioural categories were recorded for each minute of a 15-minute observation period. The total percentages were calculated, i.e., the percentage of visible penguins out of the eight under study, and the percentages for each behavioural category, based on the visible penguins.

For each category, the median and the 25th and 75th percentiles were calculated, given the asymmetry of the distribution of values, and box-and-whisker plots were presented with the distribution by days of observation and season.

The differences between the situations with and without enrichment were tested using a paired samples t-test, assuming that the distribution of differences around zero did not deviate too far from normality. The differences were studied by category of behaviour and by month. Calculations were carried out using *IBM SPSS 28*, with results considered significant if $p < 0.05$

3. Results

3.1. Activity Budget

The percentage of visible penguins per observation visually lowered across the three periods analysed (*Figure 10*). The highest percentage of visible penguins was observed on the 7th of December 2023, the 11th of January 2024 and the 1st of February 2024 (*Figure 10*). From February to March 2024, the percentage of visible penguins per observation remained relatively consistent, however, the variability of the percentage of penguins visible per observation decreased compared to the previous period (*Figure 10*). The lowest percentage of visible penguins was observed in the April to May 2024 period (*Figure 10*).

The overall trend suggests a decline in visibility from December until April.

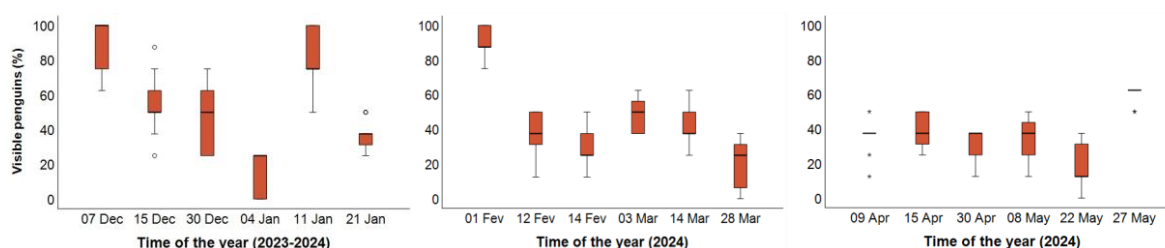


Figure 10. Percentage of visible penguins from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers.

The percentage of penguins engaged in maintenance behaviours remained visually low in December, but it increased by the end of the month before declining again in January to nearly 0% (*Figure 11*). The wide interquartile range on December 30th indicated considerable variability in the percentage of penguins exhibiting these behaviours on this particular observation (*Figure 11*). From February to March 2024, the percentage of penguins engaged in maintenance behaviours stayed visually low (*Figure 11*). The months of April and May were marked by high variability in the percentage of penguins exhibiting maintenance behaviours per observation, with a peak having occurred on the 9th of April (*Figure 11*).

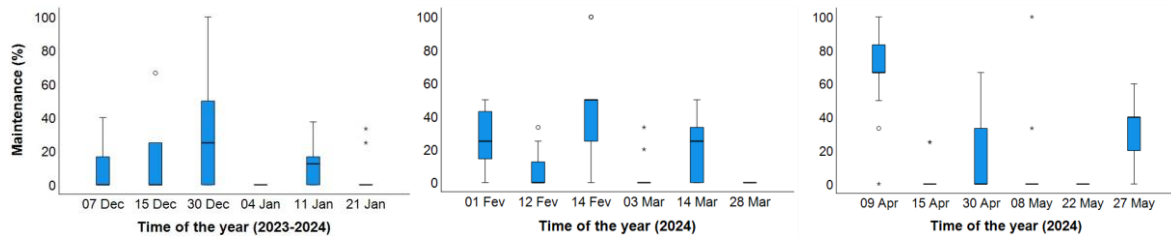


Figure 11. Percentage of visible penguins engaging in maintenance behaviours from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers.

The percentage of penguins engaging in stationary behaviours fluctuated over time between December 2023 and April 2024. During the December-January period, the percentage of visible penguins engaged in stationary behaviours was generally higher (Figure 12). Although there was a slight decline in this category of behaviours from February to March, this period exhibited the highest variability in the percentage of penguins exhibiting this behaviour per observation (Figure 12). In April-May, the percentage of visible penguins engaged in stationary behaviours further decreased, particularly in early April (Figure 12).

Overall, the results indicated a decline in the percentage of visible penguins exhibiting stationary behaviours from December 2023 to April 2024.

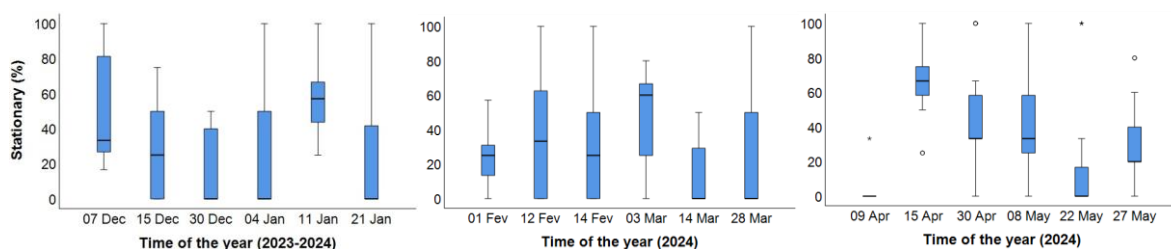


Figure 12. Percentage of visible penguins engaging in stationary behaviours from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers.

From December 2023 to January 2024, the percentage of visible penguins engaging in locomotion on land behaviours per observation was visually relatively low (Figure 13). Notably, variability in the percentage was particularly high on the 21st of January. From

February to March 2024, the percentage of visible penguins engaging in locomotion on land behaviours increased slightly (*Figure 13*). The variability in the percentage of penguins exhibiting these behaviours per observation was the highest during this period (*Figure 13*). The period from April to May contrasted with earlier months, as the percentage of penguins engaging in locomotion on land behaviours declined to nearly 0% in most observations, except on the 8th of May and the 22nd of May (*Figure 13*).

While the percentage of penguins exhibiting locomotion land behaviours was higher from February to March 2024, the overall trend indicates consistently low activity throughout seasons (*Figure 13*).

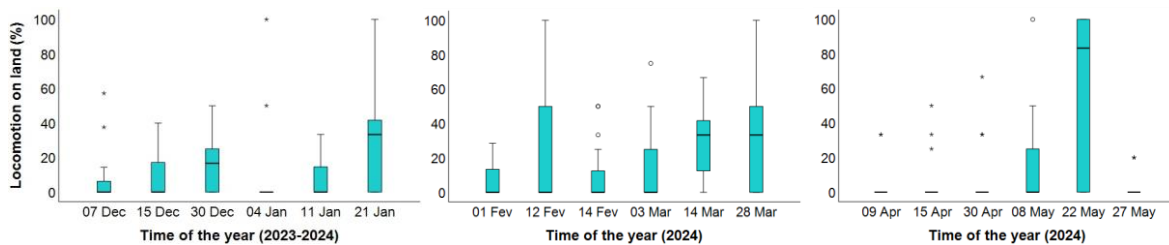


Figure 13. Percentage of visible penguins engaging in locomotion on land behaviours from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers. The star symbol represents extreme outliers while the circle represents mild outliers.

The percentage of visible penguins engaging in swimming behaviours remained visually low across the period of December 2023 to April 2024 (*Figure 14*). Two peaks of swimming behaviours occurred on the 15th and 30th of December, although the overall trend for this period suggested the majority of penguins were not exhibiting behaviours related to this category (*Figure 14*). This trend continued through most observations from February to March 2024 (*Figure 14*). An increase in the percentage of visible penguins engaged in swimming behaviours was observed in early April; however, this percentage dropped back to near 0% for the remainder of the month (*Figure 14*). In general, swimming behaviours remained uncommon.

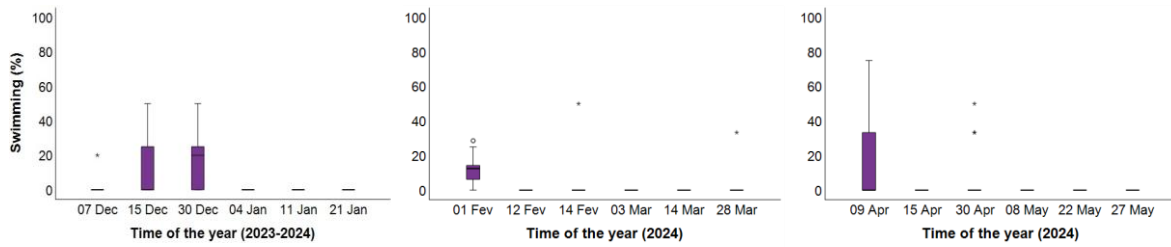


Figure 14. Percentage of visible penguins engaging in swimming behaviours from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers.

The percentage of visible penguins exhibiting social behaviours displayed trends similar to those observed in the previous category. Aside from three peaks of this percentage on December 15th, February 12th, and March 3rd, the percentage dropped to nearly 0% in the remaining observations (*Figure 15*). Overall, social behaviours were uncommon.

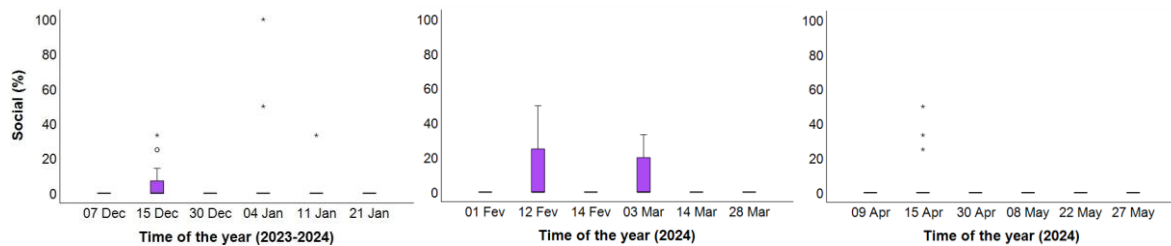


Figure 15. Percentage of visible penguins engaging in social behaviours from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers.

From December 2023 to early January 2024, the percentage of penguins engaging in environmental interaction behaviours was visually low, with almost no recorded interactions (*Figure 16*). In contrast, from February to March 2024, there was an increase in the percentage of penguins participating in environmental interaction behaviours. However, the wide interquartile ranges indicated significant variability in the percentage of penguins exhibiting this behaviour per observation (*Figure 16*). April and May were characterised by a new decline in the percentage of penguins engaging in environmental interaction behaviours (*Figure 16*). Except for May 8th and May 27th, the percentage of penguins exhibiting behaviours belonging to this category was near 0% (*Figure 16*).

Overall, the percentage of visible penguins exhibiting environmental interaction behaviours revealed fluctuating levels of engagement.

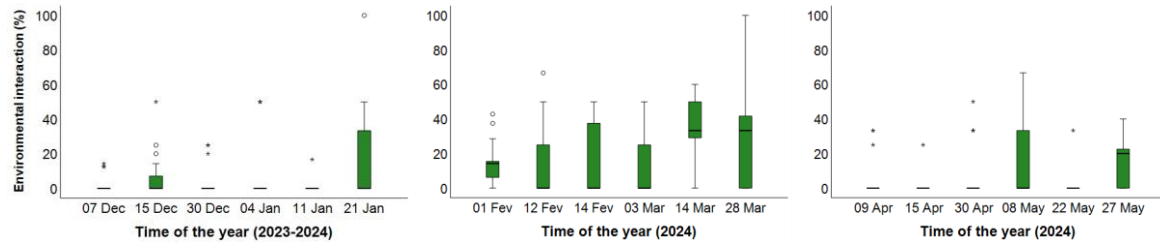


Figure 16. Percentage of visible penguins engaging in environmental interaction behaviours from December 2023 to April 2024. Each box represents the interquartile range (25th to 75th percentile), the horizontal line within the box indicates the median, and the "whiskers" correspond to the minimum and maximum values within the interquartile range. The star symbol represents extreme outliers while the circle represents mild outliers.

The other ethogram categories (*Table 1*) not presented here indicate that either their behaviours were not observed at all, or that their occurrences were extremely rare. No abnormal behaviours were detected.

3.2. Effects of Environmental Enrichment

From February to March, in the context of EE devices placed on land, the Maintenance behavioural category (*Figure 17A*) revealed a significant decrease in behaviours with the presence of EE devices [$t(77) = -3.031$, $p = 0.003$].

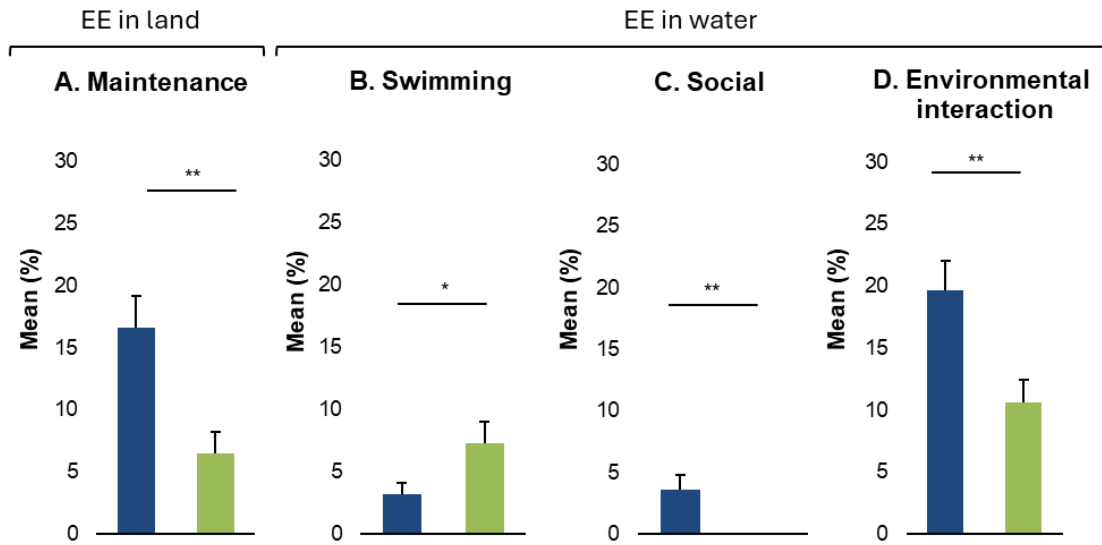
During the same period, significant differences were observed in the mean percentage of behaviours related to EE devices placed in water. The Swimming behavioural category (*Figure 17B*) showed a significant increase in behaviours with enrichment [$t(85) = 2.048$, $p = 0.044$]. Conversely, the Social behavioural category (*Figure 17C*) demonstrated a significant decrease in behaviours in the presence of enrichment [$t(85) = -3.271$, $p = 0.002$]. Similarly, the Environmental interaction category (*Figure 17D*) exhibited a significant decrease in behaviours with the presence of enrichment [$t(85) = -2.678$, $p = 0.009$].

Significant differences were noted in the mean percentage of behaviours across several categories from April to May, particularly regarding the presence of EE devices placed on land. The Maintenance behavioural category (*Figure 17E*) demonstrated a

significant decrease in behaviours with the presence of EE devices [$t(81)=-5.047, p< 0.001$]. Similarly, the Swimming behavioural category (*Figure 17F*) exhibited a significant decrease in behaviours with the presence of enrichment [$t(81)=-2.794, p=0.007$]. In contrast, the Environmental interaction category (*Figure 17G*) indicated a significant increase in behaviours with enrichment [$t(81)=2.571, p=0.012$]. Lastly, the Nesting behavioural category (*Figure 17H*) showed a significant increase in behaviours with the presence of EE devices [$t(81)=3.002, p=0.004$].

Additionally, the introduction of EE resulted in some behavioural diversity among the penguins, with new behaviours being observed. These included friendly competition for nesting materials (without showing aggressive behaviours before, during and after interactions), as several penguins were observed tugging at the same piece of artificial seaweed or attempting to steal seaweed and leaves from one another's beaks; and diving into the pool to explore and interact with EE devices placed at the bottom, although this behaviour was seen only once.

February and March



April and May

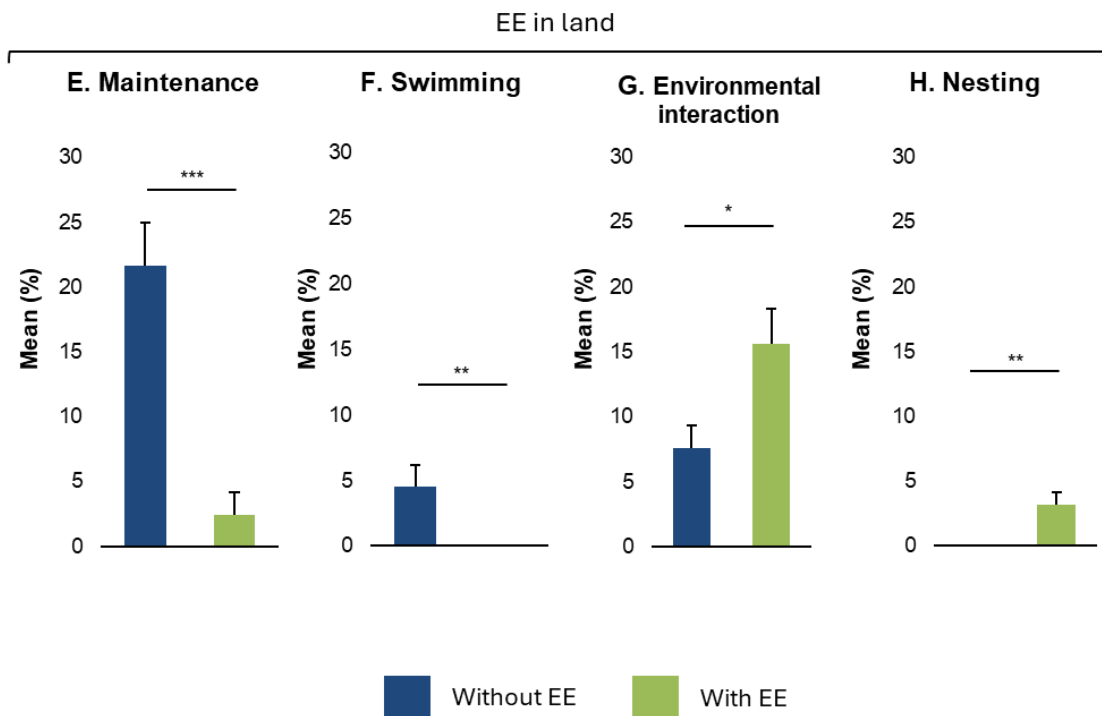


Figure 17. Effect of environmental enrichment: Comparison of significant differences in the mean percentage of behavioural categories (A-H) based on the absence (represented in blue) or presence (represented in green) of environmental enrichment (EE). Results are distinguished by the type of enrichment (aquatic or land-based). Further distinctions are made between months: February and March 2024 and April and May 2024. Statistical significance is indicated, with asterisks representing the level of significance where $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

3.3. Enclosure Use

Analysis of the enclosure use revealed consistent patterns across different conditions. Zone A (the area directly in front of the nests) consistently exhibited overutilisation (*Figure 18*) regardless of the month, the presence or absence of enrichment, or the type of enrichment (aquatic or land-based). In contrast, Zones B (the sandbank and the area beneath and in front of the inactive waterfall), D (the remaining land area of the exhibition), and E (the exhibition pool area) were consistently underutilised relative to their respective sizes (*Figure 9*).

