European Association for Aquatic Mammals

Marine Mammal Experts. Let's Share Your Stories!

El "must" de Canarias
LORO PARQUE
ANIMAL EMBASSY

52nd Annual Symposium European Association for Aquatic Mammals EAAM

Proceedings of the

EAAM 52nd Annual Symposium

Loro Parque, Tenerife, Spain

12-15 March 2024

European Association for Aquatic Mammals

Rue de la Science 14b, 1000 Brussels – Belgium – information@eaam.com

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A Letter from EAAM President

Dear Colleagues and Friends of the EAAM:

We are delighted to present the proceedings of the 52nd European Association for Aquatic Mammals (EAAM) Symposium, held at the magnificent Loro Parque in Tenerife in March 2023. This event was a remarkable gathering that spanned four days, during which experts in both ex-situ and in-situ conservation of aquatic mammals came together to engage in robust scientific discussions.

The symposium featured a diverse array of presentations, workshops, and panel discussions, highlighting the latest research findings, conservation strategies, and management practices essential to the well-being and preservation of aquatic mammals. The exchange of knowledge and ideas was truly inspiring, and we are confident that the insights gained will significantly contribute to the advancement of our collective efforts in conservation.

We would like to extend our heartfelt gratitude to Mr. Wolfgang Kiesling, our gracious host, whose dedication and support were pivotal in making this event a success. We also thank the entire organizing committee and the exceptional team at Loro Parque for their hard work and commitment.

A special thank you goes to the scientific committee, led by Manuel Garcia Hartmann, for their invaluable contributions in curating a comprehensive and thought-provoking program. Their expertise and guidance were instrumental in shaping the high quality of the scientific content presented.

Enclosed within these proceedings, you will find the scientific abstracts presented during the conference. We hope this compilation serves as a valuable resource for continued research and collaboration in the field of aquatic mammal conservation and can only encourage you to pursue these four inspiring days by reaching out authors listed in this edition.

Thank you once again to all who participated and contributed to the success of this symposium. We look forward to future gatherings where we can continue to share knowledge and work together towards our common goal of conserving aquatic mammals.

Sincerely,

Martin Böye
President of the EAAM

A Letter from EAAM Past President

March 1, 2024

Dear colleagues and friends, dear EAAM 2024 attendees,

Once again, we are gathering in our yearly meeting to share friendship and expertise. This is the 52nd edition of the EAAM Annual Symposium. The second edition hosted in person after the pandemia.

This year, Loro Parque, our host institution, has invited all of us to celebrate animal welfare, science, conservation, and education in the magnificent island of Tenerife, surrounded by nature wonders and their animal embassy.

The 2024 Organizing Committee, the EAAM Scientific Committee, and all members of the Board have worked tirelessly to provide every participant with a unique experience. We hope you enjoy the program and we are happy to see you again celebrating with us.

During the past year, EAAM has been actively involved in international matters and concluded the support to the Ukrainian marine mammals. The creation of the Marine Mammal Emergency Fund resulted in a success. Our fund has helped emergency actions involving marine mammals in France and Brazil. Notwithstanding, the political situation around Europe and the Americas has kept the Board busy monitoring, discussing, and getting involved with other organizations in order to protect the marine mammal community while promoting our role as the real experts in the matter. A lot of efforts are still necessary to educate politicians and we invite you all to participate in the actions.

I became officially a member of the Association during the last EAAM Symposium in Loro Parque 10 years ago, and is also here in Tenerife where my term as EAAM President ends. It is time for Martin Böye to step in as new EAAM President. I am sure the Association is in good hands. His enthusiasm, his French network, and his experience in the field, including many hours lobbying and dealing with activists, will continue proving his value for the European marine mammal community. It was an honor and a privilege for me to represent you during these 2 years. I will continue striving for the best for the community as Past President.

Guillermo J. Sánchez Contreras
EAAM President (2022 - 2024)

Conference Program
Wednesday - 13 March 2024

Time:	Activity:
08:30 - 09:00	Registrations
09:00 - 09:45	Official Symposium Opening
09:45 - 10:45	**Keynote Speaker – Dr. Heidi Lyn** Bottlenose Dolphins: Behavior, Enrichment Cognition and – The Value in Studying the Whole Animal
10:45 - 11:15	**Coffee Break**
VET I - CHAIR:	García-Hartmann, M.
11:15 - 11:30	Dolphins CT-Scan: Organization, Management and Realization: The Experience of Loro Parque – Grande, F.
11:30 - 11:45	Cetacean Anesthesia: Review of a Decade of Clinical Anesthesia Cases, Lessons Learned & Future Plans – Johnson, S.
11:45 - 12:00	Attempted Tooth Extraction in a Beluga Whale (*Delphinapterus leucas*) Through Medical Training – Monreal, P. T.
12:00 - 12:15	Pathological Study of an Open Patent Ductus Arteriosus (PDA) in a 20-Year-Old Killer Whale (*Orcinus orca*) – Cámara N.
12:15 - 12:25	**Short Talk** – Debosschere, Y./ Debosschere, Y.
12:25 - 12:30	PentaVet – Sastre Paleologos, M. A.
ACOUSTIC - CHAIR:	Almunia, J.
12:30 - 12:45	Bottlenose Dolphin (*Tursiops truncatus*) Whistle Behavior During Controlled Noise Presentation – Ames, A.
12:45 - 13:00	Fruitful Conservation Biology Efforts on Dolphins by Bridging Studies in the Wild and Under Human Care – Torres Ortiz, S.
13:00 - 13:15	Fjord&Baelt Centre's 25 Years – Dudzinski, K.

Conference Program
Wednesday - 13 March 2024

Time:	Activity:
13:15 - 14:30	**Lunch**
BEHAVIOUR - CHAIR:	Neves J.
14:30 - 14:45	The Impact of Calves to a Social Group – Dudzinski, K.
14:45 - 15:00	Space Use by Common Bottlenose Dolphins (*Tursiops truncatus*) with Respect to Human Presence at Bailey's Key, Roatan, Honduras – Karlin, M.
15:00 - 15:15	Pair Swimming Positions in Male-Male Bottlenose Dolphin Dyads Based on Age and Kinship and Resulting Social Implications – Themelin, M.
15:15 - 15:30	Smiling Underwater: Play, Open Mouth and Rapid Mimicry in Bottlenose Dolphins – Böye, M.
15:30 - 15:40	**Short Talk** – Arija-Hoyo, C./ Katic, R.
15:40 - 15:55	Using Computerized, Cognitive Enrichment to Engage California Sea Lions and Gain Insight into Individual differences in Learning Processes – Ramos, A.
15:55 - 16:10	Leveraging Dire Conservation Narratives for Positive Behavioural Shifts in Zoo Visitors: A Case Study from Zoomarine Algarve – Neves, J.
16:30 - 17:00	**Coffee Break**
17:00 - 19:00	**Round Table** Marine Mammal Research Network (MMRN): A New Tool to Create Collaborations Between Scientists and Facilities – Torres Ortiz, S.
20:00 - 22:00	**Conservation Evening**