Between December 2023 and March 2024, Zone C (the area on land where the penguins normally stood for the outside feeding) usage was more proportional to its size, as well as from February to March 2024 particularly during periods when aquatic enrichments were present (*Figure 18*). However, during the same timeframe, Zone C was overutilised in the absence of EE devices and underutilised when land-based enrichments were available (*Figure 18*).

From April to May 2024, Zone C was consistently underutilised (*Figure 18*), irrespective of whether enrichment was present and regardless of the type of enrichment applied (land-based or aquatic; *Table 2*). Zone E was rarely used.

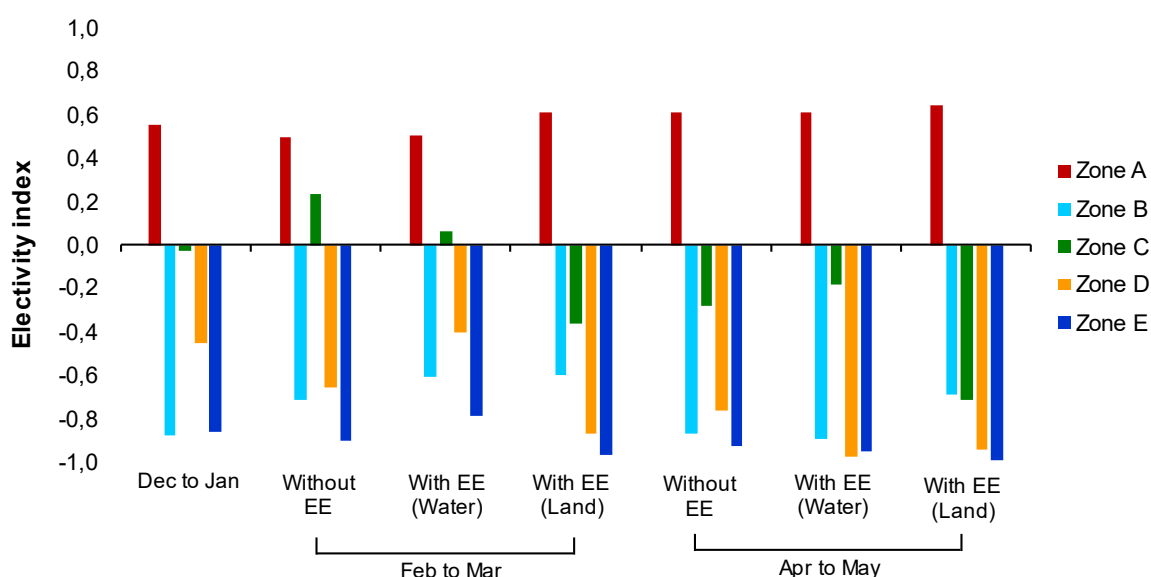


Figure 18. Variation in the Electivity index according to the different display zones (A-E), as described in *Figure 9*, and time of the year (December 2023 to January 2024, February to March 2024 and April to May 2024). A distinction is made between the presence and absence of environmental enrichment (EE) and specifies whether the EE devices were located in water or on land.

4. Discussion

4.1. Activity budget

An activity budget helps to understand how an animal or group of animals allocates its time across various behaviours and activities. This analysis offers important insights into their routines, including predominant behaviours, the presence of stereotypies, level of exploration and interaction with the environment, additionally aiding in understanding the nature of social relationships among individuals, indicating whether these relationships are tense or friendly. These behavioural patterns establish a baseline for comparison, allowing the identification of periods throughout the year when additional resources may be necessary to stimulate behaviours that are either unobserved or observed at low frequencies. This information is crucial for evaluating animal welfare and pinpointing areas that require intervention.

The activity budget of SEA LIFE Porto's Humboldt penguins was assessed precisely for this reason: by understanding how the animals behave in typical, non-enriched environments, one could objectively evaluate which types of enrichment were most effective. In this case, data collected over time provided insights into behavioural changes throughout the animal's annual cycle, helping to identify when enrichment may be most important. Due to logistical constraints, the introduction of EE could only start in late January 2024. However, activity patterns from December 2023 to January 2024 were chosen to be presented, as this timeframe offers a more comprehensive overview of the penguins' behaviour over time.

From December 2023 to April 2024, a decrease in the number of penguins visible in the outdoor area was noticeable (*Figure 10*). An overall declining trend indicated that the number of penguins outside of their nests was consistently decreasing during this period. This reduction can likely be attributed to the breeding and nesting season, which for this group began around February and extended until July. During this time, the penguins predominantly remained in their nests, with each member of a pair taking turns in the outdoor area. As such, based on preliminary in-person observations, it was uncommon to see both penguins from a pair simultaneously outside, meaning that when all pairs were breeding, only half of the group would be visible at any given time.

Maintenance behaviours showed a general decline over time, although with considerable fluctuation (*Figure 11*). Behaviours associated with locomotion on land and environmental interaction were particularly high from February to March 2024 (*Figure 13*

and *Figure 16*, respectively), a trend that is understandable considering that habitat exploration often involves animals moving more, for instance, to engage with environmental features. Throughout all observed periods, stationary behaviours were consistently the most frequently exhibited by the penguins (*Figure 12*) and swimming and social the least (*Figure 14* and *Figure 15*, respectively).

These results are not uncommon for penguins in captivity. Marshal et al. (2016) did an activity budget analysis for five colonies of *Spheniscus* penguins and, even though there were noticeable differences among the colonies, the penguins spent most of their time either out of sight or resting, with categories such as preening, terrestrial locomotion and swimming occurring in smaller percentages and varying across studies. Zhang et al. (2021) concluded that Humboldt penguins in captivity dedicated the majority of their time to stationary, locomotion and swimming behaviours, even though their frequency changed in accordance to weather conditions and visitor presence. In the current study, these factors were not considered, although it is acknowledged that they might have influenced results.

While time and activity budgets are not identical, they serve similar purposes in analysing animal behaviour. For example, both approaches examine the energy allocation of animals to different activities and are used in welfare assessments. Therefore, it seems reasonable to draw a parallel between the limitations of the two. Tallo-Parra et al. (2023) discussed some of the limitations of using a time budget to assess animal welfare, which are important to consider. The authors emphasise that determining whether a specific behaviour is beneficial or detrimental can be challenging (Tallo-Parra et al., 2023). For instance, an animal's inactivity can indicate both contentment and boredom (Tallo-Parra et al., 2023). Furthermore, the frequency with which a behaviour occurs does not necessarily correlate with its welfare implications; short-lived aggressive encounters can have severe negative consequences for animals, even if they do not happen that often (Tallo-Parra et al., 2023). Lastly, while time budgets provide insights into behavioural patterns, they may not fully capture an animal's physical well-being (Tallo-Parra et al., 2023).

Even though activity budgets are a valuable framework for analysing animal behaviour, they do have limitations. As previously discussed, activity patterns can vary both between and within species, thus being essential to consider the environmental, social and contextual factors influencing animal behaviour in order to have a more comprehensive understanding of animal welfare.

4.2. Effects of Environmental Enrichment

The results regarding the introduction of EE varied across study months. The same type of enrichment that proved to be successful in one period may not have had the same effect in the following. The only category of behaviour that exhibited significant differences with EE, both in the February-March 2024 and April-May 2024 periods was the Maintenance category (*Figure 17 A and E*).

Although a significant difference in swimming behaviour with EE was observed from February to March, this trend did not continue from April to May (*Figure 17 B and F*). In contrast, environmental interaction behaviours decreased with EE during February-March but increased in the April-May period (*Figure 17 D and G*). The lack of an increase in swimming behaviour (*Figure 17F*), combined with a rise in habitat exploration (*Figure 17G*) and nesting behaviours (*Figure 17H*) during April-May, suggests that the animals may prioritise finding nest-building materials during this time, likely due to being in their reproductive and nesting phases. In addition to the artificial algae and leaves, at least one of the land-based enrichments, the artificial plant, could be perceived as a potential source of nesting materials. Indeed, while doing a multi-zoo comparison of the behaviour and breeding success of Humboldt penguins, Marshall et al (2016) noticed a significant seasonal change in the amount of time penguins spent in water throughout their breeding cycle. During the pre-nesting period, penguins spent a significantly greater proportion of their time in the water, with a marked reduction during nesting and post-nesting phases (Marshall et al., 2016), which is consistent with the results of this study.

Overall, the penguins displayed low levels of social interactions, particularly with the presence of EE in February and March (*Figure 17C*). This decrease may be related to the fact that EE encourages an increase in solitary behaviours or redirects the focus to new activities. Most of the group's interactions occur between mated pairs, and during this period, there was a decline in the number of penguins outside their nests, which likely contributed to the reduced visibility of social behaviours.

Regarding the penguins' reaction to EE items, the artificial algae and leaves stood out as the favourite enrichment item as indicated by *ad libitum* observations. When these enrichment devices were presented, it was common to see penguins emerging from their nests to collect and transport these materials to their nests. The bird replicas combined with Peruvian booby bird sound prompted the penguins to frequently look around as if trying to locate the source of the noise. However, they did not appear to associate the sound with

the replicas themselves possibly because they were static. A further improvement to this enrichment could be trying to introduce some movement, such as hanging them so that they sway in the wind. As mentioned earlier, the artificial plant, that was intended to add hiding spots within the display, appeared to be perceived by the animals as additional nesting materials. The animals were frequently observed pulling at its branches and leaves, likely attempting to gather them for their nests. This further emphasises the importance of adjusting the provided EE items to meet the biological and ecological needs of the animals.

Aquatic enrichments did not produce the anticipated effect of increasing the swimming behaviours of the penguins. O'Brien and Cronin (2023), which used an enrichment item similar to the aquatic aerator, reported similar results to those of this study with African penguins (*Spheniscus demersus*). Their research found that this enrichment failed to increase either the number of penguins in the water or the duration of their swimming activity (O'Brien & Cronin, 2023). Despite being aware of this research prior to initiating our own, it was decided to test a similar enrichment, as different penguin species may respond differently to the same type of stimulus. In a study on Humboldt penguins, Razal and Miller (2021) concluded that only one of the enrichment items successfully increased their activity levels, while none of the other methods managed to extend the time spent in the water (Razal & Miller, 2021). *Ad libitum* observations did not reveal a preference for any of the aquatic enrichments, nor did they seem to cause any aversion in the penguins. Overall, this group of animals appeared indifferent to the aquatic enrichment items, and the primary motivator for encouraging penguins to enter the pool seemed to be other penguins already swimming there.

Nevertheless, the introduction of EE encouraged the penguins to display a broader range of behaviours, particularly fostering social interactions, with the animals competing for nesting materials. This competition may mimic natural behaviours, which can be beneficial for their overall well-being and social dynamics. Although a penguin diving into the pool to explore and interact with EE devices was observed only once, this might indicate possible curiosity to explore the pool, with swimming behaviours potentially being further encouraged by modifying or testing new designs of EE devices placed in water.

4.3. Enclosure Use

The Electivity Index shed light on the fact that relative to its size, Zone A is being overutilised by the penguins, while Zones B, D and E are not being used as much as they could be (*Figure 18*). In turn, the use of Zone C depends on the context, and it can be underutilised or used in proportion to its size (*Figure 18*). These conclusions imply that Zone A is the animals' preferred area, whereas Zones B, D and E seem to be perceived as less valuable by the penguins. Zone C occupies a middle ground and its importance to animals can change depending on the context, such as the presence or absence of EE.

Given that this study was conducted in proximity to and during the reproductive and nesting phases, the animals' preference for Zone A's use may be influenced by its closeness to the nesting sites. Furthermore, *ad libitum* observations suggested that Zone B functioned primarily as a transition area, where the animals went to search for nesting materials rather than spending extended periods of time, at least during the reproduction and nesting season. Conducting studies outside this particular period may reveal different results.

The use of each zone may vary according to the time of day. For instance, *ad libitum* observations revealed that, around feeding time, the animals predominantly stayed in Zone C, as this is where they were typically fed in the afternoon. Zhang et al. (2021) also observed shifts in behavioural patterns, noticing, for example, that Humboldt penguins increased pool use after feeding hours. Because the observation hours in this study occurred a few hours after the morning feeding and shortly before the afternoon feeding, the underutilisation of Zone E may be attributed to the timing of the observations, which may have coincided with periods when swimming behaviours typically do not occur. This rationale could likewise explain the lower activity observed in other zones.

Furthermore, the more enrichment items placed in a zone, the more likely it is that the zone will be used. Therefore, Zone C's underutilisation, despite the presence of enrichment items, may be explained by the fact that usually only one enrichment item was placed there. Moreover, the same items placed in Zone C (artificial algae and leaves) were also distributed throughout the rest of the enclosure, with larger areas receiving a proportionally greater amount. This likely contributed to Zone C being even less used on days when land-based enrichment was introduced. Additionally, since the penguins mainly frequented Zone C during feeding times, its underutilisation might suggest a reduction in the penguins' food

anticipatory behaviour. This shift may indicate that the introduction of EE redirected the penguins' attention to other activities, encouraging the use of other zones of the display.

The perimeter of Zone D is where the viewing glasses are located, making it the area closest to visitors. It is possible that the animals avoid using this area for this reason, or they restrict its use to times when visitor presence is lower. Indeed, in Little penguins (*Eudyptula minor*) the presence or absence of visitors significantly influenced the penguins' proximity to the viewing area, in this case, with a notable decrease in the distance when visitors were absent (Sherwen et al., 2015), suggesting that visitors were preventing the penguins from making full use of the exhibit. Chiew et al. (2019) reported similar findings with the same species, observing that fewer Little penguins were seen near the viewing area as visitor proximity increased. However, a contrasting study on African penguins found no difference in enclosure use with and without visitors (Williams et al., 2021), highlighting the variability in behavioural responses between different species. Additionally, although some of the studies mention the age of the penguins, none seem to explicitly address how long the animals had been in human care. This might be an important factor to consider since research shows penguins can become accustomed to the presence of humans (Ozella et al., 2014), meaning that their behaviour and responses to visitors could vary based on previous experiences.

In the wild, Humboldt penguins must forage for their food, a necessity that is eliminated in captivity where food is guaranteed. The lack of Zone E's use may be related to this, as the penguins do not rely on it for sustenance. Moreover, the influence of visitors on penguin behaviour is once again worth considering. For instance, in Little penguins, a decrease in visitor presence, or a reduction in their adverse behaviours that could be perceived as a threat, led to an increase in time spent in the pool (Sherwen et al., 2015; Chiew et al., 2019). On the contrary, higher visitor numbers were linked to a rise in pool use and associated behaviours in Gentoo penguins' (*Pygoscelis papua*) (Collins et al., 2016; Edes et al., 2021). This suggests that, depending on the species, the mere presence of visitors may be a stimulating factor. As such, the assessment of visitor numbers and their correlation with enclosure use by the animals can provide valuable insights into how human presence influences the behaviour of SEA LIFE Porto Humboldt penguins.

5. Conclusion

The activity budget analysis revealed that the overall number of visible penguins decreased as the breeding and nesting season progressed. On non-enrichment days, the group predominantly engaged in stationary behaviours, along with locomotion on land and maintenance behaviours. Additionally, environmental interaction behaviours fluctuated throughout the year, with swimming and social behaviours being the least frequently observed.

The impact of EE devices on the penguins' walking, exploratory, and swimming behaviours varied by month and type of enrichment used. Aquatic enrichment devices should be reevaluated, as they generally failed to enhance swimming behaviours. Additionally, the other enrichment items must be periodically reassessed, as the animals' personalities and needs may change with age. Over time, the penguins may habituate to EE devices, therefore diminishing their novelty and effectiveness.

The introduction of EE did not lead to an increased use of the display area. Except for Zone C, the electivity index for each area remained relatively stable over time, regardless of enrichment type. It appears that penguins tended to avoid exhibit areas near visitors, and preferred to remain closer to their nests. Further studies monitoring visitor numbers and analysing their correlation with enclosure use could provide valuable insights into how human presence affects the behaviour of this specific group.

Future studies should also investigate the effects of weather changes and visitor presence, particularly outside the reproductive season, to better understand how these factors influence the penguins' behaviour. Additionally, conducting a statistical analysis involving a larger sample size of observations could provide more robust conclusions and clearer insights into the effects of EE on their behaviour.

Chapter 2: Behavioural Training of Humboldt Penguins

1. Introduction

Maintaining animals in captivity implies including measures and precautions in their husbandry routines to safeguard their health and well-being. Animals unaccustomed to being handled often endure fear, anxiety, heightened vigilance, aggression, and the onset of stereotypic behaviours or other undesirable responses (Brando, 2012). Allowing the animals to understand the processes involved in their care and training them to willingly engage in routine tasks – such as moving between areas, remaining calm on scales, or accepting handling and treatment – can enhance their welfare, promote positive emotional states, and minimise the negative outcomes frequently associated with involuntary events, therefore granting animals more autonomy and control within their surroundings (Brando, 2012).

Operant conditioning, as described by Castro & Wasserman (2009), revolves around an animal's ability to learn the causal link between its actions and the resulting outcomes. The key idea of this concept is that behaviour is influenced and modified by its consequences (Laule et al., 2003; Castro & Wasserman 2009). Operant conditioning presents two strategies for behaviour management: positive reinforcement and negative reinforcement, both of which enhance the likelihood of a behaviour being repeated (Laule et al., 2003). While positive reinforcement involves offering a stimulus after a behaviour to encourage its repetition, negative reinforcement entails removing a stimulus following a behaviour to increase the likelihood of that behaviour recurring (Heidenreich, 2007). Negative reinforcers are generally stimuli perceived as unpleasant or aversive, whereas positive reinforcers are typically desirable or enjoyable stimuli (Heidenreich, 2007).

Positive reinforcement training (PRT) encourages animals to voluntarily get involved in activities normally seen as negative, by rewarding the animals for showcasing the desired behaviours (Brando & Norman, 2023), leading to positive behavioural changes. This allows the keeper to build a trusting relationship with the animals, encourages desired behaviours, and fosters a dynamic, engaging, and secure learning environment (Brando, 2012).