Conference Program
Thursday - 14 March 2024

Time:	Activity:
08:30 - 09:00	Registrations
09:00 - 10:00	**Keynote Speaker – Dr. Elisabetta Palagi** In Water or on Land, Play Is a Serious Matter!
WELFARE - CHAIR:	Dudzinski, K.
10:00 - 10:15	A Methodological Approach to Develop a Five Domains Model Framework to Assess the Welfare of a Captive Group of Yangtze Finless Porpoises (*Neophocaena asiaeorientalis asiaeorientalis*) – Platto, S. – Video presentation
10:15 - 10:30	Welfare Under Human Care: Behavioural Changes in Bottlenose Dolphins (*Tursiops truncatus*) Depending on External Factors and Social Dynamics in Madrid Zoo Aquarium – Serrano García, A.
10:30 - 10:45	Cognitive Enrichments – The Bridge Between Welfare and Science – Matrai, E.
10:45 - 11:00	Dolphin-WET (Welfare Evaluation Tool) – Baumgartner, K
11:00 - 11:05	American Humane – Nizan, J.
11:05 - 11:30	**Coffee Break**
VET II - CHAIR:	Sánchez Contreras, G.
11:30 - 11:45	Assessing the Neuroendocrine System of Cetaceans: A First Look into the HPA Axis of Bottlenose Dolphins and Killer Whales – Alonso-Almorox, P.
11:45 - 12:00	Endoscopic Findings in Killer Whales During Gastroscopies Performed by Voluntary Behaviour: The Experience of Loro Parque – Grande, F.
12:00 - 12:15	Understanding and Managing Fungal Infections Through an In-House Mycology Protocol in Bottlenose Dolphins (*Tursiops truncatus*) – Marques, G. N.
12:15 - 12:30	The Influence of the Nutritional Protocols and Other Factors on the Hand-Rearing of Harbour Seal (*Phoca vitulina*) Orphan Pups – Sanchez-Contreras, G.

Conference Program
Thursday - 14 March 2024

Time:	Activity:
12:30 - 12:45	Evaluation of *Erysipelothrix rhusiopathiae* Occurrences and Vaccinal Reactions in Cetaceans - Thirty-Year Retrospective Results Through Two Global Epidemiological Surveys – Lacave, G.
12:45 - 12:55	**Short Talk** – Marques, G. N./Naranjo de Torres, M.
12:55 - 13:10	**Short Talk (Video Presentation)** – Jeniu Pérez, J.
13:15 - 14:30	**Lunch**
TRAINING - CHAIR:	Issenjou, N.
14:30 - 14:45	Delphinid Creativity Under Stimulus Control – Manitzas Hill, H.
14:45 - 15:00	Redirecting an Undesired Behavior of Saltwater Ingestion in a California Sea Lion Through Positive Reinforcement and Shaping Techniques – Sousa, F./Flanagan, C.
15:00 - 15:15	Dolphin Training Applied to Scientific Projects in Cognition and Bioacoustics – Ubero Ramírez, C.
15:15 - 15:30	Voluntary Corneal Debridement in a California Sea Lion – Midwood, P.
15:30 - 15:45	**Short Talk** – Garcia, M./Lima, C./Jinariu, V.
15:45 - 15:55	**Short Talk** – Martin Deller, I./Rodriguez-Hernández, M.
15:55 - 16:00	Seafoodia – Besson, G.
16:15 - 17:00	**Coffee Break/Poster Session**
17:00 - 18:30	**Round Table** Peer-Review Publishing – Process How-To's & Recommendations – Dudzinski, K
18:30 - 20:00	Annual General Meeting
20:30 - 00:00	**Gala Dinner**

Conference Program
Friday - 15 March 2024

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Time:	Activity:
08:30 - 09:00	Registrations
09:00 - 10:00	**Keynote Speaker – Dr. Renaud de Stephanis** 30 Years of Iberian Orca Research
IN SITU:	Böye, M.
10:00 - 10:15	Presence of Bottlenose Dolphins (*Tursiops truncatus*) in the Coast of Marina Baixa (Alicante, Spain) and Associated Environmental Factors – Arija Hoyo, C.
10:15 - 10:30	25 Years of Research: A Marine Protected Area for Cetaceans and Marine Turtles in the Strait of Gibraltar – Silgado Calderón D
10:30 - 10:45	Interactions Between Orcas and Sailboats in the Strait of Gibraltar – Baringo, F.
10:45 - 11:00	Public Aquarium, Engineering, Science, Funding: A Case Study on Reducing Marine Mammal Bycatch – Culik, B.
11:00 - 11:05	**Short Talk** Whale-PAL to Successfully Prevent Orca Attacks on Pleasure Craft – Culik, B.
11:05 - 11:30	**Coffee Break**
TECH AND SCIENCE:	Almunia, J.
11:30 - 11:45	Unexpected Context-Dependent Vocal Use in Bottlenose Dolphins (*Tursiops truncatus*) Under Human Care – Gallo, A.
11:45 - 12:00	Developing AI-Based Acoustic and Video System to Study Orca Communication and Welfare – Lüke, J. P.
12:00 - 12:15	Monitoring Sound in Marine Mammal Facilities: A Practical Approach – Almunia, J.
12:15 - 12:30	Overcoming the Challenges of Blow-Sampling with UAS in Small Cetaceans – Bruck, J. – Video presentation

Conference Program
Friday - 15 March 2024

Time:	Activity:
12:30 - 12:45	Mechanical Water Disinfection – A New Way to Keep Aquariums Clean and Healthy – Fleck, N.
12:45 - 12:50	Studying Animal Behaviour with Artificial Intelligence – A Case Study on Polar Bears – Zuerl, M.
13:00 - 13:30	Official Symposium Closure
13:30 - 18:30	**Loro Parque Visit with lunch**

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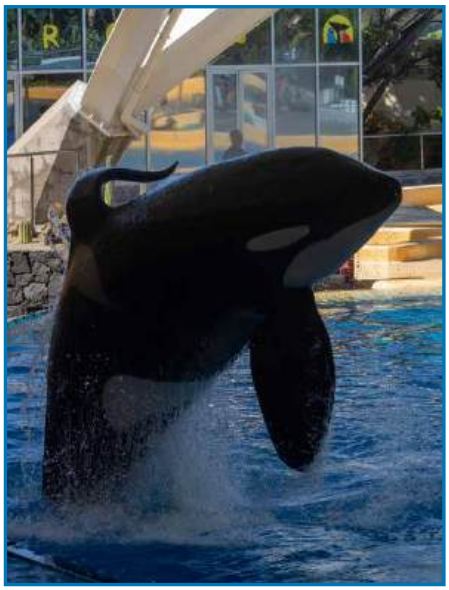
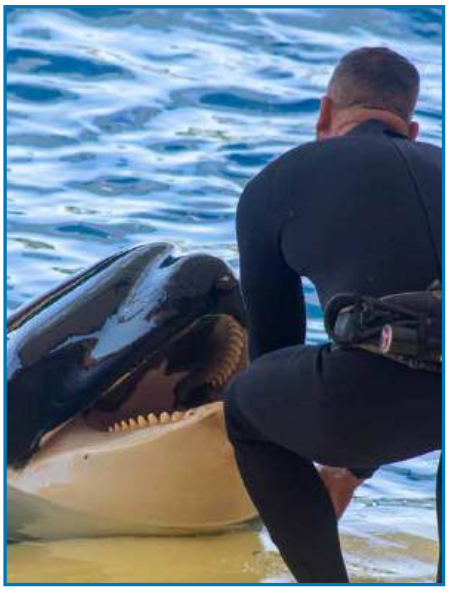
2024 KEYNOTE SPEAKERS