Specific case studies illustrate the success of PRT. For example, adult zebra sharks (*Stegostoma fasciatum*) were successfully desensitised to being in a holding tank, to the presence of trainers within the tank, and to tactile stimulation (Marranzino, 2013). This procedure not only allowed aquarists to feed them more consistently, while facilitating the

exact monitoring of what each shark was fed, but also eased their regular physical examination (Marranzino, 2013). In another instance, positive reinforcement techniques were used on Marabou storks (*Leptoptilos crumeniferus*), resulting in several positive outcomes. These included simplifying the process of relocation, minimising aggression among the storks, and consistently monitoring their health in a way that reduced stress (Miller & King, 2013). Additionally, PRT has been employed to train animals to engage in their own medical care (Booth et al., 2022), to voluntarily move to different sections of the enclosures (Bloomsmith et al., 1998; Williams et al., 2022) and to be separated from other group members (Spiezio et al., 2015). Thus, not only does PRT enhance the physical and psychological welfare of animals (Pomerantz & Terkel, 2009; Neal Webb et al., 2020) but it also improves their overall health. Its contribution to improving animal husbandry standards and animal welfare is increasingly relevant, not only making human-animal interaction easier, more positive, and safer for all those involved but also promoting the ethical treatment of animals.

The work developed with the colony of Humboldt penguins at SEA LIFE Porto, based on operant conditioning with positive reinforcement, had the specific goal of training the penguins to lie ventral side down in the keeper's arms with their legs and feet extended away from their body, as this position is comfortable for the birds (*Figure 19*; Erlacher-Reid et al., 2011; Duncan et al., 2016), facilitating veterinary and husbandry procedures and thereby reducing the associated discomfort. This training procedure attempted to develop a method that could be seamlessly integrated into the keepers' daily tasks, ensuring both consistency and ease of implementation.



Figure 19. Illustrative representation of the intended penguin behaviour (created by the author). Ideally, the penguin should briefly remain ventral side down in a keeper's arms, while spreading the legs and feet away from the body.

2. Materials and Methods

2.1. Training Procedure

A training program was established to increase penguins' cooperation and reduce any discomfort they might experience during health examinations and husbandry procedures. The goal was to help the animals acclimate to close interactions and handling by keepers, thereby facilitating these tasks. The chosen body position is commonly used for foot examinations (Duncan et al., 2016). This includes visual inspections of the plantar surface of their feet (Erlacher-Reid et al., 2011) and infrared thermography to detect any lesions on the surface of the foot (Duncan et al., 2016).

Training took place from January 2024 to May 2024, alongside the EE study, encompassing a total of 66 sessions. The penguins started to show reproductive and nesting behaviours in February, lasting approximately until July. From 1 to 4 times per week, the training sessions served as a replacement for the morning feeding, with no sessions at weekends. Exceptionally, there was one week (13th-17th May) when no training sessions were conducted.

All sessions occurred in the display's indoor area between 8 AM and 10 AM, depending on the keepers' schedules, and lasted for as long as the penguins desired to participate. Their duration ranged from less than 10 seconds to nearly two minutes per penguin, with typical group sessions averaging about 15 minutes. The length of the sessions was not taken into account when assessing the training process.

During each session, the door connecting the exhibition to the indoor area remained open, allowing the penguins to exit freely at any time, and to observe the other penguins' training. No more than 2 keepers were required for each training session: one to spatially separate the penguin in training from the rest of the group, and another to effectively interact with the penguin.

Food served as the primary reinforcer, specifically sprat and capelin, selected from the penguins' daily diet. They were always fed *ad libitum*. To keep the penguins engaged and stimulated, the type of fish offered was randomly alternated. In the first session, the fish were cut in half to attempt to prolong each penguin's interaction with the keeper. However, this method was not repeated because the penguins had difficulty in correctly positioning the halved fish in their mouths and throats, making it much harder for them to catch and swallow the food. Consequently, they quickly gave up and abandoned the session.

Each training session was recorded using a Sony HDR-CX405 camera (Sony Corporation, 1-7-1 Konan Minato-ku Tokyo, 108-0075 Japan), allowing for later review and

the creation of detailed summaries. These summaries included the duration of training for each penguin, the highest step achieved in each session, an assessment of their responsiveness (covering motivation levels, aggressiveness, restlessness, and distraction), and whether they performed the intended behaviours and how effectively. Additional relevant details were also noted to ensure comprehensive descriptions of each session, aiding in better evaluating each animal's progress. A total of 66 videos, varying in duration according to the length of the corresponding training session, were reviewed. Each penguin's session was assessed and rated as "Terrible," "Bad," "Good," or "Excellent," with the criteria for each classification outlined in *Table 3*. While these criteria provide a foundational framework, they can be adjusted to account for the dynamics and personalities of different penguin groups. In some sessions, the penguins displayed behaviours characteristic of multiple ratings, in which case the final rating was based on the category with the most checked behaviours.

Table 3. Criteria used to classify each penguin's training sessions as "Terrible", "Bad", "Good", "Excellent", or "Did not enter the indoor area".

CLASSIFICATION OF TRAINING SESSIONS					
	Terrible	Bad	Good	Excellent	
Entering the indoor area	The penguin refuses to enter or leaves the indoor area within 2 seconds.	The penguin enters the indoor area but stays at least one metre from the keeper, showing reluctance to approach.	The penguin enters the indoor area and stays within one metre of the keeper without significant distress ¹ .	The penguin enters the indoor area without hesitation and immediately stands next to the keeper.	Did not enter the indoor area
Proximity to the keeper	Refuses to go near the keeper.	Appears tense and uncomfortable ¹ to approach, remaining at the one-metre boundary from the keeper.	Shows some wariness ² but overall remains calm, standing near ($\leq 1\text{m}$) the keeper for at least half of the session.	Stays next to the keeper without any signs of distress ¹ or wariness ² for the entirety of the session.	

¹ Shows signs of distress. The penguin's body is tense, its feathers are fluffed up, and it has little interest in eating. When on the keeper's lap, it might be possible to notice its heartbeat increase.

² The penguin might lower its chest closer to the ground. Its feathers are fluffed up (particularly noticeable on the neck and surrounding areas), and the animal moves slowly and assesses the keeper's every movement. It hesitates before making movements and looks around a lot as if evaluating everything that is happening.

³ Beaking, bobby jabbing, biting, threatening vocalisations, hitting with the flipper, etc.

⁴ Does not show signs of distress. The penguin's body is relaxed, with smooth feathers, and it eats with interest. When on the keeper's lap, no heartbeat changes are noticeable.

Table 3 (continued).

CLASSIFICATION OF TRAINING SESSIONS					
	Terrible	Bad	Good	Excellent	
Touch response	Struggles or acts aggressively ³ when the keepers lift their hand or touch it.	Moves away when the keepers lift their hand or touch it. Warily ² eats from a distance ($\geq 1\text{m}$) or leaves the indoor area without returning.	Allows touches but may step back (remaining at $\leq 1\text{m}$ of the keeper). Tolerates light pets rather than firm pressure.	The penguin is comfortable ⁴ with being touched, remaining in the desired position throughout the training session.	Did not enter the indoor area
Feeding behaviour	Eats reluctantly or not at all, failing to maintain the desired position.	Stays on high alert during feeding, eating quickly (each bite under 2 seconds) often from a distance ($\geq 1\text{m}$), and runs away immediately after finishing.	Though tense or restless, it eventually calms down and continues to eat, whether that is near or further from the keeper ($\leq 1\text{m}$), or after being in the keeper's lap. Sometimes runs away but returns and may allow more touches.	Eats normally and comfortably ⁴ , both before and after being touched or being in the keeper's lap. The penguin might come back into the indoor area more than once.	

¹ Shows signs of distress. The penguin's body is tense, its feathers are fluffed up, and it has little interest in eating. When on the keeper's lap, it might be possible to notice its heartbeat increase.

² The penguin might lower its chest closer to the ground. Its feathers are fluffed up (particularly noticeable on the neck and surrounding areas), and the animal moves slowly and assesses the keeper's every movement. It hesitates before making movements and looks around a lot as if evaluating everything that is happening.

³ Beaking, bobby jabbing, biting, threatening vocalisations, hitting with the flipper, etc.

⁴ Does not show signs of distress. The penguin's body is relaxed, with smooth feathers, and it eats with interest. When on the keeper's lap, no heartbeat changes are noticeable.

Table 3 (continued).

CLASSIFICATION OF TRAINING SESSIONS					
	Terrible	Bad	Good	Excellent	
General behaviour	Fidgets, attempts to escape, shows visible signs of distress ¹ or aggression ³ , and resists the training process.	The penguin is more focused on the keeper's actions than on obtaining food. Remains wary ² throughout training, and if it eventually lies on the keeper's arms, it remains tense and restless, running away within a second when placed on the floor.	Although wary ² of the keeper, the penguin prioritizes food and does not rush to leave the indoor area after eating. It calms down in the keeper's arms after a brief struggle, remaining there for a few seconds. When placed on the floor, it does not run away but shows little interest in further interaction.	The penguin explores the indoor area or continues its daily routine, such as swimming or preening after eating. It engages with the keepers, following them around or showing curiosity about its surroundings. It does not run away when the keepers pass by in the indoor area and even allows touches after the session is over.	Did not enter the indoor area

¹ Shows signs of distress. The penguin's body is tense, its feathers are fluffed up, and it has little interest in eating. When on the keeper's lap, it might be possible to notice its heartbeat increase.

² The penguin might lower its chest closer to the ground. Its feathers are fluffed up (particularly noticeable on the neck and surrounding areas), and the animal moves slowly and assesses the keeper's every movement. It hesitates before making movements and looks around a lot as if evaluating everything that is happening.

³ Beaking, boddy jabbing, biting, threatening vocalisations, hitting with the flipper, etc.

⁴ Does not show signs of distress. The penguin's body is relaxed, with smooth feathers, and it eats with interest. When on the keeper's lap, no heartbeat changes are noticeable.

To facilitate the monthly weighing process, and before the beginning of the training sessions, the penguins had already learned to enter the indoor area and voluntarily step onto a scale during their morning feedings. Given this circumstance, another signal was required to clarify to the animals that, despite these procedures occurring in the same location, they were intended to elicit different behaviours. To address this, a green and blue board (*Figure 20*) was introduced as a visual cue as research suggests that animals of this

species seem able to discriminate well between wavelengths in the blue-green region of the spectrum (Bowmaker & Martin, 1985). The goal was for the penguins to associate the board with lying in the keeper's arms while being lifted off the ground. This cue was used consistently in every training session and was not employed in any other context, making it exclusively associated with eliciting this specific behaviour during training.

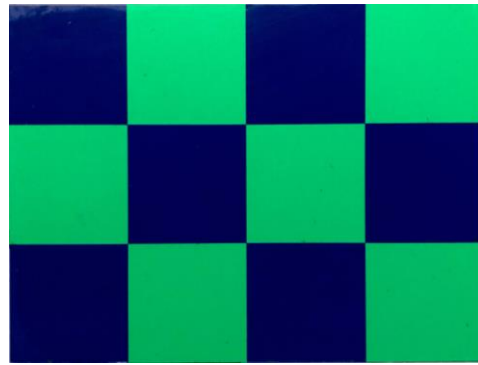


Figure 20. The checkered green and blue board used as a visual cue in every training session. The board was 40 cm long and 30 cm high. Each square was 10 x 10 cm.

When the penguins showed reluctance to participate in the training session, they were immediately fed either in their nests or in the exhibit's outdoor area but only once the participating penguins had completed their session. On days without training sessions, their routine involved stepping onto the scale during the morning weights, followed by the afternoon group feedings.

2.2. Training steps: Description, Goals, and Challenges

The training process was adjusted to each penguin's character, employing a dynamic and flexible approach that progressed gradually to avoid discouraging the animals from participating in the training sessions or entering the indoor area at all. This process involved making daily adjustments and continuously evaluating the animals' performance and the suitability of each training step, adjusting the chosen approach as necessary to respect each animal's limits.

Nine predefined steps (*Figure 21*) were established, with the keeper advancing to the next step when the penguin showed signs of tolerating the proximity and level of touch

involved. If needed, the keeper would revert to earlier steps. To ensure consistency throughout the training sessions, it was always the same keeper engaging and physically interacting with the penguins. Certain training steps could not be completed during sessions when only one keeper was present.



Step 1: The penguin enters the indoor area and remains within 1m of the keeper and the sign.



Step 2: While in the indoor area, the bird stands still next to the keeper.



Step 3: The penguin allows to be touched in any part of its body.



Step 4: The penguin allows touches with a little bit more pressure (as if doing a massage on their back) or allows it to be held back by its belly.



Step 5: The penguin allows it to be held by both sides as if the keeper is going to pick it up, but without lifting it.



Step 6: The penguin allows to be held while the keeper raises it until it is on its tiptoes.



Step 7: The penguin calmly lies on the keeper's arms.

Step 8: The penguin calmly lies on the keeper's arms and extends its legs and feet.

Step 9: The penguin calmly lies on the keeper's arms and extends its legs and feet as the keeper lifts and stands.

Figure 21. Humboldt penguins' training steps. Steps 8 and 9 are yet to be reached, therefore visual evidence was not possible to be taken.

Step 1. The goal for this step was for the penguins to enter the indoor area. To be considered a success, the penguins should stay within one metre of both the keeper and the visual cue. Often, the penguins would stand at the entrance of the indoor area but without entering. On those occasions, the keeper would feed them near the entrance and offer food gradually closer to herself and the visual cue.

Step 2. The objective of this step was to acclimate the penguins to being close to the keeper without perceiving her as a threat. The penguin standing still next to the keeper was interpreted as a sign that it felt secure enough to approach her.

Step 3. The purpose of this step was to desensitise the penguins to physical contact. The main challenge encountered was that some penguins would step back and refrain from approaching as soon as they saw the keeper slowly lifting her hand. However, as the sessions progressed, most of the penguins felt secure enough to allow touches.

Step 4. This step aimed to acclimate the penguins to experiencing touch with more pressure, simulating the sensation they would feel when being picked up. As in the previous step, the penguins would occasionally move away, eating further away from the keeper or even leaving the indoor area altogether.

Step 5. The goal of this stage was to accustom the penguins to feeling the keeper's hands on both sides of their bodies and to get them used to the position they would be in when being picked up to be cradled.

Step 6. This step aimed to acclimate the penguins to the sensation of being lifted without doing so. Due to the awkward positioning, it was common at first to see the penguins lifting and lowering their feet, becoming somewhat restless as they were unsure of how to stand properly. Eventually, this behaviour subsided.

Step 7. This step was seen as a milestone, bringing the penguins close to the final position. It was a critical step as if the penguins were not at ease with being picked up, there was a high risk of them regressing in progress. Success was defined by the penguin staying calm, without showing signs of restlessness or stress. The main challenge was to calm the penguins so they would remain still.

Step 8. For this step to be successful, the penguins needed to extend their legs and feet and stay still for a few seconds. Since this step has not been tried yet, the main

challenges are hard to pinpoint. However, because lying down with their legs and feet fully extended is not a natural position for penguins, it is anticipated that this will be a challenging step, with the penguins likely to move their feet frequently. This step may require a second keeper's help to extend the penguin's legs and feet while it rests on the first keeper's lap.

Step 9. Although not a crucial step, this phase aimed to acclimate the penguins to being on the keeper's lap at a higher height from the ground than usual. This would facilitate certain procedures, such as medical examinations on different parts of their bodies, by ensuring the penguins remained calm and cooperative. It is anticipated that the penguins will become more restless when they feel at a higher distance from the ground, therefore it may be useful to subdivide this step, gradually keeping the penguin at higher heights.

For every step, the penguins were initially offered one or two fish, serving a dual purpose: to ensure they received their daily vitamins and to encourage them to approach the keeper. During a few sessions, an alternative to Step 6 was tested, where the penguins were lifted a few centimetres off the ground. This was not considered a viable step, as the penguins appeared uncomfortable, likely due to their awkward positioning and uncertainty about how to place themselves. Occasionally, for Step 7, after placing the penguins on the ground they were briefly held back (the keeper extended her arm and positioned it in front of the penguin), to ensure they noticed the reward (food). If the penguin still wanted to leave after seeing the reward, it would be released immediately. Even though momentarily delaying the penguins' release after a potentially stressful situation might add to the stress, it was attempted as a way to help the penguins associate a positive outcome with the process. Indeed, there were occasions when, after being cradled, the penguin initially wanted to leave but opted to stay and eat upon noticing the reinforcer.

Appendix D presents a training flowchart that outlines some measures to help penguins gradually adjust to each step. These measures and recommendations were based on the keeper's experience with this particular group, reflecting decisions made specifically for these animals. Since different animals may respond differently, the process should be adjusted accordingly.

While no specific verbal cues were established beforehand, it was common for the keeper to speak with the penguins, calling them to enter the indoor area and providing explanations regarding her forthcoming actions or desired behaviours. Before the beginning of this work, these animals already responded to their names, often going into the indoor

area to eat when called, demonstrating a certain level of associative learning and recognition.

2.3. Statistical Analysis

Statistical analysis was conducted to determine if there were significant differences in training progress between sexes. This analysis was performed using *IBM SPSS (Statistical Package for the Social Sciences) Statistics 28* software. Normality and equality of variances were evaluated using a Shapiro-Wilk test and a Levene's test, respectively. As the data did not meet these assumptions, a Mann-Whitney U test was used to compare the highest training step achieved by males and females during the training sessions (n=66). The significance level was set at $\alpha=0.05$.

3. Results

Since the first session, the penguins entered the indoor area spontaneously, as illustrated in *Figure 22A-D*, schematised according to the pairings within the group. The penguins were already accustomed to the area from previous training involving stepping onto a scale, therefore it was not a new or unfamiliar environment.

Of the eight penguins trained, three reached Step 7 (*Figure 22B-D*), four approached the keeper and allowed to be touched (Step 4, *Figure 22A-C*), while one remained wary and made minimal progress (Step 3, *Figure 22D*), continuing to be highly suspicious of the keeper throughout the process. Training was a back-and-forth process, involving daily adjustments based on each penguin's behaviour. A penguin that resisted being touched in one session might have become relaxed enough to be cradled on the keeper's lap in the next. *Appendix E* highlights the variability in the responses to training, from session to session.

The impact of the reproductive cycle phase on training was noticeable (indicated in *Figure 22* as Step 0, which denotes the days on which the penguins did not participate in training). During this period, several penguins were reluctant to leave their nests and enter the indoor area for multiple consecutive sessions. This reluctance made it challenging to maintain consistency in the training process, potentially slowing the animals' progress. Furthermore, after some penguins began being fed in their nests, and the remaining individuals appeared to realise they could obtain food without participating in training

sessions, several penguins started refusing to do so, opting instead to remain at their nests to receive their food.