Let People Know We Are Part of the Solution



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Keynote Speaker

Wednesday, 13 March 2024

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Bottlenose Dolphins: Behavior, Enrichment Cognition and Communication – The Value in Studying the Whole Animal

Heidi Lyn
Comparative Cognition Lab

https://c3polab.org/

Heidi Lyn is an Associate Professor and Joan M. Sinnott chair of Psychology at The University of South Alabama where she studies nonhuman animal cognition and communication. She is internationally known, having held positions at UCLA, the New York Aquarium and St. Andrews University. Her research on a variety of species, including dolphins, dogs, monkeys, and otters, has been published in such journals as Psychological Science, Animal Cognition, and Developmental Science and featured in books as well as Scientific American and Science magazines. She will be speaking on Bottlenose dolphins: behavior, enrichment, cognition and communication - the value in studying the whole animal.

Keynote Speaker

Thursday, 14 March 2024

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In Water or on Land, Play Is a Serious Matter!

Elisabetta Palagi, PhD
Associate Professor – Ethology Unit
Department of Biology – University of Pisa

Elisabetta Palagi is Associate Professor at the University of Pisa. She has been studying social mammals including humans since 1992. Her studies have been carried out under both controlled and wild conditions. She holds a master's degree in biology, a Ph.D. in evolutionary biology, and a solid publication record on a wide array of topics bridging sociobiology, comparative psychology, and anthropological sciences. Among others, she has demonstrated individual recognition in lemurs and their use of multimodal signaling. She has also extensively investigated the functions and evolutionary significance of play, conflict management and resolution in social groups, and the behavioral patterns underlying emotional contagion and empathic abilities in human and nonhuman animals. Outside the primate order, she is focusing on dogs, wolves, spotted hyenas, meerkats, horses, seals and dolphins.

Keynote Speaker

Friday, 15 March 2024

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30 Years of Iberian Orca Research

Renaud de Stephanis
Doctor

Renaud de Stephanis is a prominent researcher in the field of cetacean conservation and study, with a particular focus on the Strait of Gibraltar. His work has been pivotal in understanding the dynamics of various cetacean species in this critical area, including orcas, fin whales, sperm whales, and pilot whales.

Academic Achievements and Scientific Contributions

With over 100 published papers and authorship of 10 books, de Stephanis has demonstrated an exceptional commitment to research and scientific dissemination. He has led studies on cetacean behaviour, migratory patterns, and spatial distribution, uncovering critical aspects of their interactions with human activities such as maritime traffic and whale-watching tourism.

Impact on Conservation Policies and Protected Areas

De Stephanis has been a key driving force in developing strategies to mitigate human impacts on cetaceans. His research has significantly influenced policy formulation and has been instrumental in creating marine protected areas in Spain, particularly in Andalusia. His work has led to the declaration of 3 Sites of Community Importance (SCIs) in the region, showcasing his ability to balance cetacean protection with sustainable marine environment use.

Notable Initiatives and Leadership in Projects

Renaud has been the initiator of the Iberian orca conservation plan, a pioneering project in the region. Additionally, he is involved in over 50 research projects, underscoring his role as a leader and collaborator in the scientific community.

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2024 ABSTRACTS

Let People Know We Are Part of the Solution






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Oral Presentation

Dolphins CT-scan: organization, management and realization: the experience of Loro Parque

Grande F. (1), Ubero Ramírez, C.(1), Martín Hernández, M. (1), Lacave, G. (2), Báez Díaz, Y. (1), Dreisorner, C. (1), Gallardo Coll, I. (1), Hernández Grillo, A. (1), Encinoso, M. (3)

(1.) Loro Parque y Loro Parque Fundación, Avenida Loro Parque s/n – 38400 Puerto de la Cruz –Tenerife - España (veterinario@loroparque.com)

(2.) Marine Mammal Veterinary Services, 8310 Bruges, Belgium

(3.) Radiology Service Responsible of Veterinary Hospital ULPGC – Faculty of Veterinary Medicine – University of Gran Canaria – España

Since June 2023, Loro Parque has been equipped with a modern CT-scan machine characterized by a 90 cm gantry and a table capable of supporting up to 300 kg. This has allowed Loro Parque to be one of the few zoos in Europe equipped with this modern and important diagnostic tool. Furthermore, this tool represents a further step forward in ensuring animal welfare. The CT scan machine has now become a tool for daily use, allowing an improvement from a diagnostic and therapeutic point of view for Loro Parque animals.

CT scan examinations were carried out on some dolphins: this involved a complex organization of the complete procedure, from the voluntary restraint of the animal to its transport to the CT room. This study describes the steps related to the preparation, organization, management and realization of this kind of exam.

Oral Presentation

Cetacean Anesthesia: Review of a Decade of Clinical Anesthesia Cases, Lessons Learned & Future Plans

James E. Bailey* (1), Shawn P. Johnson (2)

(1.) Innovative Veterinary Medicine, 101 Marketside AVE, STE 404-402, Ponte Vedra, FL 32081 USA, innovative (veterinary.medicine@gmail.com)

(2.) Sea Change Health, 596 W. McKinley Ave, Sunnyvale, California, 94086 USA

Historical difficulty with anesthesia cetaceans had caused hesitation in performance of often medically necessary procedures, which promoted negative outcomes and myths and misconceptions about anesthesia of cetaceans. Although still applied relatively infrequently, improvement in methods of anesthetic management of cetaceans has made it an acceptable risk, even for elective procedures in cetaceans.

Some generalities will be shared from review of 50 anesthesia events involving bottlenose and Pacific white-sided dolphins. In brief, pre-anesthetic sedation begins with benzodiazepines alone (diazepam, midazolam), or in combination with opioids (butorphanol, meperidine). Ultrasound-guided cannulation of the lateral subcutaneous caudal vein enables delivery of propofol for rapid orotracheal intubation. The typical subject of over 150 kg and 25 years age minimally received either 0.25 mg/kg diazepam PO or 0.08 mg/kg IM midazolam for sedation, 3-4 mg/kg propofol IV for induction, and an end-expired sevoflurane concentration between 1.8%-2.0% for maintenance of anesthesia > 1.5 hours duration with 1 hour recovery. Conventional Controlled Mechanical Ventilation (CMV) was applied with variable success, but the alternative mode of Apneustic Anesthesia Ventilation (AAV) proved more effective. Direct arterial blood pressure monitoring and blood gas were obtained from ultrasound-guided cannulation of the median artery of the pectoral flipper. Descriptive statistic will be shared.