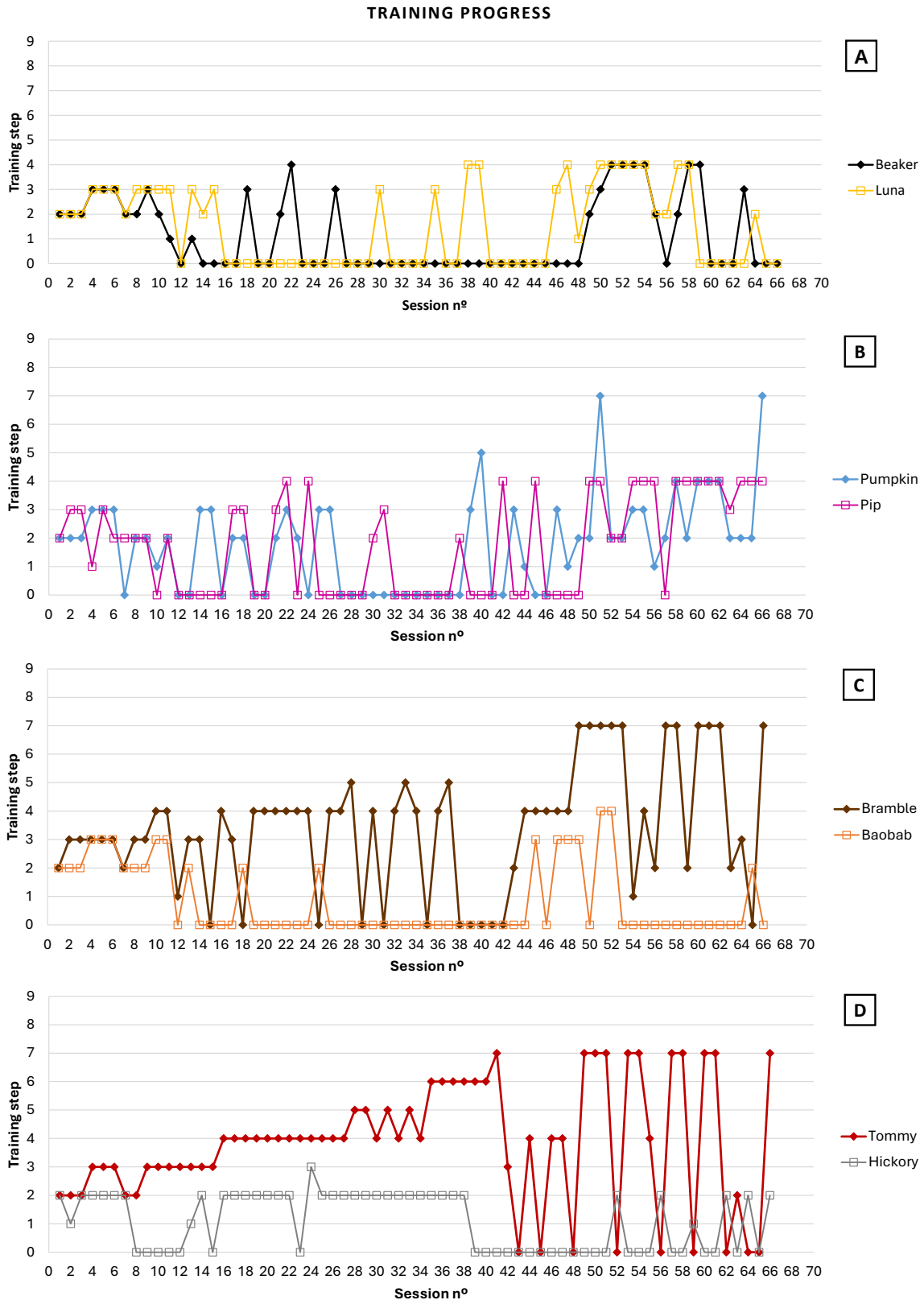


Figure 22. Penguins' training progress: highest step achieved in each session for the different penguin pairs. The sessions where the penguins did not participate in training are marked as Step 0. **(A)** Beaker and Luna, **(B)** Pumpkin and Pip, **(C)** Bramble and Baobab, and **(D)** Tommy and Hickory.

The penguins occasionally refused to participate in the training sessions because they preferred one type of fish over another. As training sessions progressed, it became clear that capelin was not sufficiently motivating for most penguins to consistently engage in the sessions. When capelin was the chosen reinforcer, the penguins frequently approached the keeper but then turned away and left, leading to disruptions in the consistency of the sessions. Additionally, it was observed that when the penguins had consecutive days without training, such as over weekends, their progress was hindered, and they frequently regressed to earlier steps.

Based on in-person observations, the penguins seemed to understand the meaning of the visual cue, frequently hesitating to enter the indoor area or retreating when they saw it. On days when only the scale was present and no training was scheduled, they often entered the training area with greater enthusiasm. This behaviour suggests that the penguins may have been successfully trained to voluntarily participate in weighing, or that they may have associated the board with physical contact and thus appeared less motivated when it was present.

The statistical analysis revealed a significant difference between females' and males' highest steps achieved during training sessions ($U=46068.500$, $p<0.001$). Males had a higher mean rank than females, suggesting they reached higher training steps (*Figure 23*). *Ad libitum* observations indicated that females often remained cautious and avoided physical contact, whereas males appeared to tolerate training better.

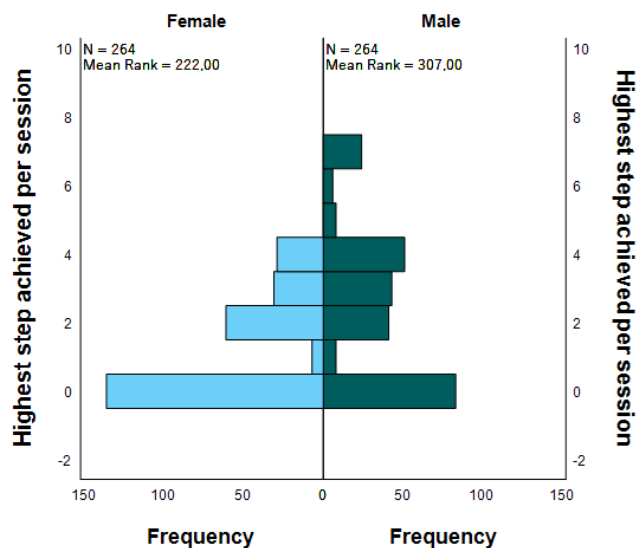


Figure 23. Independent-samples Mann Whitney U test. Frequency of the highest training step (*Figure 21*) achieved per session by the penguins, categorised by sex. A total of 66 training sessions were conducted for the four male and four female penguins.

Ad libitum observations revealed a noticeable improvement in the penguins' comfort with physical touch and proximity to keepers during (Appendix E) and after training sessions. In the indoor area, outside of training sessions, the penguins appeared to exhibit a more relaxed behaviour when keepers walked by. They also appeared more comfortable with light touches on their backs when being guided to the outdoor area, remaining calm without signs of fear or distress. This behaviour contrasted with their reactions in the outdoor area, where the penguins frequently moved away from keepers.

4. Discussion

The work developed at SEA LIFE Porto demonstrated that penguins under professional care can be successfully trained for veterinary and husbandry purposes. However, training can be a time-consuming process, with penguins' ability to learn fluctuating both over time and among individuals. Even though the ultimate goal of achieving the desired lying position has not yet been reached, it took at least four months for three penguins to reach a stage where they allowed the keeper to cradle them. Due to the variability in each penguin's response to training, it is difficult to predict how much additional time will be necessary to achieve the final goal.

It is not uncommon for animals of this species to have a lengthy training process. In Rößler et al (2022), the training process, which involved conditioning to a whistle, desensitisation to the training area, and habituation to training tools, needed at least 700 trials before meeting the necessary criteria to proceed with psychoacoustic measurements. Unlike the penguins in the Rößler et al (2022) study, which had never been trained and were completely naïve to the process, the penguins at SEA LIFE Porto had prior training experience, potentially making them more accustomed to this process and accelerating their progress in learning this new behaviour.

On the contrary, it is also important to consider that training adult animals can often be more lengthy than training younger individuals. While the species differ, research on the common marmoset (*Callithrix jacchus*) has shown that age significantly influences learning ability (Glavis-Bloom et al., 2022). Older marmosets require more time to reach the same level of task comprehension as younger individuals (Glavis-Bloom et al., 2022). Even after acquiring a basic understanding of the task, ageing marmosets exhibited a slower learning rate, indicating a decline in their ability to learn and adapt to new challenges efficiently

(Glavis-Bloom et al., 2022). The authors also concluded that working memory capacity was compromised with age (Glavis-Bloom et al., 2022), which suggests that similar age-related factors may influence training in other species, including penguins, possibly making the process more difficult and time-consuming for adults than younger animals.

Another relevant factor to consider is the influence of the reproductive cycle on the penguins' trainability. In this study, particularly during the nesting phase, few penguins ventured away from their nests, making it impossible to proceed with training. During consecutive sessions, several penguins did not go through the training process, which may have hindered overall progress. A similar situation occurred during the research by Rößler et al (2022). The birds became sexually mature and underwent various moulting bouts, which had "strong negative effects on the concentration of penguins", consequently impacting their willingness to participate in the training (Rößler et al., 2022, p.6). Understanding how the reproductive cycle impacts penguin behaviour can help trainers adjust their methodology accordingly to maximise success, therefore being a key point to consider when designing a training program.

The personality of each penguin and how it influenced training was an additional aspect that stood out. Personality can be defined as "those characteristics of individuals that describe and account for temporally stable patterns of affect, cognition, and behaviour" (Gosling, 2008, p. 986). There is significant evidence that animals have personalities (Gosling & Vazire, 2002) and research on the topic is rapidly increasing (Gosling, 2008). It is therefore important to understand how this set of characteristics might influence the animals' response, in this case, in the training context. Quintavalle Pastorino et al. (2019) concluded that three species of penguins – Gentoo penguins (*Pygoscelis papua*), King penguins (*Aptenodytes patagonicus*) and Northern Rockhopper penguins (*Eudyptes moseleyi*) – retain species-specific behavioural individuality for certain personality traits, even though the three live in proximity, share the same enclosure, and for most of the year undergo identical management procedures. Indeed, depending on the species and for several of the examined personality traits, variations in their expression were identifiable (Quintavalle Pastorino et al., 2019) indicating a behavioural heterogeneity both within and between species. Regarding the penguin group at SEA LIFE Porto, distinct personalities were associated with varying responses to training. For example, Hickory, previously described as presenting a cautious character, allowed physical contact only on one occasion (Figure 22D). For the remaining sessions, Hickory resisted touch and showed consistent apprehension throughout each training interaction. Opposites really do attract, since its partner Tommy, who exhibited a more responsive and curious character, had

markedly different training outcomes. Tommy was among the most consistent penguins and demonstrated particularly promising progress in its training (*Figure 15D*). Even among closely paired individuals, personality differences can lead to significantly different levels of engagement and success in training, highlighting the broader implications of personality on training effectiveness.

Quintavalle Pastorino et al. (2019) noted additional differences between sexes, with both Gentoo and Northern Rockhopper male penguins being significantly more hostile toward keepers and observers and, even though not statistically significant, more likely to play than females. The findings of this study indicate that males tolerated training more easily than females, reaching significantly higher steps during training sessions. Subjective observations revealed that females were generally more cautious and averse to physical contact, whereas males demonstrated greater tolerance throughout the training process. This suggests that sex could be a key factor influencing training progress, particularly within this group. Understanding its impact on training potential can provide valuable insights for optimising protocols not only for this population but also across other groups.

These results emphasise the diverse range of factors that can impact the training process, shedding light on the discrepancies observed in the trainability of the penguins at SEA LIFE Porto. Each penguin reacted differently to each training step, with some advancing more quickly while others either stalled or did not progress at all. It was not uncommon to see progress followed by setbacks, even when the animals had shown no signs of distress in previous sessions. This variability further underscores the importance of tailoring the training to the factors that influence the penguins' response, be they individual differences or other external factors.

During the breeding and nesting period, many penguins chose to stay in their nests. Those who ventured into the training area appeared to have realised that they would still be fed at the nest without needing to participate in the training sessions, leading some to stop attending these. To address this issue, it would be advisable to have a longer separation between regular feeding and training. Melfi et al. (2019) suggest feeding several hours after training to maintain the two activities distinct.

Ad libitum observations suggested that penguins became more comfortable with physical touch and proximity to keepers during and after training sessions, particularly indoors, where they showed reduced avoidance and tolerated light touches. However, in the outdoor area, the penguins exhibited more avoidance behaviour, often moving away

from keepers. This disparity in behaviour may reflect territoriality, with the penguins perceiving the outdoor environment as their domain and thus reacting more defensively toward human presence. This suggests that the penguins may adjust more easily to human interactions in controlled environments, as long as they occur outside their territory.

5. Conclusion

The training process was designed to be highly adjustable, seamlessly fitting into the centre's existing management routines. This flexibility allowed the keepers to incorporate training activities into their daily workload, making the process more practical. Despite this adjustability, the training methods remained consistent throughout, with continuous assessments of both the animals' performance and the suitability of each training step. Adjustments were made as needed to respect each animal's boundaries. Training sessions should continue until every animal in the group consistently performs the intended behaviour.

Based on this training attempt, it is recommended to use sprat as the primary reinforcer on training days, while alternating between sprat and capelin for non-training days. Sprats are not only preferred by penguins but are also smaller in size, requiring them to consume more of this fish to feel satiated, therefore prolonging the feeding period and extending the duration of the training session. Likewise, it is recommended to extend the separation between training sessions and regular feeding times to enhance efficiency. For practical implementation, training should be carried out first, followed by maintenance of the displays and nests, and finally feeding. This approach ensures a clear temporal separation between the two activities without significantly disrupting the keepers' routines.

While this work has provided useful insights into the penguins' behaviour and their responses to training, future studies could further explore the impact of prior training on the learning abilities of Humboldt penguins. This knowledge would help determine whether early training provides a foundational advantage and how training at different stages of life can affect the duration and success of the whole process. Additional studies on the age-related learning times in penguins, as well as sex-related trainability differences, would be beneficial to understanding the matter more comprehensively. It would also be interesting to observe how the training process unfolds outside of the reproductive and nesting

seasons. With the penguins consistently leaving their nests and approaching the indoor area, the training process is expected to progress much more quickly.

In conclusion, it is important to reiterate that this work was meant to outline a flexible training approach rather than establish strict rules. This method was designed to adjust to keepers' daily routines so that it can hopefully be adopted by other zoos and aquariums, especially when time is limited. While this process was not perfect and there is room for improvement, it serves as a foundational training approach that can be refined based on the experiences and setbacks reported here. The effort at SEA LIFE Porto demonstrates that with patience and consistency, training can be both straightforward and efficient.

Final Remarks

This Dissertation has briefly explored how EE's introduction influenced penguins' behaviour in captivity. Reflecting on the objectives outlined in the introduction, the effectiveness of EE devices varied by season. Swimming behaviours had a slight increase with enrichment, even though this did not translate to the whole period of observations. Walking behaviours remained consistent throughout time, although low, and exploratory behaviours suffered marked fluctuations in accordance with the time of the year. Slight differences in enclosure use were noticed, although not for all zones.

Determining whether the training process qualified as enrichment is challenging. A more empirical analysis, with results based on statistically significant data would be essential to make a definitive assessment. Nonetheless, the animal-keeper relationship appeared to have improved during this process, with the penguins gradually becoming more comfortable with touch. This process marked a promising initial step toward establishing a formalised animal training program at SEA LIFE Porto.

If all goes well, public awareness will continue to grow, making the work developed at SEA LIFE Porto increasingly vital. These efforts not only enhance the welfare of the animals but also serve as an example for other institutions in demonstrating a commitment to prioritising animal care. Increased public awareness may create positive pressure to allocate more resources to animal welfare and, given the complexity and time-consuming nature of initiatives such as environmental enrichment, animal training, and behaviour assessment, it is becoming increasingly clear that institutions need dedicated teams to address these important issues.

Bibliographical References

- Alfaro-Shigueto, J., Mangel, J. C., Pajuelo, M., Dutton, P. H., Seminoff, J. A., & Godley, B. J. (2010). Where small can have a large impact: Structure and characterization of small-scale fisheries in Peru. *Fisheries Research*, 106(1), 8–17. <https://doi.org/10.1016/j.fishres.2010.06.004>.
- Ancel, A., Beaulieu, M., & Gilbert, C. (2013). The different breeding strategies of penguins: A review. *Comptes Rendus Biologies*, 336(1), 1–12. <https://doi.org/10.1016/j.crvi.2013.02.002>.
- Anderson, R. C., & Wood, J. B. (2001). Enrichment for Giant Pacific Octopuses: Happy as a Clam? *Journal of Applied Animal Welfare Science*, 4(2), 157–168. https://doi.org/10.1207/s15327604jaws0402_10.
- Arechavala-Lopez, P., Cabrera-Álvarez, M. J., Maia, C. M., & Saraiva, J. L. (2021). Environmental enrichment in fish aquaculture: A review of fundamental and practical aspects. *Reviews in Aquaculture*, 14(2), 704–728. <https://doi.org/10.1111/raq.12620>.
- Barber, J. C. E. (2009). Programmatic approaches to assessing and improving animal welfare in zoos and aquariums. *Zoo Biology*, 28(6), 519–530. <https://doi.org/10.1002/zoo.20260>.
- Bain, M. (2019). Overview of Animal Training: A Welfare Perspective. *Encyclopedia of Animal Behavior*, 203–213. <https://doi.org/10.1016/b978-0-12-809633-8.20850-9>.
- Beaulieu, M. (2024). Capturing wild animal welfare: a physiological perspective. *Biological Reviews*, 99(1), 1–22. <https://doi.org/10.1111/brv.13009>.
- Beausoleil, N. J., Swason, J. C., McKeegan, D. E. F., & Croney, C. C. (2023). Application of the Five Domains Model to food chain management of animal welfare: opportunities and constraints. *Frontiers in Animal Science*, 4:1042733. <https://doi.org/10.3389/fanim.2023.1042733>.
- Binding, S., Farmer, H., Krusin, L., Cronin, K. (2020). Status of animal welfare research in zoos and aquariums: Where are we, where to next? *Journal of Zoo and Aquarium Research*, 8(3), 166–174. <https://doi.org/10.19227/jzar.v8i3.505>.
- BirdLife International. (2020). *Spheniscus humboldti*. The IUCN Red List of Threatened Species 2020: e.T22697817A182714418. <https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T22697817A182714418.en>.
- Bloomsmith, M. A., Stone, A. M., & Laule, G. E. (1998). Positive reinforcement training to enhance the voluntary movement of group-housed chimpanzees within their enclosures. *Zoo Biology*, 17(4), 333–341. [https://doi.org/10.1002/\(sici\)1098-2361\(1998\)17:4%3C333::aid-zoo6%3E3.0.co;2-a](https://doi.org/10.1002/(sici)1098-2361(1998)17:4%3C333::aid-zoo6%3E3.0.co;2-a).
- Booth, D., Kamau, A., Kayondo, H., Sumaya, A. M., & Ashraf, M. W. (2022). Training a Giraffe (*Giraffa camelopardalis reticulata*) for Voluntary Foot Radiographs at Dubai Safari Park. *Journal of Zoological and Botanical Gardens*, 3(4), 688–698. <https://doi.org/10.3390/jzbg3040051>.
- Bowmaker, J. K., Martin, G. R. (1985). Visual pigments and oil droplets in the penguin, *Spheniscus humboldti*. *Journal of Comparative Physiology A*, 156, 71–77. <https://doi.org/10.1007/bf00610668>.
- Brambell Report (1965). *Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems*. Her Majesty's Stationery Office, London, United Kingdom. Retrieved March 2, 2024, from <https://archive.org/details/b3217276x/mode/2up?q=discipline>.
- Brando, S. I. C. A. (2012). Animal Learning and Training: Implications for Animal Welfare. *Veterinary Clinics of North America: Exotic Animal Practice*, 15(3), 387–398. <https://doi.org/10.1016/j.cvex.2012.06.008>.
- Brando, S., & Norman, M. (2023). Handling and Training of Wild Animals: Evidence and Ethics-Based Approaches and Best Practices in the Modern Zoo. *Animals*, 13(14), 2247. <https://doi.org/10.3390/ani13142247>.
- Brereton, J. E. (2020). Directions in animal enclosure use studies. *Journal of Zoo and Aquarium Research*, 8(1), 1–9. <https://doi.org/10.19227/jzar.v8i1.330>.