Oral Presentation

Attempted tooth extraction in a beluga whale (*Delphinapterus leucas*) through medical training

Monreal-Pawlowsky, T.* (1), Burdick, C. (2), Whiton, J. (2, 3), Sverrisdóttir, A. L. (4), Garðarsdóttir, S. D. (4), Velázquez-Urgel, I. (5)

(1.) International Zoo Veterinary Group, Station House, Parkwood Street, Keighley, West Yorkshire, BD21 4NQ, United Kingdom (t.monreal@izvg.co.uk)

(2.) SEA LIFE Trust Beluga Whale Sanctuary, Ægisgata 2, 900 Vestmanneyjar, Iceland

(3.) Big Bear Alpine Zoo, 42801 Moonridge Rd, Big Bear Lake, CA 92315, USA

(4.) Dýralaeknathjónusta Sudurlands, Studlum, 816. Ölfus, Iceland

(5.) Ars Veterinaria, Carrer del Cavallers, 37, 08034 Barcelona, Spain

A female beluga whale (Delphinapterus leucas) suffered from horizontal alveolar bone loss, periapical lucencies and an enlarged periodontal space in the second and third right and left mandibular teeth; the first ones were missing. It also had periodontitis of her fourth right mandibular tooth. The tooth presented vertical alveolar bone destruction that could be seen in latero-lateral extra- and intra-oral radiographs, as well as movement. As restraint and conventional approach was not possible, medical training was attempted to remove the teeth that were causing localized swelling, discomfort, and potential infection. An oral exam was done, and pictures of the mouth, intra-oral radiographs and thermal images taken. Dental charts and a detailed training plan were developed, and an extraction attempt done. Intraligamental lidocaine dental block was injected after desensitising the area with topical benzocaine. Conventional closed technique was used for the extraction. The manoeuvre was unsuccessful. After that it was decided to allow it to be expelled on its own as it was loose despite the ligament preventing total extraction, which happened a few weeks later. The area healed up well and follow up X-rays and thermal images detected no further alveolar bone loss and no swelling of the area.

Oral Presentation

Pathological study of an open Patent Ductus Arteriosus (PDA) in a 20-year-old Killer Whale (*Orcinus orca*).

Câmara, N.* (1, 2, 3), Grande, F. (2), Arbelo, M. (1), Sierra, E. (1), Rivero, M. (1), Castro, A. (1), Bernaldo de Quirós, Y. (1); Fiorito, C. (1), Suárez-Santana, C. (1), Felipe-Jiménez, I. (1), Alcaraz-Rico, L. (1), Colom-Rivero, A. (1), Navarro-Sarmiento, J. (1), Alonso-Almorox, P. (1), Molpeceres-Diego, I. (1), Marrero-Ponce, L. (1), Suárez-González, Z. (1), Grandía Guzmán, R. (1), Fernández, A. (1)

(1.) Veterinary Histology and Pathology, Institute of Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas of Gran Canaria. Campus Universitario Cardones de Arucas, Trasmontaña s/n, 35413 Arucas, Las Palmas of Gran Canaria, Spain.

(2.) Loro Parque Foundation. Avenida Loro Parque, s/n, 38400 Puerto de la Cruz, Tenerife, Spain.

(3.) The Oceanic Platform of the Canary Islands (PLOCAN). Carretera de Taliarte, s/n, 35214 Telde, Las Palmas of Gran Canaria, Spain.

(4.) Câmara, N. (nakita.camara@plocan.eu; nakita.camara@ulpgc.es; kita_camara@hotmail.com)

The ductus arteriosus is an anatomical structure present in the fetal heart which closes during the first months of life, in the case of humans, to ensure the proper function of the cardiovascular system. In cetaceans, more specifically in bottlenose dolphins, this structure is open at time of birth, stays open in yearlings for months, and closes during the first years of life. Therefore, reported cases of a patent ductus arteriosus (PDA) in juveniles and/or adult cetaceans are extremely rare, either in wildlife or in captivity (under human care).

Here we describe the diagnosis of a PDA in a 20-year-old female killer whale (*Orcinus orca*).

A complete pathological study was carried out. Necropsy and histology showed that the animal suffered a cardiovascular anomaly from birth classified as congenital/development heart defect. She presented a large and open PDA associated with lesions consistent with severe chronic pulmonary hypertension connected with chronic heart lesions affecting systemically to other organs which ultimately led to heart failure and death.

To our best knowledge, this is the first time where an open PDA and the associated chronic systemic pathology, is reported in a 20-year-old killer whale (*Orcinus orca*) which was born in captivity.

Short Talk Presentation

Treatment of periapical abscesses in Californian sea lions (Zalophus californianus)

Debosschere, Y.* (1), Quiévy, A. (1)

(1.) Pairi Daiza, Domaine du Cambron 7940 Brugelette, Belgium (yves@vetdent.be)

Marine mammals often face dental issues, yet little is known about dental pathology. In Sea Lions (*Zalophus californianus*) the most common lesion encountered, both in the wild and in captivity, is attrition/abrasion. A study of Sinai et al. on 1085 wild specimens showed that 65.7% were affected by some form of tooth wear.

Labraga-Martagon et al. suggested that mechanical abrasion (chewing) and acid demineralization, likely from regurgitation, were the main contributions for this phenomenon. Environmental stressors are known to impact their chewing behaviour, hence influencing tooth wear. While noise's effects on wild animals are well-researched (Southall et al.), captive animal noise standards are almost absent, despite similar consequences.

In the wild, 81.7% of adult sea lions (> 8 years) exhibit tooth wear, compared to 46.4% of young adults (5-8 years) and 35.5% of juveniles (< 5 years). Interestingly, even older captive sea lions often display abrasive tooth worn down already at a young age.

These case reports aim to explore the underlying reasons for this phenomenon and determine the most effective approach to addressing this pathology or, where possible, preventing it.

Short Talk Presentation

Complicated root fractures causing chronic jaw pain in a common bottlenose dolphin (*Tursiops truncates*)

Debosschere, Y.* (1)

(1.) Veterinary Dental Services, Drogenboomstraat, 62 8790 Waregem, Belgium & Pairi Daiza, Domaine du Cambron 7940 Brugelette, Belgium (yves@vetdent.be)

Although oral pathology presents as a frequent occurrence among captive cetaceans (Jett *et al,* 2017), there is a dearth of documentation regarding such conditions, particularly in relation to treatment and the consequences of dental pathology. Across all thecodonts, despite potential anatomical variations, oral conditions exhibit similar evolutionary patterns, allowing for the use of the human model as a comparative reference.

In cases of complicated root fracture, the crown remains visibly detached yet mobile, completely separated from the root. Conversely, in a complicated crown-root fracture, the crown remains partially attached to the root. Both types of fractures occur beneath the gumline, posing challenges for visual diagnosis. Left untreated, these lesions lead to chronic inflammation and pain.

In the human model, Trigeminal Nerve Pain (TNP) resulting from root fractures or cracks is extensively documented. Similar consequences are anticipated in cetaceans, inducing severe discomfort and pain, thereby impacting the animal's overall well-being. Given the substantial role of a dolphin's mandible in bone-conducted source positioning (Reinwald et al, 2018), heightened attention is warranted when managing oral pathology.

This case report aims to address the underlying causes of TNP and elaborate on the treatment of root fractures in bottlenose dolphins.