- Brereton, J. E., & Fernandez, E. J. (2022). Which index should I use? A comparison of indices for enclosure use studies. *Animal Behavior and Cognition*, 9(1), 119–132. <https://doi.org/10.26451/abc.09.01.10.2022>.
- Broom, D. M. (1988). The scientific assessment of animal welfare. *Applied Animal Behaviour Science*, 20(1-2), 5–19. [https://doi.org/10.1016/0168-1591\(88\)90122-0](https://doi.org/10.1016/0168-1591(88)90122-0).
- Browning, H. (2023). Welfare comparisons within and across species. *Philosophical Studies*, 180, 529–551. <https://doi.org/10.1007/s11098-022-01907-1>.
- Capdeville, J., & Veissier, I. (2001). A Method of Assessing Welfare in Loose Housed Dairy Cows at Farm Level, Focusing on Animal Observations. *Acta Agriculturae Scandinavica, Section A — Animal Science*, 51(sup030), 62–68. <https://doi.org/10.1080/090647001316923081>.
- Carenzi, C., & Verga, M. (2009). Animal welfare: review of the scientific concept and definition. *Italian Journal of Animal Science*, 8(sup1), 21–30. <https://doi.org/10.4081/ijas.2009.s1.21>.
- Carlstead, K., Seidensticker, J., & Baldwin, R. (1991). Environmental enrichment for zoo bears. *Zoo Biology*, 10(1), 3–16. <https://doi.org/10.1002/zoo.1430100103>.
- Castro, L., Wasserman, E. A. (2009). Animal learning. *Wiley Interdisciplinary Reviews: Cognitive Science*, 1(1): 89–98. <https://doi.org/10.1002/wcs.18>.
- Chiew, S. J., Butler, K. L., Sherwen, S. L., Coleman, G. J., Fanson, K. V., & Hemsworth, P. H. (2019). Effects of regulating visitor viewing proximity and the intensity of visitor behaviour on little penguin (*Eudyptula minor*) behaviour and welfare. *Animals*, 9(6), 285. <https://doi.org/10.3390/ani9060285>.
- Cohn, J. P. (1988). Captive breeding for conservation: Despite success stories, this strategy remains controversial within the conservation community. *BioScience*, 38(5), 312–316. <https://doi.org/10.2307/1310732>.
- Coker, R. E. (1919). Habits and economic relations of the guano birds of Peru. *Proceedings of the United States National Museum*, 56(2298), 449–511.
- Coleman, K., & Novak, M. A. (2017). Environmental Enrichment in the 21st Century. *Institute for Laboratory Animal Research Journal*, 58(2), 295–307. <https://doi.org/10.1093/ilar/ilx008>.
- Collins, C. K., Quirke, T., Overy, L., Flannery, K., & O'Riordan, R. (2016). The effect of the zoo setting on the behavioural diversity of captive gentoo penguins and the implications for their educational potential. *Journal of Zoo and Aquarium Research*, 4(2), 85–90. <https://doi.org/10.19227/jzar.v4i2.158>.
- Cooke, S. J., Blumstein, D. T., Buchholz, R., Caro, T., Fernández-Juricic, E., Franklin, C. E., Metcalfe, J., O'Connor, C. M., St. Clair, C. C., Sutherland, W. J., & Wikelski, M. (2014). Physiology, Behavior, and Conservation. *Physiological and Biochemical Zoology*, 87(1), 1–14. <https://doi.org/10.1086/671165>.
- Conde, D. A., Flesness, N., Colchero, F., Jones, O. R., & Scheuerlein, A. (2011). An Emerging Role of Zoos to Conserve Biodiversity. *Science*, 331(6023), 1390–1391. <https://doi.org/10.1126/science.1200674>.
- Crawford, R., Ellenberg, U., Frere, E., Hagen, C., Baird, K., Brewin, P., Crofts, S., Glass, J., Mattern, T., Pompert, J., Ross, K., Kemper, J., Ludynia, K., Sherley, R.B., Steinfurth, A., Suazo, C.G., Yorio, P., Tamini, L., Mangel, J.C., Bugoni, L., Uzcátegui, G.J., Simeone, A., Luna-Jorquera, G., Gandini, P., Woehler, E.J., Pütz, K., Dann, P., Chiaradia, A., & Small, C. (2017). Tangled and drowned: a global review of penguin bycatch in fisheries. *Endangered Species Research*, 34, 373–396. <https://doi.org/10.3354/esr00869>.
- Croxall, J.P., Davis, L.S. (1999). Penguins: paradoxes and patterns. *Marine Ornithology*, 27, 9–11.
- Culik, B. (2001). Finding food in the open ocean: foraging strategies in Humboldt penguins. *Zoology*, 104(3-4), 327–338. <https://doi.org/10.1078/0944-2006-00038>.
- Culik, B., Hennicke, J., & Martin, T. (2000). Humboldt penguins outmanoeuvring El Niño. *Journal of Experimental Biology*, 203(15), 2311–2322. <https://doi.org/10.1242/jeb.203.15.2311>.
- Dantzer, R., & Mormède, P. (1983). Stress in Farm Animals: A Need for Reevaluation. *Journal of Animal Science*, 57(1), 6–18. <https://doi.org/10.2527/jas1983.5716>.

- Dawkins, M. S. (2003). Behaviour as a tool in the assessment of animal welfare. *Zoology*, 106(4), 383–387. <https://doi.org/10.1078/0944-2006-00122>.
- De la Puente, S., Bussalleu, A., Cardeña, M., Valdés-Velásquez, A., Majluf, P., & Simeone, A. (2013). Humboldt Penguin. In: *Penguins—Natural History and Conservation*, P. G. Borboroglu & P. D. Boersma (Eds.), University of Washington Press, 265–283, ISBN: 978-0-295-99284-6.
- Dockès, A. C., & Kling-Eveillard, F. (2006). Farmers' and advisers' representations of animals and animal welfare. *Livestock Science*, 103(3), 243–249. <https://doi.org/10.1016/j.livsci.2006.05.012>.
- Duncan, A. E., Torgerson-White, L. L., Allard, S. M., Schneider, T. (2016). An Evaluation of Infrared Thermography for Detection of Bumblefoot (pododermatitis) in Penguins. *Journal of Zoo and Wildlife Medicine* 47(2), 474–485. <https://www.jstor.org/stable/24774001>.
- Edes, A. N., Baskir, E., Bauman, K. L., Chandrasekharan, N., Macek, M., & Tieber, A. (2021). Effects of Crowd Size, Composition, and Noise Level On Pool Use in a Mixed-Species Penguin Colony. *Animal Behavior and Cognition*, 8(4), 507–520. <https://doi.org/10.26451/abc.08.04.05.2021>.
- Eggleton, P., & Siegfried, W. R. (1979). DISPLAYS OF THE JACKASS PENGUIN. *Ostrich*, 50(3), 139–167. <https://doi.org/10.1080/00306525.1979.9634105>.
- Ellenberg, U., Mattern, T., Seddon, P. J., & Jorquera, G. L. (2006). Physiological and reproductive consequences of human disturbance in Humboldt penguins: The need for species-specific visitor management. *Biological Conservation*, 133(1), 95–106. <https://doi.org/10.1016/j.biocon.2006.05.019>.
- Erlacher-Reid, C., Dunn, J. L., Camp, T., Macha, L., Mazzaro, L., & Tuttle, A. D. (2011). Evaluation of potential variables contributing to the development and duration of plantar lesions in a population of aquarium-maintained African penguins (*Spheniscus demersus*). *Zoo Biology*, 31(3), 291–305. <https://doi.org/10.1002/zoo.20395>.
- Fanning, L., Larsen, H., & Taylor, P. S. (2020). A Preliminary Study Investigating the Impact of Musical Concerts on the Behavior of Captive Fiordland Penguins (*Eudyptes pachyrhynchus*) and Collared Peccaries (*Pecari tajacu*). *Animals*, 10(11), 2035. <https://doi.org/10.3390/ani10112035>.
- Farm Animal Welfare Council (FAWC). (1993). Report on Priorities for Animal Welfare Research and Development. Retrieved March 3, 2024, from <https://edepot.wur.nl/134980>.
- Farm Animal Welfare Council (FAWC). (2007). FAWC Report on Stockmanship and Farm Animal Welfare Retrieved March 3, 2024, from https://assets.publishing.service.gov.uk/media/5a7eb072e5274a2e8ab47927/FAWC_report_on_stockmanship_and_farm_animal_welfare.pdf.
- Farm Animal Welfare Council (FAWC). (2009). Farm Animal Welfare in Great Britain: Past, Present and Future. Retrieved March 3, 2024, from <https://www.ongehoord.info/wp-content/uploads/2017/12/11-1.pdf>.
- Fernandez, E.J. (2022). Training as enrichment: A critical review. *Animal Welfare*, 31(1), 1–12. <https://doi.org/10.7120/09627286.31.1.001>.
- Fernandez, E. J., Myers, M., & Hawkes, N. C. (2021). The Effects of Live Feeding on Swimming Activity and Exhibit Use in Zoo Humboldt Penguins (*Spheniscus humboldti*). *Journal of Zoological and Botanical Gardens*, 2(1), 88–100. <https://doi.org/10.3390/jzbg2010007>.
- Glavis-Bloom, C., Vanderlip, C. R., & Reynolds, J. H. (2022). Age-Related Learning and Working Memory Impairment in the Common Marmoset. *Journal of Neuroscience*, 42(47), 8870–8880. <https://doi.org/10.1523/JNEUROSCI.0985-22.2022>.
- Goodenough, A. E., Sewell, A., & McDonald, K. (2023). Behavioural patterns in zoo-housed Humboldt penguins (*Spheniscus humboldti*) revealed using long-term keeper-collected data: validation of approaches and improved husbandry. *Applied Animal Behaviour Science*, 258, 105811. <https://doi.org/10.1016/j.applanim.2022.105811>.

- Gosling, S. D. (2008). Personality in Non-human Animals. *Social and Personality Psychology Compass*, 2(2), 985–1001. <https://doi.org/10.1111/j.1751-9004.2008.00087.x>.
- Gosling, S. D., & Vazire, S. (2002). Are we barking up the right tree? Evaluating a comparative approach to personality. *Journal of Research in Personality*, 36(6), 607–614. [https://doi.org/10.1016/s0092-6566\(02\)00511-1](https://doi.org/10.1016/s0092-6566(02)00511-1).
- Hays, C. (1986). Effects of the 1982–1983 El Nino on Humboldt penguin colonies in Peru. *Biological Conservation*, 36(2), 169–180. [https://doi.org/10.1016/0006-3207\(86\)90005-4](https://doi.org/10.1016/0006-3207(86)90005-4).
- Heidenreich, B. (2007). An Introduction to Positive Reinforcement Training and Its Benefits. *Journal of Exotic Pet Medicine*, 16(1), 19–23. <https://doi.org/10.1053/j.jepm.2006.11.005>.
- Heidenreich, B., Pedersen, A., Mackie, J., Harding, L. (2023). *EAZA Animal Training Guidelines – 1st Edition*. European Association of Zoos and Aquaria. Amsterdam, The Netherlands.
- Hennicke, J. C., & Culik, B. M. (2005). Foraging performance and reproductive success of Humboldt penguins in relation to prey availability. *Marine Ecology Progress Series*, 296, 173–181. <https://doi.org/10.3354/meps296173>.
- Herling, C., Culik, B. M., & Hennicke, J. C. (2005). Diet of the Humboldt penguin (*Spheniscus humboldti*) in northern and southern Chile. *Marine Biology*, 147, 13–25. <https://doi.org/10.1007/s00227-004-1547-8>.
- Hill, S.P. & Broom, D.M. (2009). Measuring zoo animal welfare: theory and practice. *Zoo Biology*, 28(6), 531–544. <https://doi.org/10.1002/zoo.20276>.
- International Union for Conservation of Nature (IUCN). (2023). *The IUCN Red List of Threatened Species*. Retrieved January 10, 2024, from <https://www.iucnredlist.org/>.
- Jerez-Cepa, I., & Ruiz-Jarabo, I. (2021). Physiology: An Important Tool to Assess the Welfare of Aquatic Animals. *Biology*, 10(1), 61. <https://doi.org/10.3390/biology10010061>.
- Keulartz, J. (2015). Captivity for Conservation? Zoos at a Crossroads. *Journal of Agricultural and Environmental Ethics*, 28, 335–351. <https://doi.org/10.1007/s10806-015-9537-z>.
- Kresnye, K.C., Chung, C.-F., Martin, C.F., & Shih, P.C. (2022). Survey on the Past Decade of Technology in Animal Enrichment: A Scoping Review. *Animals*, 12(14), 1792. <https://doi.org/10.3390/ani12141792>.
- Laule, G. E., Bloomsmith, M. A., Schapiro, S. J. (2003). The Use of Positive Reinforcement Training Techniques to Enhance the Care, Management, and Welfare of Primates in the Laboratory. *Journal of Applied Animal Welfare Science*, 6(3), 163–173. https://doi.org/10.1207/s15327604jaws0603_02.
- Lawrence, A. B., Vigors, B., & Sandøe, P. (2019). What Is so Positive about Positive Animal Welfare?—A Critical Review of the Literature. *Animals*, 9(10), 783. <https://doi.org/10.3390/ani9100783>.
- Luna-Jorquera, G., Garthe, S., Sepulveda, F. G., Weichler, T., & Vásquez, J. A. (2000). Population Size of Humboldt Penguins Assessed by Combined Terrestrial and At-Sea Counts. *Waterbirds*, 23(3), 506–510. <https://doi.org/10.2307/1522191>.
- Maple, T.L., & Perdue, B.M. (2013). Environmental Enrichment. In: *Zoo Animal Welfare*. Springer, Berlin, Heidelberg, *Animal Welfare*, 14, 95–117. https://doi.org/10.1007/978-3-642-35955-2_6.
- Marashi, V., Barnekow, A., Ossendorf, E., & Sachser, N. (2003). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Hormones and Behavior*, 43(2), 281–292. [https://doi.org/10.1016/s0018-506x\(03\)00002-3](https://doi.org/10.1016/s0018-506x(03)00002-3).
- Marranzino, A. (2013). The Use of Positive Reinforcement in Training Zebra Sharks (*Stegostoma fasciatum*). *Journal of Applied Animal Welfare Science*, 16(3), 239–253. <https://doi.org/10.1080/10888705.2013.798555>.
- Marshall, A. R., Deere, N. J., Little, H. A., Snipp, R., Goulder, J., & Mayer-Clarke, S. (2016). Husbandry and enclosure influences on penguin behavior and conservation breeding. *Zoo Biology*, 35(5), 385–397. <https://doi.org/10.1002/zoo.21313>.

- Mason, G. J. (1991). Stereotypies and suffering. *Behavioural Processes*, 25(2-3), 103–115. [https://doi.org/10.1016/0376-6357\(91\)90013-P](https://doi.org/10.1016/0376-6357(91)90013-P).
- Mason, G. J. (2010). Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution*, 25(12), 713–721. <https://doi.org/10.1016/j.tree.2010.08.011>.
- Melfi, V. (2013). Is training zoo animals enriching? *Applied Animal Behaviour Science*, 147(3-4), 299–305. <https://doi.org/10.1016/j.applanim.2013.04.011>.
- Melfi, V. A., & Ward, S. J. (2019). Welfare Implications of Zoo Animal Training. In: *Zoo Animal Learning and Training*, V.A. Melfi, N.R. Dorey and S.J. Ward (Eds.), 271–288. <https://doi.org/10.1002/9781118968543.ch11>.
- Mellen, J., & Sevenich MacPhee, M. (2001). Philosophy of environmental enrichment: Past, present, and future. *Zoo Biology*, 20(3), 211–226. <https://doi.org/10.1002/zoo.1021>.
- Mellor, D. J. (2017). Operational Details of the Five Domains Model and Its Key Applications to the Assessment and Management of Animal Welfare. *Animals*, 7(8), 60. <https://doi.org/10.3390/ani7080060>.
- Mellor, D. J., Beausoleil, N. J., Littlewood, K. E., McLean, A. N., McGreevy, P. D., Jones, B., & Wilkins, C. (2020). The 2020 Five Domains Model: Including Human–Animal Interactions in Assessments of Animal Welfare. *Animals*, 10(10), 1870. <https://doi.org/10.3390/ani10101870>.
- Mellor, D. J., Hunt, S. & Gusset, M. (Eds). (2015). *Caring for Wildlife: The World Zoo and Aquarium Animal Welfare Strategy*. Gland: WAZA Executive Office, ISBN: 978-2-8399-1695-0.
- Merlin Entertainments (SEA LIFE). (n.d.). *Explore | Official SEA LIFE Website*. Retrieved January 10, 2024, from <https://www.visitsealife.com/explore/>.
- Merlin Entertainments. (2024a). *SEA LIFE Porto | Grupos*. Retrieved January 10, 2024, from <https://www.visitsealife.com/porto/visitar/bilhetes-e-passes/grupos/>.
- Merlin Entertainments. (2024b). *SEA LIFE Porto | As nossas Espécies*. Retrieved January 10, 2024, from <https://www.visitsealife.com/porto/descobrir/mapa-sea-life-porto/as-nossas-especies/>.
- Merritt, K., & King, N. E. (1987). Behavioral sex differences and activity patterns of captive humboldt penguins (*Spheniscus humboldti*). *Zoo Biology*, 6(2), 129–138. <https://doi.org/10.1002/zoo.1430060204>.
- Miller, R., & King, C. E. (2013). Husbandry training, using positive reinforcement techniques, for Marabou stork *Leptoptilos crumeniferus* at Edinburgh Zoo. *International Zoo Yearbook*, 47(1), 171–180. <https://doi.org/10.1111/izy.12001>.
- Miller, L. J., Lauderdale, L. K., Bryant, J. L., Mellen, J. D., Walsh, M. T., & Granger, D. A. (2021). Behavioral diversity as a potential positive indicator of animal welfare in bottlenose dolphins. *PLOS ONE*, 16(8), e0253113. <https://doi.org/10.1371/journal.pone.0253113>.
- Montecino, V., & Lange, C. B. (2009). The Humboldt Current System: Ecosystem components and processes, fisheries, and sediment studies. *Progress in Oceanography*, 83(1-4), 65–79. <https://doi.org/10.1016/j.pocean.2009.07.041>.
- Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, 102(3-4), 262–302. <https://doi.org/10.1016/j.applanim.2006.05.032>.
- Muhammad, M., Stokes, J. E., & Manning, L. (2022). Positive Aspects of Welfare in Sheep: Current Debates and Future Opportunities. *Animals*, 12(23), 3265. <https://doi.org/10.3390/ani12233265>.
- Neal Webb, S. J., Bridges, J. P., Thiele, E., Lambeth, S. P., Schapiro, S. J. (2020) The implementation and initial evaluation of a physical therapy program for captive chimpanzees (*Pan troglodytes*). *American Journal of Primatology*, 82(3), e23109. <https://doi.org/10.1002/ajp.23109>.
- Newberry, R. C. (1995). Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science*, 44(2-4), 229–243. [https://doi.org/10.1016/0168-1591\(95\)00616-z](https://doi.org/10.1016/0168-1591(95)00616-z).