Oral Presentation

Bottlenose dolphin (*Tursiops truncatus*) whistle behavior during controlled noise presentation

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Literature on the Lombard response in cetaceans is increasing, yet findings from these studies have led to confusion on the degree to which species can compensate for increasing noise in their environment. Further, no studies have investigated the degree to which any cetacean species increases the sound pressure of vocalizations in response to controlled increases in noise. In this study, we trained two bottlenose dolphins (*Tursiops truncatus*) to whistle when requested while stationed underwater on a bite plate and presented with broadband (3 - 43 kHz) white noise. We played back four levels of noise (96, 108, 120, 132 dB) in random order for 14s, requesting whistle production approximately in the interim of each level presentation. For one dolphin, we found a slight response consistent with what has been reported for terrestrial mammals and some cetaceans. For the other dolphin, there was no response; however, we hypothesize that this was due to changes in training methods that led the animal to produce its whistle at maximum levels that could not be increased, regardless of noise presentation. This indicates a potential ceiling at which bottlenose dolphins can no longer compensate for noise increasing in their environment.

Oral Presentation

Fruitful conservation biology efforts on dolphins by bridging studies in the wild and under human care

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The International Union for the Conservation and Nature (IUCN) identifies 25% of marine mammals as at risk of extinction. Studying marine mammals in the wild involves numerous challenges compared to terrestrial mammals due to their mobility and lack of continuous visibility of these animals.

Facilities that host marine mammals provide a unique opportunity for scientists to better understand these animals under controlled conditions. Since 2021, the University of Southern Denmark started a collaboration with the dolphin department of Loro Parque. This resulted in the initiation and completion of six research projects in the topics of hearing and echolocation. In the first project we tested and validated the use of an adapted tag to measure hearing in freely moving animals. This validation enabled scientist to measure hearing in wild odontocetes for the first time, which opens venues for implementing these techniques in larger species like killer whales. The second and third project tested the capabilities and thresholds in a target discrimination task of the dolphins' echolocation abilities.

This paper will present the results of the three research projects and their implications for conservation as well as a better knowledge on the sensory capabilities of dolphins.

Video

Fjord&Baelt Centre's 25 Years

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Aquatic Mammals journal's Historical Perspectives (HP) series began in 2008, with interviews and essays from 15 colleagues who'd spent their careers studying or caring for a variety of aquatic mammals. Since then, ~100 interviews have been completed with colleagues from Europe, North America, Asia, and South America, as well as having nearly three dozen essays published in the journal. At the end of 2022, we added the first facility HP essay and completed a ~10-minute video compilation for the Fjord&Baelt Centre's 25th year anniversary. The journal continues to complete interviews for the HP series, even though the last few years were impacted by the pandemic. And, with the Fjord&Baelt essay and video, we present the inaugural avenue for facilities to participate in the journal's HP series. We hope you enjoy this video and maybe consider participating with the journal's HP series in the future.

Link: https://vimeo.com/792009078

Oral Presentation

The Impact of Calves to a Social Group

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For 22+ years, a longitudinal study of a bottlenose dolphin breeding group in managed care has been conducted with a focus on inter-individual behavioral exchanges. A subset of these data was analyzed to examine the influence of calves (*n* = 6, 3 <1 y, 2 2-y, 1 3-y) on the social group. Using three, 30-min sessions of underwater video, social groups were event sampled for the presence and absence of calves. The social groups were coded for activity level, group size, degree of spatial spread, and behavioral context. The presence of calves was correlated with higher levels of activity and larger group size. As calves aged, activity level increased, although unexpectedly group spread decreased with older calves. This may be related to more interactions that were affiliative in nature versus play, swimming, or agonistic actions. These results suggest that while offspring are enriching to a mother, they most certainly positively impact an entire social group. Additional analyses are ongoing to further refine these findings.

Oral Presentation

Space use by common bottlenose dolphins (*Tursiops truncatus*) with respect to human presence at Bailey's Key, Roatan, Honduras

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The purpose of this research was to measure dolphin spatial patterns with respect to human presence using Geographic Information Systems (GIS). From June 2021-March 2023, 18-21 bottlenose dolphins were observed from a dock that encompassed the perimeter of the lagoon at Bailey's Key, Roatan, Honduras. Observations were associated with a researcher in the water to observe and video-record dolphin behaviors. Research assistants collected panoramic photographs panning the lagoon on 1-min intervals during Baseline, Before, During, and After sessions; sessions were associated with the researcher's in-water sessions. Geographic coordinates were assigned to dolphin locations and analyzed using Getis-Ord Gi* statistic. Spatial statistics identified significant hotspots of dolphin clusters ($p < 0.10$) during every session and across seasons. These hotspots always included 2 adjacent regions associated with guest activity – a covered bench and boat dock, where visitors enter and exit Bailey's Key. Cold spots were identified Before, During, and After at western and southern regions, which were farthest from guest activity. These results suggest no significant change in dolphin clustering patterns due to a researcher in the lagoon and indicate a strong preference by the dolphins to spatially cluster in regions associated with guest presence.

Oral Presentation

Pair swimming positions in male-male bottlenose dolphin dyads based on age and kinship and resulting social implications.

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A managed-care group of bottlenose dolphins is part of a 22+ years longitudinal study on social behaviors. From these data, 59 underwater sessions (~30min each) were analyzed to explore effects of age and kinship on male-male dyads in their use of pair swimming positions (PSP) and potential social implications. Videos were event sampled for all types of PSP between males, which were coded based on each individual position relative to their partner on three axes. PSP durations of each dyad combination were used for analysis: 47min of PSP were recorded and seven types of PSP were identified. Infant position (IP) was the most observed, representing half of our dataset. Only unrelated dyads were observed in PSP, even though a few males were related. Dyads involving an older male (A-Adult or S-Subadult) with a younger male (Subadult, J-Juvenile, C-Calf) were the most observed, with IP representing 70% of their PSP. The IP 'Mom' role was assumed by the older male in AS/AJ male dyads, and by the younger male in SJ/SC dyads. These results offer insight into mentoring between males as they become sexually and socially mature, with adults and subadults potentially playing a different role for younger individuals.

Oral Presentation

Smiling underwater: play, open mouth and rapid mimicry in bottlenose dolphins

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Play is a widespread behavior present in phylogenetically distant taxa. This activity, especially in its social form, requires complex communicative exchanges. Such communication has been primarily studied in terrestrial mammalian species, with marine species largely neglected. Here, we focus on visual communication during play in bottlenose dolphins (Tursiops truncatus). We found that the Open Mouth (OM) display was emitted more often during social than during solitary play. Importantly, it also occurred more often when the sender was in the receiver's visual field, suggesting that animals are attentive to the playmate's attentional state. Detecting an OM evoked the same facial expression in the receiver and, when present in both players, such rapid mimicry made play sessions more reciprocal. Our results strikingly match those obtained on cooperative social primates and carnivores. It is difficult to know whether such similarities derive from shared evolutionary pathways (homology) or from evolutionary convergence (homoplasy), as both has been suggested for play behavior in general. However, the pervasive presence of OM signals and rapid mimicry in the mammal phylogenetic tree indicates the relevance of such visual mechanisms in shaping complex communication.

Short Talk Presentation

Parameters linked to maternal-filial behaviours between a female bottlenose dolphin (*Tursiops truncatus*) and her five years old daughter

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The maternal-filial bond has an important role not only in the development of calves during the infancy, but also in the learning of social and hunting skills. Because of that, the duration of this bond varies significantly even within the same species. However, knowledge is lacking about the factors that motivate maternal-filial behaviour in non-infant calves.

The present study focuses on the behaviour of a five year old female bottlenose dolphin that showed a great maternal-filial bond with her mother, including late lactation. To understand it, observations and ethograms were conducted over 6 months in Mundomar Benidorm, recording behavioural and environmental information, and different variables linked to daily activities. The results showed significant correlations between maternal-filial behaviours and the frequency of other conducts such as resting, locomotion or playing and affiliative behaviours. Equally, the maternal-filial behaviour rate was affected by factors like the presence of new behaviours during the training sessions conducted before the ethograms, the presence or absence of agonistic conducts or the fact of the park was open or closed to the public.

These results allow us to better understand the incentives behind the maternal-filial behaviours in a non-infant female in human care.

Short Talk Presentation

One step further: bitable enrichment devices against destructive biting behavior and agonistic interactions among bottlenose dolphins (*Tursiops truncatus*), a follow-up

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A low occurrence of rake marks in bottlenose dolphins under human care is desirable, as it has been proposed as a positive welfare indicator. In a preliminary study, we found that bitable enrichment devices could reduce rake marks in bottlenose dolphins. Three years ago, Dolfinarium Harderwijk made the decision to remove biting enrichment devices in response to dental problems, as well as the concern of destructive behavior due to oral fixation towards other environmental objects. However, trainer observations recorded an increase in rake mark occurrences among a group of male dolphins, as well as an increased oral fixation towards their environment. Despite the absence of bitable enrichment, dolphins continued to experience dental problems. As a follow-up to our initial study, we implemented a new type of bitable enrichment devices (Kong toys) in this group of males, along with using Rake Marks Quantification (RQM) method daily to assess their effect. Preliminary results indicate that the novel rings may have effect in preventing destructive behavior towards the environment, and play behavior is observed more frequently. Diversifying environmental enrichment may redirect unwanted biting behavior to bitable rings and promote affiliate behaviors, improving overall welfare.

Oral Presentation

Using computerized, cognitive enrichment to engage California sea lions and gain insight into individual differences in learning processes.

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Cognitive enrichment is a crucial aspect of animal welfare, particularly in managed care settings. In the absence of the variability and challenges found in natural environments, animals may experience stress, boredom, and overall diminished well-being. The Enclosure Video Enrichment (EVE) system, developed at the US Navy Marine Mammal Program (MMP), serves as a computerized cognitive enrichment tool, enabling important questions about animal cognition to be explored (Winship et al., 2023). Three male sea lions were trained to interact with EVE, adapting methods that have been previously successful with no-human primates. After mastering an initial training game, these animals progressed to more complex games involving correct and incorrect choices. The animals were required to make decisions and learn from outcomes withing the game, independent of trainer guidance. Gameplay strategies were assessed in the initial training game before and after the introduction of failure. Personality surveys of the three focal animals were completed by trainers to evaluate the characteristics present in each individual and were related to the frequency of gameplay strategies incorporated by each animal. The results of these comparisons are discussed within the framework of considering unique differences of each animal and the importance of animal welfare.

Oral Presentation

Leveraging dire conservation narratives for positive behavioural shifts in zoo visitors: a case study from Zoomarine Algarve.

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Zoos and aquariums have shifted their conservation education approach from using doom and gloom examples to highlighting success stories and positive efforts in breeding and reintroduction. While dire examples can effectively highlight the urgency of conservation issues, an overemphasis on negativity might risk desensitizing or overwhelming audiences. But can small bits of dire information positively affect the visitors' pro-conservation behavioural intentions? Our study investigated the effect of including the Vaquita's dire conservation situation in an animal-visitor interaction programme at Zoomarine Portugal. Over 121 days, 391 participants were surveyed in a non-paired, pre/post methodology, using the Conservation Caring scale, adapted from Skibins et al. (2013). Three types of knowledge questions (conservation, non-related and biology) were added to test for information retention. The study revealed small yet significant increases in some measures among those exposed to Vaquita information. This demonstrated a positive shift in attitudes toward conservation, showcasing how highlighting critical conservation examples nudged participants towards pro-conservation behavioural intentions regarding bottlenose dolphins. The findings suggest that integrating a single dire conservation example can effectively influence public attitudes and behavioural intentions positively, signaling a potential avenue for promoting conservation efforts in zoos and aquariums.

Round Table

Marine mammal research network (MMRN): a new tool to create collaborations between scientists and facilities.

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Research plays a vital role at expanding knowledge and advancing our understanding of the world. Research is essential for the conservation of species as many populations in the wild are declining.

Formed in 2021 after a round table discussion at the EAAM conference, The Marine Mammal Research Network (MMRN) is a diverse group of leading marine mammal experts from around the world. Research interests include a broad range of topics: animal welfare science, communication and acoustics, physiology, behaviour, cognition, ecology, and conservation.

The goal of the MMRN is to broaden the scope and impact of research with marine mammals in human care by establishing cooperative relationships between facilities and scientists, which can enhance and grow scientific communication. We believe that helping create these connections will benefit the field of marine mammal science and directly impact the enrichment and wellbeing of the animals with which we work. These collaborations can result in translational benefits for the conservation and welfare of marine mammals in the wild.

The goal of this round table is to discuss how collaborative research between scientists and facilities can be beneficial for both parties and how this collaboration can be achieved.

Video Presentation

A methodological approach to develop a Five Domains Model framework to assess the welfare of a captive group of Yangtze Finless Porpoises (*Neophocaena asiaeorientalis asiaeorientalis*)

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Globally, animal welfare science has seen a substantial growth as our knowledge of animal cognition, behavior, and well-being has progressed. Welfare assessments, which encompass systematic observations and measurements of physiological, behavioral, and environmental indicators, have become crucial tools for identifying potential risks to the well-being of the animals under human care. These tools can help to implement targeted management protocols to enhance the animals' living conditions. A Five Domains Model (FDM) framework was developed to assess the welfare of a captive group of Yangtze Finless porpoises (*Neophocaena asiaeorientalis asiaeorientalis*) held at the Baiji Dolphinarium (Chinese Academy of Sciences, Wuhan, P.R. China). The following steps were considered to develop the FDM framework: 1) the literature review of potential welfare indicators for the considered species; 2) the review from the panel of experts to validate the selected indicators; 3) the determination of the framework to structure the welfare assessment tool; 4) the development of a scoring system; 5) the evaluation of the validity, practicality, and reliability of the tool, (6) the final development and 7) the implementation. The steps 1 to 5 have already been completed, and they will be presented and discussed, including the challenges encountered during the framework development.

Oral Presentation

Welfare under human care: behavioural changes in bottlenose dolphins (*Tursiops truncatus*) depending on external factors and social dynamics in Madrid Zoo Aquarium

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Zoos are committed to fully understand the behavioural, emotional, and physical needs of the animals under their care to guarantee their welfare. Therefore, an observational project was developed at Madrid Zoo Aquarium including 8 bottlenose dolphins (*Tursiops truncatus*). This project comprised 189 multifocal ethograms conducted from May to November 2023, considering different schedules, previous activities, and environmental factors.