- O'Brien, S. L., & Cronin, K. A. (2023). The Impacts of a Commercial Bubble Curtain on Zoo-Housed African Penguin (*Spheniscus demersus*) Swimming Behavior. *Journal of Zoological and Botanical Gardens*, 4(3), 567–577. <https://doi.org/10.3390/jzbg4030040>.
- Ozella, L., Favaro, L., Carnovale, I., & Pessani, D. (2014). Pond Use by Captive African Penguins (*Spheniscus demersus*) in an Immersive Exhibit Adjacent to Human Bathers. *Journal of Applied Animal Welfare Science*, 18(3), 303–309. <https://doi.org/10.1080/10888705.2014.977384>.
- Paredes, R., & Zavalaga, C. B. (2001). Nesting sites and nest types as important factors for the conservation of Humboldt penguins (*Spheniscus humboldti*). *Biological Conservation*, 100(2), 199–205. [https://doi.org/10.1016/S0006-3207\(01\)00023-4](https://doi.org/10.1016/S0006-3207(01)00023-4).
- Paredes, R., Zavalaga, C. B., & Boness, D. J. (2002). Patterns of Egg Laying and Breeding Success in Humboldt Penguins (*Spheniscus Humboldtii*) at Punta San Juan, Peru. *The Auk*, 119(1), 244–250. <https://doi.org/10.1093/auk/119.1.244>.
- Paredes, R., Zavalaga, C. B., Battistini, G., Majluf, P., & McGill, P. (2003). Status of the Humboldt Penguin in Peru, 1999-2000. *Waterbirds*, 26(2), 129–138. [https://doi.org/10.1675/1524-4695\(2003\)026\[0129:SOTHPI\]2.0.CO;2](https://doi.org/10.1675/1524-4695(2003)026[0129:SOTHPI]2.0.CO;2).
- Patrick, P. G., Matthews, C. E., Ayers, D. F., & Tunnicliffe, S. D. (2007). Conservation and Education: Prominent Themes in Zoo Mission Statements. *The Journal of Environmental Education*, 38(3), 53–60. <https://doi.org/10.3200/joe.38.3.53-60>.
- Porcher, J. (1997). La relation de communication entre l'éleveur et ses animaux: un domaine encore à explorer. *Courrier de l'environnement de l'INRA*, 32, 51–62.
- Pomerantz, O., Terkel, J. (2009) Effects of positive reinforcement training techniques on the psychological welfare of zoo-housed chimpanzees (*Pan troglodytes*). *American Journal of Primatology*, 71(8), 687–695. <https://doi.org/10.1002/ajp.20703>.
- Quintavalle Pastorino, G., Preziosi, R., Faustini, M., Curone, G., Albertini, M., Nicoll, D., Moffat, L., Pizzi, R., & Mazzola, S. (2019). Comparative Personality Traits Assessment of Three Species of Communally Housed Captive Penguins. *Animals*, 9(6), 376. <https://doi.org/10.3390/ani9060376>.
- Quispe, R., Lerma, M., Luna, N., Portflitt-Toro, M., Serratosa, J., & Luna-Jorquera, G. (2020). Foraging ranges of Humboldt Penguins *Spheniscus humboldti* from Tilgo Island: the critical need for protecting a unique marine habitat. *Marine Ornithology*, 48, 205-208.
- Razal, C., & Miller, L. (2021). The effect of different types of environmental enrichment on Humboldt penguin *Spheniscus humboldti* behaviour. *Journal of Zoo and Aquarium Research*, 9(4), 281–286. <https://doi.org/10.19227/jzar.v9i4.411>.
- Reisfeld, L., Barbirato, M., Ippolito, L., Cardoso, R. C., Nichi, M., Sgai, M. G. F. G., & Pizzutto, C. S. (2013). Reducing bumblefoot lesions in a group of captive Magellanic penguins (*Spheniscus magellanicus*) with the use of environmental enrichment. *Pesquisa Veterinária Brasileira*, 33(6), 791–795. <https://doi.org/10.1590/S0100-736X2013000600017>.
- Rößler, H., May, A., Dähne, M., & Beaulieu, M. (2022). Long and winding road: Training progress and trainability variation across a psychoacoustic experiment in penguins. *Applied Animal Behaviour Science*, 256, 105764. <https://doi.org/10.1016/j.applanim.2022.105764>.
- Romero, P., Restitutti, F., McGill, N., Hoey, S., & Bennett, R. C. (2023). Anaesthetic management and complications of a Humboldt penguin (*Spheniscus humboldti*) undergoing diagnostic imaging. *Irish Veterinary Journal*, 76(29). <https://doi.org/10.1186/s13620-023-00256-7>.
- Schwartz, M. K., Boness, D.J., Schaeff, C.M., Majluf, P., Perry, E.A., & Fleischer, R.C. (1999). Female-solicited extrapair matings in Humboldt penguins fail to produce extrapair fertilizations. *Behavioral Ecology*, 10(3), 242–250. <https://doi.org/10.1093/beheco/10.3.242>.

- Sherwen, S. L., Magrath, M. J. L., Butler, K. L., & Hemsworth, P. H. (2015). Little penguins, *Eudyptula minor*, show increased avoidance, aggression and vigilance in response to zoo visitors. *Applied Animal Behaviour Science*, 168, 71–76. <https://doi.org/10.1016/j.applanim.2015.04.007>.
- Seier, J. V., Dhansay, M. A., & Davids, A. (2005). Risks associated with environmental enrichment: intestinal obstruction caused by foraging substrate. *Journal of Medical Primatology*, 34(3), 154–155. <https://doi.org/10.1111/j.1600-0684.2005.00105.x>.
- Simeone, A., & Luna-Jorquera, G. (2012). Estimating rat predation on Humboldt Penguin colonies in north-central Chile. *Journal of Ornithology*, 153, 1079–1085. <https://doi.org/10.1007/s10336-012-0837-z>.
- Simeone, A., Araya, B., Bernal, M., Diebold, E. N., Grzybowski, K., Michaels, M., Teare, J. A., Wallace, R. S., & Willis, M. J. (2002a). Oceanographic and climatic factors influencing breeding and colony attendance patterns of Humboldt penguins *Spheniscus humboldti* in central Chile. *Marine Ecology Progress Series*, 227, 43–50. <https://doi.org/10.3354/meps227043>.
- Simeone, A., Wilson, R.P., Knauf, G., Knauf, W. & Schützendübe, J. (2002b). Effects of attached data-loggers on the activity budgets of captive Humboldt penguins. *Zoo Biology*, 21(4), 365–373. <https://doi.org/10.1002/zoo.10044>.
- Simeone, A., & Bernal, M. (2000). Effects of Habitat Modification on Breeding Seabirds: A Case Study in Central Chile. *Waterbirds*, 23(3), 449–456. <https://doi.org/10.2307/1522182>.
- Simeone, A., Bernal, M. & Meza, J. 1999. Incidental mortality of Humboldt Penguins *Spheniscus humboldti* in gill nets, central Chile. *Marine Ornithology*, 27 (1), 157–161.
- Sivak, J., Howland, H. C. & McGill-Harelsstad, P. 1987. Vision of the Humboldt penguin (*Spheniscus humboldti*) in air and water. *Proceedings of the Royal Society B*, 229, 467–472. <http://doi.org/10.1098/rspb.1987.0005>.
- Smith, K. M., Karesh, W. B., Majluf, P., Paredes, R., Zavalaga, C., Reul, A. H., Stetter, M., Braselton, W. E., Puche, H., & Cook, R. A. (2008). Health Evaluation of Free-Ranging Humboldt Penguins (*Spheniscus humboldti*) in Peru. *Avian Diseases*, 52(1), 130–135. <http://www.jstor.org/stable/27565711>.
- Spiezio, C., Piva, F., Regaiolli, B., & Vaglio, S. (2015). Positive reinforcement training: a tool for care and management of captive vervet monkeys (*Chlorocebus aethiops*). *Animal Welfare*, 24(3), 283–290. <https://doi.org/10.7120/09627286.24.3.283>.
- Sroka, M. G. U., Ambree, O., Dohmen, C., Palme, R., Kaiser, S., & Richter, S. H. (2024). Personality matters – The interplay between consistent individual differences and mouse welfare in female C57BL6/J mice. *Frontiers in Animal Science*, 5:1423814. <https://doi.org/10.3389/fanim.2024.1423814>.
- Tallo-Parra, O., Salas, M., & Manteca, X. (2023). Zoo Animal Welfare Assessment: Where Do We Stand? *Animals*, 13(12), 1966. <https://doi.org/10.3390/ani13121966>.
- Taylor, S. S., Leonard, M. L., & Boness, D. J. (2001). Aggressive Nest Intrusions by Male Humboldt Penguins. *The Condor*, 103(1), 162–165. <https://doi.org/10.1093/condor/103.1.162>.
- Taylor, S. S., Leonard, M. L., Boness, D. J., & Majluf, P. (2002). Foraging by Humboldt penguins (*Spheniscus humboldti*) during the chick-rearing period: general patterns, sex differences, and recommendations to reduce incidental catches in fishing nets. *Canadian Journal of Zoology*, 80(4), 700–707. <https://doi.org/10.1139/z02-046>.
- Taylor, S. S., Leonard, M., Boness, D. J., & Patrícia Majluf. (2004). Humboldt Penguins *Spheniscus Humboldtii* change their foraging behaviour following breeding failure. *Marine Ornithology*, 32(1), 63–67.
- Trathan, P. N., García-Borboroglu, P., Boersma, D., Bost, C.-A., Crawford, R. J. M., Crossin, G. T., Cuthbert, R. J., Dann, P., Davis, L. S., De La Puente, S., Ellenberg, U., Lynch, H. J., Mattern, T., Pütz, K., Seddon, P. J., Trivelpiece, W., & Wienecke, B. (2015). Pollution, habitat loss, fishing, and climate change as critical threats to penguins. *Conservation Biology*, 29(1), 31–41. <https://doi.org/10.1111/cobi.12349>.

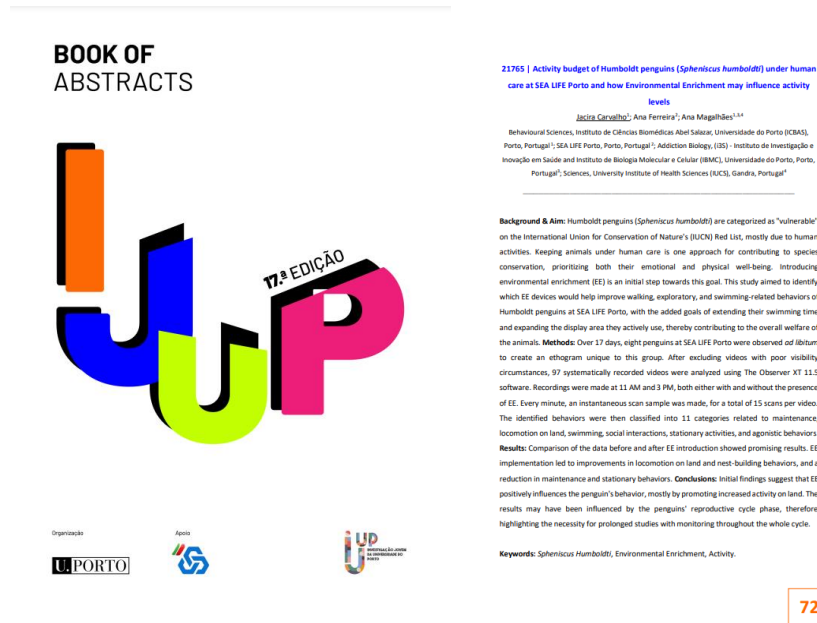
- Tribe, A., & Booth, R. (2003). Assessing the Role of Zoos in Wildlife Conservation. *Human Dimensions of Wildlife*, 8(1), 65–74. <https://doi.org/10.1080/10871200390180163>.
- Vanderploeg, H. A., & Scavia, D. (1979). Two Electivity Indices for Feeding with Special Reference to Zooplankton Grazing. *Journal of the Fisheries Research Board of Canada*, 36(4), 362–365. <https://doi.org/10.1139/f79-055>.
- Wallace, R. S. (2014). Sphenisciformes (Penguins). In: *Fowler's Zoo and Wild Animal Medicine*, R. E. Miller & M. E. Fowler, Elsevier Saunders, 8, 82-88. ISBN: 978-1-4557-7397-8.
- Wallace, R. S., Dubach, J., Michaels, M. G., Keuler, N. S., Diebold, E. D., Grzybowski, K., Teare, J. A., & Willis, M. J. (2008). Morphometric Determination of Gender in Adult Humboldt Penguins (*Spheniscus humboldti*). *Waterbirds*, 31(3), 448–453. <https://www.jstor.org/stable/25148354>.
- Wallace, R.S., & Araya, B. (2015). Humboldt Penguin *Spheniscus humboldti* population in Chile: counts of moulting birds, February 1999–2008. *Marine Ornithology*, 43, 107–112.
- Ward, S. J., Hosey, G., Williams, E., & Bailey, R. (2024). Enrichment and animal age, not biological variables, predict positive welfare indicators in zoo-housed carnivores. *Applied Animal Behaviour Science*, 270, 106006. <https://doi.org/10.1016/j.applanim.2023.106006>.
- Weimerskirch, H., Bertrand, S., Silva, J., Bost, C., & Peraltila, S. (2012). Foraging in Guanay cormorant and Peruvian booby, the major guano-producing seabirds in the Humboldt Current System. *Marine Ecology Progress Series*, 458, 231–245. <https://doi.org/10.3354/meps09752>.
- Wells, D. L. (2009). Sensory stimulation as environmental enrichment for captive animals: A review. *Applied Animal Behaviour Science*, 118(1-2), 1–11. <https://doi.org/10.1016/j.applanim.2009.01.002>.
- Westlund, K. (2014). Training is enrichment—And beyond. *Applied Animal Behaviour Science*, 152, 1–6. <https://doi.org/10.1016/j.applanim.2013.12.009>.
- Williams, E., Carter, A., Rendle, J., & Ward, S. J. (2021). Understanding impacts of zoo visitors: Quantifying behavioural changes of two popular zoo species during COVID-19 closures. *Applied Animal Behaviour Science*, 236, 105253. <https://doi.org/10.1016/j.applanim.2021.105253>.
- Williams, S. E., & Hoffman, E. A. (2009). Minimizing genetic adaptation in captive breeding programs: A review. *Biological Conservation*, 142(11), 2388–2400. <https://doi.org/10.1016/j.biocon.2009.05.034>.
- Williams, M. L., Torrini, L. A., Nolan, E. J., & Loughman, Z. J. (2022). Using Classical and Operant Conditioning to Train a Shifting Behavior in Juvenile False Water Cobras (*Hydrodynastes gigas*). *Animals*, 12(10), 1229. <https://doi.org/10.3390/ani12101229>.
- Wolfensohn, S., Shotton, J., Bowley, H., Davies, S., Thompson, S., & Justice, W. S. M. (2018). Assessment of Welfare in Zoo Animals: Towards Optimum Quality of Life. *Animals*, 8(7), 110. <https://doi.org/10.3390/ani8070110>.
- Yeates, J. W., & Main, D. C. J. (2008). Assessment of positive welfare: A review. *The Veterinary Journal*, 175(3), 293–300. <https://doi.org/10.1016/j.tvjl.2007.05.009>.
- Zavalaga, C. B., & Paredes, R. (1997). Sex Determination of Adult Humboldt Penguins Using Morphometric Characters. *Journal of Field Ornithology*, 68(1), 102–112. <https://www.jstor.org/stable/4514199>.
- Zavalaga, C. B., & Alfaro-Shigueto, J. (2015). Unveiling an Important Humboldt Penguin (*Spheniscus humboldti*) Breeding Colony in Perú and the Need for Its Protection Against the Potential Impact of Guano Harvest. *Waterbirds*, 38(3), 302–307. <https://doi.org/10.1675/063.038.0311>.
- Zhang, J., Quirke, T., Wu, S., Li, S., & Butler, F. (2021). Impact of Weather Changes and Human Visitation on the Behavior and Activity Level of Captive Humboldt Penguins. *Pakistan Journal of Zoology*, 53(2), 591–602. <https://doi.org/10.17582/journal.pjz/20191227111202>.

Appendices

Appendix A

Abstract books from the conferences attended while writing this Dissertation, along with the posters and PowerPoint presented.