Statistical processing was carried out, showing a great number of relationships both between the behaviour of different animals and in relation with environmental factors. On one hand, male behaviour had a significant effect on females, varying according to their social status. For example, aggregation of some submissive females was observed in response to male sexual behaviours ($W=14,17$, $p<0,001$ and $W=10,37$, $p=0,001$). On the other hand, activities like environmental enrichment or training sessions had a significant effect on animals behaviour, while other factors such as the number of people attending Madrid Zoo Aquarium affected no animal behaviour at all.

The results of this study provided the dolphinarium team with a better understanding of the social dynamics of the group, as well as the factors which affected the animals in order to improve their welfare.

Oral Presentation

Cognitive enrichments – the bridge between welfare and science

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Cognitive enrichments provide novel opportunities for linking welfare and science. They allow the assessment of activity and the investigation of species-specific cognitive processes.

We developed a series of cognitive enrichments focusing on cooperation in a group of male Indo-Pacific bottlenose dolphins. The devices were constructed of PVC pipes, caps and rope handles, and provided in between feeding sessions without specific training regarding their manipulation. In order to investigate the welfare impact of these novel enrichments, seven welfare indicators were monitored for a 3-year observation period and compared between ‘Session days’ (when the cognitive enrichments were utilised) and ‘Non-session days’ (only regular enrichments were used).

The analyses revealed that positive welfare indicators were significantly increased, while negative welfare indicators were significantly decreased on Session days compared to Non-session days. The project was launched in 2016, and due to its success, it continues to develop further. Until today, over 300 sessions have been conducted, and no sessions have been recorded without interactions, proving the value of the devices to the dolphins. Five research papers were published on the outcomes of cognitive research and their welfare impact. We believe that cognitive enrichments are the key to advancing welfare and science simultaneously in cetaceans.

Oral Presentation

Dolphin-WET (Welfare Evaluation Tool): presentation of the final tool

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In 2018 the welfare committee of the EAAM has set up a group of experts on welfare science, cetacean biology and zoo animal medicine with the goal of developing a welfare evaluation tool for bottlenose dolphins (*Tursiops truncatus*). The approach was to create a species-specific welfare assessment tool, which offers a holistic approach to the assessment of dolphin welfare. After six years this tool has now been completed. It contains five principles and encompasses 49 indicators based on the Five Domains Model. 37 indicators are animal-based and 12 are resource-based. Depending on the indicator, they are assessed using a two- or three-level scoring system. This tool is intended for internal use and should be applied on a regular basis in order to better recognize changes on an individual basis. The application is to be facilitated by the development of an app, which also helps to obtain a quick and clear evaluation of the data. The Dolphin-WET is seen as a living document, whenever there are new findings and publications, these are to be integrated into the tool. This matrix can hopefully also be used as a basis for assessing the welfare of other marine mammal species in the future.

Oral Presentation

Assessing the neuroendocrine system of cetaceans: a first look into the HPA axis of bottlenose dolphins and killer whales.

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The growing scientific interest in the health and welfare of cetaceans, as important species both in the context of ecosystem balance and environmental indicators and as animals kept under human care, has given rise to a myriad of studies in different veterinary fields. The main component in the regulation and development of the stress response system, among many other physiological and behavioural processes, is the Hypothalamic-Pituitary-Adrenal Axis (HPA Axis) in the neuroendocrine system. This often-forgotten system is made up of a set of neurons, glands, and non-endocrine tissues that play a critical role in the stress adaptation processes an animal undergoes, therefore being potential indicators of both health and welfare.

As a first step to shed light on the intrinsically complex HPA Axis, better understand its functioning, and improve its evaluation, we have firstly standardized a post-mortem methodological assessment of this system's components for cetaceans, and secondly studied the distinct histological, immunohistochemical, and pathological features of its different components in necropsied common bottlenose dolphins (*Tursiops truncatus*) and killer whales (*Orcinus orca*) that had been kept under human care. This is also the first thorough morphological description of both the pituitary gland and adrenal glands of killer whales.

Oral Presentation

Endoscopic findings in killer whales during gastroscopies performed by voluntary behaviour: the experience of Loro Parque.

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A preventative medicine program is one of the key factors in health evaluation for ensuring the welfare of marine mammals under human care. Gastroscopy plays an important role in modern-day cetaceans preventative medicine and it can be performed routinely using trained responses that enable medical procedures.

A flexible Storz video-gastroscope (length 3.20 m; diameter 1.3 cm) was used to perform several gastroscopies by voluntary behaviour in three killer whales. It was possible to check the forestomach and the entrance of the second stomach. Moreover, this study has allowed to evaluate some differences of the forestomach of these large animals. So, this presentation provides additional and important data on anatomy findings of this part of digestive system during the gastroscopy in killer whales.

Oral Presentation

Understanding and managing fungal infections through an in-house mycology protocol in bottlenose dolphins (*Tursiops truncatus*)

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Fungal pneumonia is a very increasing disease in both managed and free-ranging dolphins. However, the low sensitivity of certain diagnostic techniques commonly used in general practice, such as direct examination of biological samples, may lead to a delayed diagnosis and critically worsen the overall prognosis of fungal infections.

Accordingly, we developed an in-house mycology protocol for a group of twenty-six bottlenose dolphins, housed at Zoomarine, for screening and follow-up purposes. This protocol included a systematic and critical control approach in sampling, inoculation, incubation, fungal phenotypic identification, and susceptibility testing, in which all steps were thoroughly scrutinized.

Clinical diagnosing and assessment of treatment needs considered EORTC/MSG criteria used in human medicine. This was based on a multimodal diagnostic approach, including behavioural data, direct microscopy, culture, haematology, imaging, serology, and molecular methods.

This hands-on culturing protocol is an example for practitioners who intend to develop in-house laboratory settings that are time and cost effective. In our experience this mycology protocol was easily included in our medical surveillance programme, allowing economic, swift, and reliable results, and permitting an early diagnosis and thorough follow-up of infections by agents such as Cunninghamella bertholletiae, Aspergillus fumigatus, Aspergillus flavus, Penicillium spp., Candida sp., and Trichosporon mucoides.

Oral Presentation

The influence of the nutritional protocols and other factors on the hand-rearing of harbour seal (*Phoca vitulina*) orphan pups

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One of the greatest challenges working with orphan seal pups is to provide them with proper nourishment. To establish the best feeding option for these individuals at the Sealcentre Pieterburen (SP) in the Netherlands, three different feeding protocols were evaluated. The first one was a simple herring-based formula successfully used at the SP for years. The remaining two were different schemes of a salmon-based emulsion. The aim of this study was not only to compare both the growth rate and survivorship of the seal pups fed with the different protocols, but also to evaluate the influence of other physical and environmental factors over them. The results showed that one of the salmon-based emulsion schemes was as effective as the herring-based formula over the growth rate and survivorship, while the other one resulted significantly less effective (H=12,89, p=0,002). However, the weight of the pups at arrival stood out among any other factors because of its influence over both growing rate (H=8,59, p=0,01) and survivorship (F=7,9, p=0,005). These results allow for improving feeding protocols and understanding of seal pups needs.