1. Investigação Jovem da Universidade do Porto 17ª Edição. Porto, Portugal



2. Universities Federation for Animal Welfare – International Animal Welfare Conference 2024. Porto, Portugal



3. 2nd Regional meeting of ISAE - International Society for Applied Ethology. Porto, Portugal



2nd Regional meeting of ISAE - International Society for Applied Ethology
12 July 2024 | ICBAS, Porto, Portugal

PROGRAM

9.30 Opening and welcome

9.45-10.30 Plenary lecture | Fish welfare: an ethological approach
João Saraiva, University of Algarve

10.45-11.15 Coffee

11.15-12.15 1st session with talks from abstracts

11.15 Behaviour as key to improve laboratory zebrafish welfare: a tale from environmental enrichment and analgesia
Ana M Valentim, I3S/IBMC

11.30 Does improper stunning influence the animal-based measures of consciousness in slaughtered lambs? (online presentation)
Marta Comin, Università degli Studi di Milano

11.45 Heat Stress in Modicana Dairy Cows: Physiological Indicators and the Evaluation of New Markers for Enhanced Welfare Assessment (online presentation)
Daniela Abreu, Università di Messina

12.00 Tiredy unloading to save mouse pups
Gabriela Morello, IS

12.15-12.30 Lunch

13.30-14.00 Plenary lecture | Indicators for Animal Welfare Assessment: The Past, the Present and the Future
Pol Lönch, Universitat Autònoma de Barcelona

14.00-15.15 2nd session with talks from abstracts

14.00 Courtship and nesting period behaviors of a mallard duck population residing in an urban park in northern Portugal
Luís Eduardo Santos, CIBIO/ Faculdade de Ciências da Universidade do Porto

14.15 Training of Humboldt penguins (*Spheniscus humboldti*) at SEA LIFE Porto for medical care
Jacira Carvalho, ICBAS

Training of Humboldt penguins (*Spheniscus humboldti*) at SEA LIFE Porto for medical care

J. Carvalho¹, A. Ferreira², T. Monreal-Pastorowsky³, A. Magalhães^{1,4,5}

¹Behavioural Sciences, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS), Porto, Portugal
²SEA LIFE Porto, Porto, Portugal
³International Zoo Veterinary Group (IZVIG), United Kingdom
⁴Addiction Biology, i3S - Instituto de Investigação e Inovação em Saúde and Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal
⁵Sciences, University Institute of Health Sciences (IUCS), Gandra, Portugal

ap20190555@edu.icbas.up.pt

The International Union for Conservation of Nature (IUCN) Red List details Humboldt penguins (*Spheniscus humboldti*) as "vulnerable," mostly due to the detrimental effects of human activities. One of the methods to support the conservation of the species is to keep animals under human care. However, in many cases, with captivity also comes increased inactivity, one of the factors that ultimately might lead to ulcerative pododermatitis, commonly known as bumblefoot. Regular examinations of the animals' feet are crucial to ensure they do not have this issue, which implies lifting the penguins off the ground, a quick but perhaps stressful process. This was exemplified by the Humboldt penguins at SEA LIFE Porto, who exhibited signs of discomfort when lifted from the ground. A training procedure was initiated to acclimate the animals to laying in a handler's arms, ventral side down, while completely extending the legs and feet away from their bodies. Over 99 days, one to five training sessions were conducted weekly, within the technical area of the exhibit, allowing the penguins to decide how long they desired to remain inside. For every training session, a checkered green and blue board was used as a visual cue, with the goal being for the penguins to associate the board with being picked up and placed in a handler's arms. Sprat (Spratulus sprattus) and capelin (Mallotus villosus) were the main reinforcers used to motivate the animals to continue interested in the training sessions. Nine steps were predefined, with the handler progressing through them as soon as the penguins demonstrated no hesitation in performing each step. Even though the final laying position was not yet achieved, noticeable progress has been made, particularly evident in the increased comfort level of the animals with touch. Moving forward, it is essential to maintain focus on session continuation until the final objective is attained.

4. Abstract submitted to the Animal Behaviour Live 5th Annual Conference. Online

BEHAVIORAL PATTERNS OF HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*) IN CAPTIVITY: THE IMPACT OF ENVIRONMENTAL ENRICHMENT ON ACTIVITY LEVELS

Jacira Carvalho (1), Ana Ferreira (2), Ana Magalhães (1,3)

- (1) Behavioural Sciences, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
- (2) SEA LIFE Porto, Porto, Portugal
- (3) Addiction Biology, i3S - Instituto de Investigação e Inovação em Saúde and Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal

Since the year 2000, Humboldt penguins (*Spheniscus humboldti*) have been listed as "vulnerable" on the International Union for Conservation of Nature (IUCN) Red List. One approach to aid in species conservation is maintaining the animals in captivity. However, it is vital to ensure their overall well-being, both mentally and physically, therefore, ongoing efforts to enhance captive animal's quality of life are crucial. Applying environmental enrichment (EE) is a fundamental starting point in this effort. This study sought to determine which (EE) items could enhance the walking, exploratory, and swimming behaviors of Humboldt penguins at SEA LIFE Porto. Additional objectives included increasing their swimming duration and expanding the display area they actively use. Observations of eight penguins were carried out *ad libitum* over 17 days to develop an ethogram specific to this group. Recordings were made at 11 AM and 3 PM, with and without EE, and after removing videos with poor visibility, 80 videos were systematically analyzed using The Observer XT 11.5 software. An instantaneous scan sample was made every minute, and the behaviors were classified into 11 categories, including maintenance, nest-building, locomotion on land, swimming, social interactions, stationary activities, and agonistic behaviors. The introduction of EE resulted in statistically significant changes, such as increased land locomotion and nest-building behaviors, while maintenance and stationary behaviors decreased. Preliminary findings indicate that EE has a positive impact on penguin activity, particularly on land. These results may have been influenced by the penguins' reproductive cycle, highlighting the need for extended research with ongoing monitoring throughout the cycle.

- Poster presented at conference nº1

Activity Budget of Humboldt Penguins (*Spheniscus humboldti*) Under Human Care at SEA LIFE Porto and How Environmental Enrichment May Influence Activity Levels

Jacira Carvalho (jac211910553@edu.uep.pt), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Portugal
Ana Ferreira (ana.ferreira@medvet.fam.ucp.pt), SEA LIFE Porto, Portugal
Ana Regalado (anaregalado@uep.pt), Instituto de Investigação e Inovação em Saúde (I3S), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, University Institute of Health Sciences (UIHS), Portugal

Introduction

One of the *ex-situ* measures taken for species conservation involves keeping the animals under human care which, undoubtedly, implies prioritizing both their physical and emotional well-being, with the implementation of environmental enrichment (EE) being a key factor contributing to the achievement of this objective (Mieske et al., 2022; Miranda et al., 2023). In alignment with this goal, various EE devices were introduced within the Humboldt penguin (*Spheniscus humboldti*) exhibit at SEA LIFE Porto. These devices were chosen to enhance the penguins' overall welfare by addressing their ethological requirements and stimulating increased levels of physical activity both on land and in water.

Aim

- Identify which EE devices help improve the penguins' walking, exploratory, and swimming-related behaviors.
- Extend the penguins' swimming duration and expand the display area they actively use.

Methodology

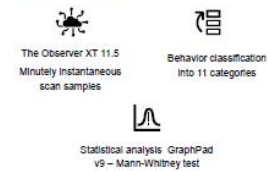
1. AD LIBITUM OBSERVATIONS



2. SYSTEMATIC OBSERVATIONS

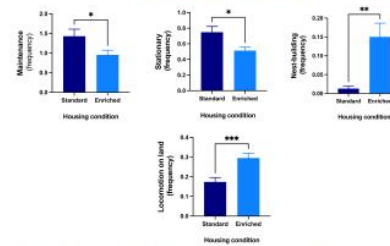


3. DATA ANALYSIS



Results

SIGNIFICANT DIFFERENCES



Figures 1, 2, 3, 4. Frequency of maintenance, stationary, nest-building, and locomotion on land behaviors, with and without enrichment. Significant differences ($P < 0.05$).

NO SIGNIFICANT DIFFERENCES

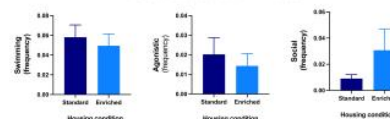


Figure 5,6,7. Frequency of swimming, agonistic and social behaviors, with and without enrichment. No significant differences ($P > 0.05$).

Conclusion

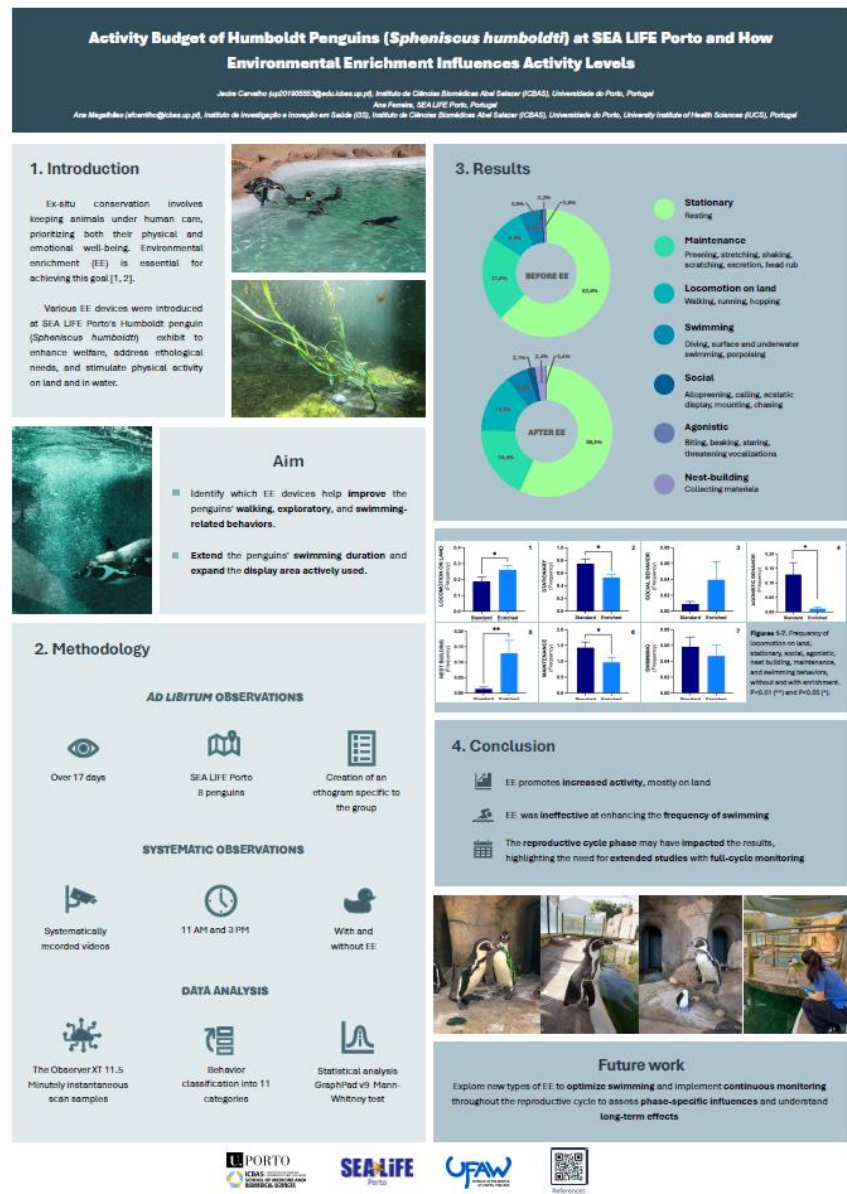


- EE promotes increased activity, mostly on land.
- Results may have been impacted by the reproductive cycle phase, emphasizing the need for prolonged studies with monitoring throughout the whole cycle.



References
Mieske, A., Hildebrandt, U., Richter-Verlinden, C., Hest, C., Hildebrandt, K., Kahl, P., Hest, J., Hildebrandt, J., Hildebrandt, G., Kahl, J., Hildebrandt, L., & Hildebrandt, A. (2022). Effect of social housing on the effect of environmental enrichment on the welfare of stationary fish and shellfish: Studies in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Journal of Fish Diseases*, 45(1), 1-15.
Miranda, R., Santana, R., Costa, M., Pires de Castro, A., & Vilhena, A. (2023). The Role of Zoo and Aquariums in a Changing World. *Annual Review of Animal Biosciences*, 11, 337-360. <https://doi.org/10.1093/aar/abz005>

- Poster presented at conference nº2



- PowerPoint presented at conference nº 3



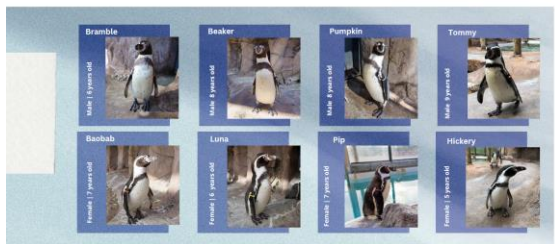
Threats



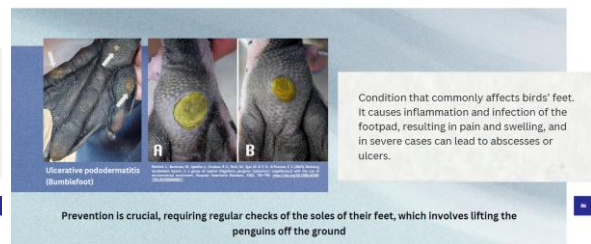
Conservation Efforts



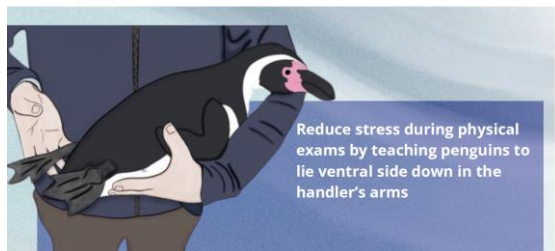
Meet the Group



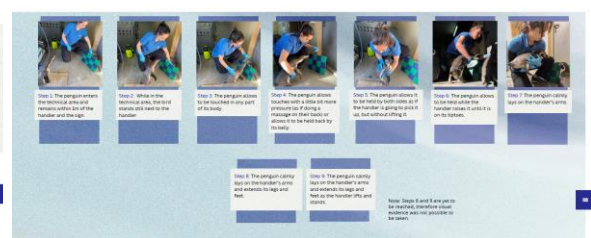
Importance of Training



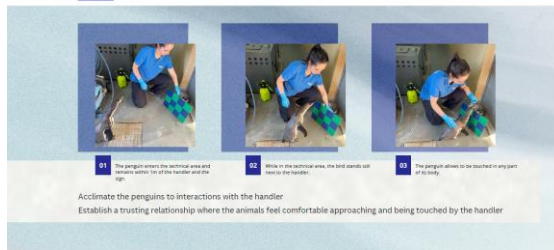
Goal – Welfare Improvement



Training Steps



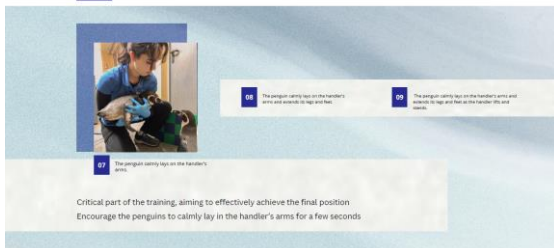
Training Steps and Objectives



Training Steps and Objectives



Training Steps and Objectives



Training Process Overview



Training Process Overview

Training site:
Exhibition's technical area with the door open, allowing penguins to leave freely

Handlers required:
Not more than 2 - one to separate the penguin in training from the group, and the other to interact with it

To build trust and consistency:
Always the same handler interacting with the penguins

Recording and later review and classification of each session



Training Process Overview

Previous training:
Entering the technical area and voluntarily step onto a scale

To avoid confusion:
Introduction of a new signal - clarify that different behaviors were expected since both events occurred in the same location

Board's characteristics based on:
Colors that the species can easily distinguish
Checked pattern to help penguins that might struggle with color differentiation

Visual cue present in every session



Training Process Overview

Steps 5 and 6:
One handler would carefully hold the penguin while the other handler fed it. The penguin focused on the food, allowing the touch to happen more easily.

Step 7:
Occasional need to briefly restrain the penguins, by not immediately releasing them into the outdoor area, so they would notice the reward. Immediate release of the penguin if it still didn't want to participate

By momentarily stopping the penguin from leaving (B), and after it sees the food reward (B-C), the penguin decides to stay and look for food (D).



If I had to describe training in 3 words...

PRACTICAL FLEXIBLE FRUSTRATING



Results

Back-and-forth process

- ~2 penguins reached near-final position (Step 7)
- ~5 penguins showed varying progress
- ~1 penguin showed minimal progress

Behavioral Observations

- ~More willingness to participate in training when the technical area door was open
- ~Noticeable improvement in the penguins' comfort with physical touch and close interactions with the handlers




Results - Factors Influencing Training Outcomes

The mature age of the penguins potentially made training more difficult due to pre-established habits and possible reluctance to change their routines

Difference between genders. Males tolerated training better than females, who often stayed cautious and avoided the handler and physical contact

Individual personalities → Different progression rates → Curious vs Reserved



Results - Training Challenges

Difficulty in maintaining training consistency

Breeding phase: from February to July → reluctance to leave their nests

Preference for sprat over capelin: → capelin wasn't seen as a good enough reward → difficult training sessions when capelin was the only fish available




The penguin wanted to leave once it understood the food was capelin.

Conclusion

Reinforcer on training sessions:
Sprat

On non-training days:
Capelin randomly alternated with sprat

Sprat, not only is preferred by the penguins but it is also smaller, extending feeding and consequently prolonging the training session



Conclusion

Separate training sessions from regular feeding

- Start with training
- Follow with maintenance of the displays and nests (>30min)
- End with feeding the non-participating animals

Ensures clear temporal separation of the two events without major disruption to keepers' routine



Conclusion

Adaptable, flexible, and practical approach to training

- Use in other zoos and aquariums with limited time for training
- Not a perfect process, leaving room for improvement
- The group made noticeable progress
- Current outcomes provide an excellent starting point for others who want to replicate it and avoid the same setbacks





THANK YOU!
Questions?



Appendix B

Ethogram of Humboldt Penguins' behaviour at SEA LIFE Porto

Table 4. SEA LIFE Porto Humboldt penguins' ethogram.

Category	Behaviour	Definition
Maintenance	Preening	The bird uses its beak to gently nibble the feathers, oiling, cleaning, and aligning them. It is done both on land and in water.
	Stretching	Elongation of the body. When lying down, the penguin extends its flippers and neck toward the sky. Occasionally, the penguin can also extend its legs and feet away from the body. When standing upright, the penguin can lean forward, while elongating its neck and extending the flippers slightly upward and back.
	Flipper flapping	Quick, short movements of the flippers, characterised by rapid opening and closing.
	Shaking	Quick side-to-side movement of the body or a part of the body, sometimes accompanied by an elongated neck. This behaviour is commonly observed shortly after the bird exits the water, likely to help dry its feathers. It can be performed both on land and while swimming in the water.
	Scratching	The bird vigorously rubs its flippers, beak or feet against its body. This behaviour is observed both on land and in water.
	Sneezing	Sudden expulsion of air through the bill.
	Excretion	The penguin leans slightly forward, raising its tail and releasing excrement. It is often followed by a shake of the tail.
	Yawning	Opening of the beak and inhalation of air, with the penguin's chest visibly inflating.

Table 4 (continued).

Category	Behaviour	Definition
Maintenance	Coughing	The bird forcefully exhales air, often accompanied by an acute sound.
	Head rub	The penguin bends its neck forward or backward so that its head rubs against its body. It is done both on land and in water.
Stationary	Lying down	The focal penguin is resting in a ventral position, either sleeping or passively observing its surroundings while occasionally raising its head. Its feet are placed underneath its belly, and its flippers rest on the sides of the body, parallel to the ground. Rarely, the penguin may be seen lying down with its feet and legs extended away from its body.
	Standing in an upright position	Apart from occasionally glancing around or raising its head, the penguin in focus is standing upright, either sleeping or motionless with open eyes. Its flippers rest at its sides, perpendicular to the ground, with the neck in a relaxed position.
	Crouching	The penguin is neither standing up nor lying down. Instead, it adopts a forward-leaning posture, lowering its chest toward the ground while maintaining its flippers tucked close to its sides. Occasionally, the penguin can be observed assuming this posture while leaning against a wall.
	Slender posture	While standing upright, the bird extends its neck upward toward the sky.
	Evasive posture	The penguin stands with its head turned away from conspecifics. It can also slightly retract and arch its neck backwards, with its beak pointed toward the sky. Its flippers rest at its sides.
	Standing upright with the neck retracted	The bird stands still in an upright position, with its neck retracted onto the shoulders. Its flippers are positioned at its sides.

Table 4 (continued).

Category	Behaviour	Definition
Locomotion on land	Walking	Waddling in an upright posture, with the neck in a relaxed position. For an animal to be considered as having walked, it must move both legs; movement with only one leg does not qualify as walking, since walking must result in a change of position on the ground.
	Running	Waddling in an upright posture, but with speeds higher than when walking.
	Hopping	The penguin in focus leans forward and propels its body into the air with a short jump, either upward or downward.
	Slender walk	Waddling with the neck elongated.
	Retracted walk	Waddling with the neck retracted.
Postural Transitions	Motion of lying/looking down	The penguin gradually lowers its head, leaning forward until the front of its body either makes contact with the ground (lying down) or is directed toward the ground (looking down).
	Motion of standing/looking up	The penguin is lying and gradually raises the front of its body until it is fully supported on its feet (standing up) or, while standing up, lifts its head to look upward if it was previously directed toward the ground.
Swimming	Diving	The penguin approaches the edge of the pool, leaning toward the water and letting itself fall inside the pool. Short in duration.
	Surface swimming	The bird floats on the water's surface, belly down. Its head may be either submerged, while it exhales, or held above the water, with its feet and/or flippers gently moving.

Table 4 (continued).

Category	Behaviour	Definition
Swimming	Underwater swimming	Swimming beneath the water's surface, sometimes at the bottom of the pool.
	Porpoising	The penguin repeatedly leaps from and into the water while swimming at higher speeds.
	Swimming on their side/back	The penguin floats on the water's surface, rolling onto its side or back. It frequently shakes the lower part of its body and uses its feet to move from one spot to another in the pool.
	Getting out of the pool	Accelerated swimming, with head underwater, to create momentum to exit the pool. The bird can land vertically on its feet, or ventrally when it has a lot of velocity.
Social	Allopreening	The penguin in focus preens another. Mutual allopreening can also take place when birds preen each other at the same time.
	Calling	The penguin can be up or lying on the ground, emitting lower and shorter vocalisations with the head directed toward the sky or leaned back. Not specifically directed at another penguin.
	Ecstatic display	While standing in an upright position, the penguin turns the inside of the flippers outward and extends them backwards, raising its chest and directing its beak toward the sky. When lying on the ground, it arches its flippers and head toward the sky. Initially, the penguin lifts and drops its chest silently or emits short and low sounds, eventually taking deep breaths, intermittently braying loudly with its beak wide open and head directed toward the sky. The penguin may also move its flippers back and forth. This behaviour can also be observed as a mutual ecstatic display when several birds engage in it simultaneously.

Table 4 (continued).

Category	Behaviour	Definition
Social	Soundless ecstatic display	The penguin assumes the same posture as during the ecstatic display but does not vocalise.
	Flipper patting	A penguin approaches another from behind, quickly fluttering its flippers against the other bird's body.
	Mounting	One penguin lies down ventrally while another lies on top of its back. The penguin on top vibrates its head against the other's neck, taps its flippers against its sides, and moves its feet along its lower back in a digging-like motion. The penguin underneath may raise its tail and head.
	Lying down together	The penguins lie down together, side by side. One can rest its head on the other's neck or back.
	Rhythmical vocalisations	A penguin performs low, rhythmical vocalisations, producing a steady, repeated sound.
	Bowing	The penguin keeps its beak closed and its neck slightly arched upward while leaning forward. Sometimes, the beak is held very close to the ground in a substantially low bow. This behaviour is typically performed in the presence of or directed toward another penguin. The body posture resembles the crouched position described earlier but in this case, the body, especially the neck, leans much further forward.
	Chasing	It can occur in water or on land. In the water, the penguin swims quickly after another. On land, the bird runs after another.
	Neck intertwine	Two penguins intertwine their necks, a behaviour observed in various contexts, such as during allopreening or amid agonistic fights.

Table 4 (continued).

Category	Behaviour	Definition
Agonistic	Staring	A penguin may gaze sideways at another bird with its head tilted, or it may move its head from side to side, alternating its gaze between one eye and the other (alternate head stare). Only seen performed on land.
	Beaking	While standing upright, the penguins clench and interlock their beaks, sometimes tilting their heads and intently jabbing, twisting, and pulling each other's bills. Only seen performed on land.
	Boddy jabbing	The penguin closes its beak and uses it to jab at another penguin's body.
	Biting	The bird opens its beak, quickly and strongly gnawing at another penguin.
	Beak slapping	A penguin repeatedly hits its beak against another penguin's beak, rapidly shaking its head from side to side. During this interaction, the beaks are typically closed.
	Threatening vocalisations	The penguin arches its flippers backward while leaning forward toward another individual. It stretches its neck, opens its beak, and emits strident and continuous vocalisations.
Environmental interaction	Observing	The penguin gazes specifically at an object or individual, positioning its head and body toward the target. For an observation to be classified as exploratory, the penguin must move its head from side to side, up and down, or forward and backward at least three times within three seconds.
	Touching	The bird touches an item or individual with its beak.
	Lifting	The penguin raises an object from the ground using its beak.

Table 4 (continued).

Category	Behaviour	Definition
Environmental interaction	Nudging	The penguin uses its beak to gently push an object.
	Tugging	The bird pulls on an object with its beak, using back-and-forth or side-to-side motions.
	Vocalising	Vocalisations directed toward an object or individual, with the animal typically orientating its head and body toward the target.
	Approaching	The bird moves toward an object or an individual, generally aligning its head and body in the direction of the target.
	Exploratory walking	A penguin walks while scanning its environment, adjusting its head and body orientation to assess environmental cues.
Human-animal engagement	Engaging with visitors	Approaching visitors near the glass, watching and/or following their movements, shadows, etc.
	Engaging with keepers/aquarium staff	Observe, avoid, or interact with the keepers or aquarium staff, occasionally displaying social or agonistic behaviours toward them.
Feeding	Individual	In the outdoor area, feeding events take place at least 2 metres away from other penguins, with no interactions observed between penguins. In the indoor area, the penguin in focus waits for its turn and voluntarily moves to and climbs onto a scale before its feeding begins.
	Group	The penguin can either patiently wait in the water or in a calmer place of the exhibition for the food to be thrown at them, or it can compete for food, stealing fish from another penguin's beak, pushing, and pecking at another.

Table 4 (continued).

Category	Behaviour	Definition
Feeding	Regurgitation	After swallowing the food, the food is returned to the bird's mouth through contractions of the chest.
Nesting	Transporting materials into the nest	The penguin transports items discovered in the exhibit back to its nest using its beak.
	Nest scraping	While in the nest, the penguin moves its feet in a digging motion, either against the ground or the nest's walls.
	Vibratory head shake	A penguin shakes its head, which appears to be vibrating. This behaviour typically occurs while the bird is lying down or bowing and may be accompanied by rhythmic vocalisations.
With no apparent motive or function	Mat lifting and dropping	The penguin lifts and drops a corner of the nest's mat. It can be performed only once or repeatedly.
Imperceptible		The penguin is visible but its behaviours are not discernable.
Not visible		The penguin is out of sight, staying inside the nest or in the camera's blind spots.
Other		Any behaviour not listed above.

Appendix C

Photographs of the introduced environmental enrichment devices.



Figure 24. Sprinkler. Used to create the effect of rain on the pool's surface



Figure 25. Aquatic aerator. Produced either jets or air bubbles in the water, depending on the air output pressure of the compressor.



Figure 26. Artificial algae, with approximately one meter in length. The algae were attached to a net and anchored with two weights. This setup prevented the algae from floating to the surface while allowing for easy removal at the end of each observation.



Figure 27. Artificial algae and a net with fish, suspended from the bridge (not represented in the picture). A small weight attached to the net kept it upright in the waterline, mimicking the movement of swimming fish as the net swayed with the water's agitation.

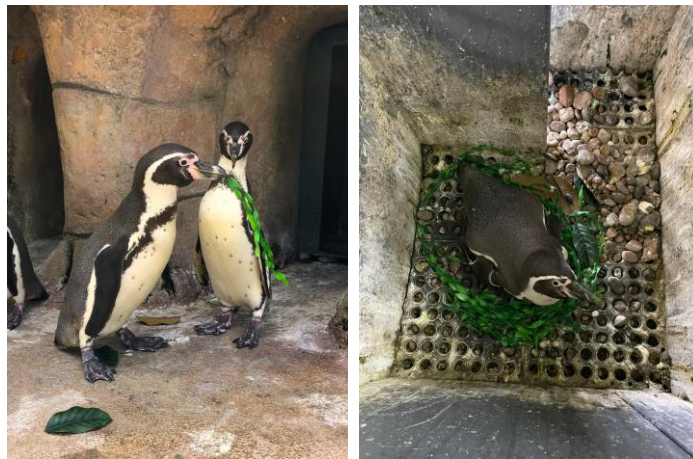


Figure 28. Artificial algae and leaves. On the left, a penguin is seen carrying the materials to the nest, while on the right, one of the “structures” built by a penguin pair using the provided materials is visible.



Figure 29. Artificial plant. Approximately one metre tall, designed to mimic natural foliage.



Figure 30. 3D printing of the Peruvian booby bird (*Sula variegata*). On the left one of the penguins is seen interacting with it.



Figure 31. Two of the feeding balls used with artificial algae (on the left) and fish (on the right). The remaining feeding balls were similar in design but contained either rocks or a rattle.

Appendix D

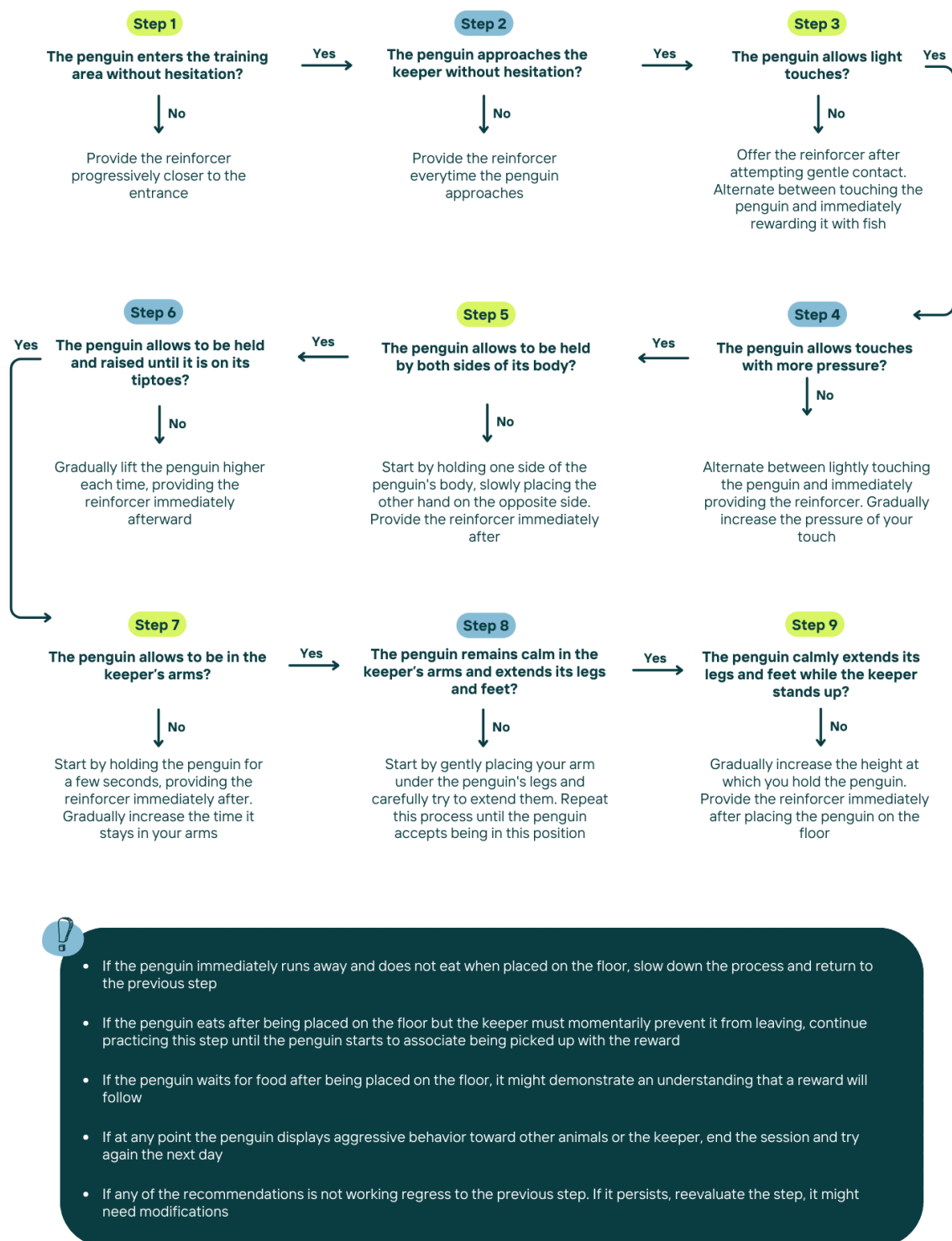


Figure 32. Training steps flowchart. The method and sequential decision-making process for training the penguins are outlined.

Appendix E

Table 5. Session classification for each Humboldt penguin at SEA LIFE Porto. The columns contain the penguins' names, while the rows represent the training session number. The colours are attributed based on Table 3, portraying the criteria for session classification. Green = "Excellent"; Yellow = "Good"; Orange = "Bad"; Grey = "Did not enter the training area".

	Pip	Pumpkin	Luna	Beaker	Baobab	Bramble	Hickery	Tommy
1	Green	Yellow	Green	Yellow	Green	Green	Yellow	Green
2	Yellow	Yellow	Green	Yellow	Orange	Yellow	Orange	Yellow
3	Yellow	Yellow	Yellow	Orange	Yellow	Yellow	Yellow	Yellow
4	Green	Yellow	Green	Yellow	Yellow	Green	Orange	Orange
5	Green	Green	Yellow	Yellow	Green	Green	Yellow	Green
6	Green	Yellow	Orange	Yellow	Yellow	Green	Yellow	Green
7	Green	Grey	Green	Yellow	Yellow	Green	Yellow	Green
8	Yellow	Yellow	Green	Yellow	Yellow	Green	Orange	Yellow
9	Yellow	Orange	Green	Yellow	Yellow	Green	Orange	Yellow
10	Grey	Yellow	Green	Yellow	Yellow	Green	Grey	Green
11	Green	Yellow	Yellow	Orange	Green	Green	Grey	Yellow
12	Grey	Grey	Grey	Grey	Grey	Orange	Grey	Yellow
13	Grey	Grey	Yellow	Orange	Yellow	Yellow	Orange	Orange
14	Grey	Yellow	Green	Grey	Grey	Green	Yellow	Green
15	Grey	Yellow	Yellow	Grey	Grey	Grey	Grey	Yellow
16	Grey	Grey	Grey	Grey	Grey	Green	Orange	Yellow
17	Green	Yellow	Grey	Grey	Grey	Yellow	Orange	Orange
18	Orange	Yellow	Grey	Orange	Yellow	Grey	Orange	Yellow
19	Grey	Grey	Grey	Grey	Grey	Green	Orange	Yellow
20	Grey	Grey	Grey	Grey	Grey	Green	Yellow	Green
21	Yellow	Yellow	Grey	Yellow	Grey	Green	Orange	Green
22	Green	Yellow	Grey	Yellow	Grey	Green	Yellow	Green
23	Grey	Yellow	Grey	Grey	Grey	Green	Grey	Green
24	Yellow	Grey	Grey	Grey	Grey	Green	Orange	Green
25	Grey	Orange	Grey	Grey	Yellow	Grey	Yellow	Green
26	Grey	Yellow	Grey	Yellow	Grey	Green	Yellow	Green
27	Grey	Grey	Grey	Grey	Grey	Yellow	Orange	Green
28	Grey	Grey	Grey	Grey	Grey	Green	Orange	Green
29	Grey	Grey	Grey	Grey	Grey	Grey	Orange	Green
30	Green	Grey	Yellow	Grey	Grey	Green	Yellow	Green
31	Green	Grey	Grey	Grey	Grey	Grey	Yellow	Green
32	Grey	Grey	Grey	Grey	Grey	Green	Orange	Green
33	Grey	Grey	Grey	Grey	Grey	Green	Yellow	Green
34	Grey	Grey	Grey	Grey	Grey	Green	Yellow	Green
35	Grey	Grey	Yellow	Grey	Grey	Grey	Yellow	Green
36	Grey	Grey	Grey	Grey	Grey	Green	Yellow	Green

Table 5 (continued).

	Pip	Pumpkin	Luna	Beaker	Baobab	Bramble	Hickery	Tommy
37								
38								
39								
40								
41								
42								
43								
44								
45								
46								
47								
48								
49								
50								
51								
52								
53								
54								
55								
56								
57								
58								
59								
60								
61								
62								
63								
64								
65								
66								