Oral Presentation

Evaluation of *Erysipelothrix rhusiopathiae* occurrences and vaccinal reactions in cetaceans - Thirty year retrospective results through two global epidemiological surveys

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Erysipelas has always been a threat to marine mammal collections and the strategies to address the situation have varied over the years.

In a first global survey (97 facilities - 1384 animals), 22 fatal and 47 non-fatal cases were reported to have occurred between 1990 and 2000, all in non-vaccinated animals or animals vaccinated once.

However, fear of vaccination remains present. To better assess the epidemiology of the disease and appreciate the extend of vaccinal reactions, a second global survey, covering the years 2001-2020, was undertaken.

It had a 68,6% retour answer (140/204 facilities), totalizing 2267 cetaceans. 108 cases of erysipelas (4,8%), with 52 fatal (48%) and 56 non-fatal cases (52%) were reported, either in non-vaccinated animals, in animals vaccinated only once and in two instances in an animal vaccinated twice.

The results showed notable geographical differences in the types of erysipelas vaccines. For 47 institutions the main reasons not to vaccinate are the fear of (fatal) anaphylactic reactions and the reported high rates of vaccine reactions. The decision whether to vaccinate or not after a fatal case differs grandly between small and larger facilities. The most interesting result is that few reactions were in fact reported aside of the classical common side-effects of vaccines (8 facilities). Three facilities observed transient lethargy and irregular breathing in eight animals, from which only one had been treated. A link between the type of vaccine and side-effect could be established. One fatal anaphylactic reaction was described to have occurred in 1989 though local anesthesia had been added to the vaccine at the time. No case had occurred in regularly vaccinated animals.

Short Talk Presentation

Clinicopathological and medical management of a gastric adenocarcinoma in a grey seal (*Halichoerus grypus*) under professional care

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A 33-year-old female grey seal (Halichoerus grypus) presented with inappetence and gradual weight loss. Medical management included blood analysis (haematology and biochemistry), imaging (radiographic and ultrasound studies), and faecal analysis, along with multimodal support therapy throughout the course of the clinical signs. Although the clinical condition periodically improved, six months after the initial presentation a follow-up blood analysis showed a marked neutrophilic leucocytosis (22.800 × 106/L), along with a severe hyporexia, one episode of haemorrhagic vomiting, and increasing general discomfort. Ultrasonographic examination revealed a heterogenous echogenicity structure with anechoic pockets in the cranial abdomen. As the animal was no longer responsive to support therapy and considering her age and overall prognosis, the seal was humanely euthanized based on welfare grounds.

A full post mortem examination was carried out, revealing a marked thickening of the distal oesophagus, cardias, and gastric fundus, along with multiple nodular lesions involving the omentum. Histopathological examination revealed a metastatic gastric adenocarcinoma.

The literature documenting pinniped neoplasms is expanding gradually, although reports on gastric neoplasia are still rare. To the authors' knowledge, this is the first complete clinical and pathological report of a gastric carcinoma in a grey seal.

Short Talk Presentation

The use of SDMA in bottlenose dolphins (*Tursiops truncatus*) as renal biomarker: preliminary results

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Symmetric dimethylated arginine (SDMA) is an amino acid used as renal biomarker measuring the protein breakdown product excreted exclusively through the kidneys, making possible to establish an indirect estimation of glomerular filtration rate. It is not influenced by extrarenal factors, thus is more sensitive than creatinine and more specific than urea to assess renal function. Studies in dogs, cats, horses and certain wild species reported an earlier detection of renal failure or chronic kidney disease using SDMA together with serum creatinine and urea. However, little information is available regarding marine mammals, only some reports on manatees (Trichechus manatus latirostris) and Californian sea lions (Zalophus californianus). This work presents first results about the use of SDMA obtained within a year in a small group of bottlenose dolphins of different ages with the aim of encouraging other institutions to include SDMA in their routine check-ups, report its possible utility as an accurate diagnostic tool to assess renal function earlier, and eventually establish possible reference interval.

Video Presentation

Management of a pyometra in a bottlenose dolphin (*Tursiops truncantus*)

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In a routine blood examination in September 2023, a behaviourally normal 47-year-old female bottlenose dolphin (*Tursiops truncatus*) had an alarming increase in white blood cells. After extensive diagnostic work, ultrasonographic findings suggested a pyometra. Her medical treatment required her temporary separation from the rest of the group including her 5-year-old nursing calf. Considering this, we decided to implement a specially designed training program that allowed us to maintain vital welfare behaviours like oral hydration, ultrasound examination and blood sampling and train for new medical behaviours like urine sampling and intramuscular injections. We also started training several new behaviours not related to her medical treatment to keep her mentally stimulated. During this time of isolation from other dolphins, we also modified our enrichment program so that multiple elements were always available, and they were changed more frequently trying to ensure her welfare during this period. We are happy to report that she is now in good condition, has been reintroduced with the rest of the group and is continuing to be monitored weekly.

Oral Presentation

Delphinid creativity under stimulus control

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A method used to measure creativity in humans was applied to behaviors of bottlenose dolphins (n = 12, 7.5) and killer whales (n = 9, 5.4) while under stimulus control for "innovate". Four devices associated with creativity were evaluated for behaviors correctly produced during test sessions: fluency, flexibility, originality, and elaboration. The following qualitative comparisons were found: both delphinids produced different frequencies (fluency) and types of behaviors with different levels of energy (flexibility). Motor behaviors and vocalizations were produced more frequently for both delphinids. Single and complex behaviors were produced by all animals with killer whales producing more complex behaviors more frequently overall than dolphins. Elaboration was more difficult for dolphins as compared to the killer whales because of acceptable criteria. Individual animals invented behaviors throughout the training periods and a handful during testing (originality). The high engagement of the animals showed how stimulating this cognitive task was for them regardless of reinforcement timing, amount, and type. Training history appeared to have little effect on individual behaviors produced during test sessions. Ultimately, this concept demonstrated that both delphinids produced "creative" behaviors that could be characterized systematically while also providing enriching interactions for both animals and trainers.

Oral Presentation

Redirecting an undesired behavior of saltwater ingestion in a California sea lion through positive reinforcement and shaping techniques

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A neutered 24-year-old male California sea lion (*Zalophus californianus),* housed at Zoomarine Portugal*, w*as occasionally seen drinking salt water since 2007, with a significant increase in frequency the last 4 years.

Considering the animal's advanced age, its medical history of chronic gastritis and the possible negative health consequences of saltwater ingestion, trainers and veterinarians decided to address this particular undesired drinking behaviour.

Through training based on positive reinforcement and shaping techniques, the sea lion was conditioned to do the same drinking behaviour but in different places in the habitat where freshwater was available. The main aim was not to suppress the behaviour itself but to redirect it. As a result, he is now seen drinking freshwater from the drinking fountains, present in every habitat, without the need for an S'd and only occasionally from the pool, mitigating the possible health issues caused by saltwater ingestion in large quantities.

For many years we have trained voluntary medical and husbandry behaviors with all the pinnipeds in our zoological collection as part of our preventive medicine program, this is yet another example of how important those behaviors are in maintaining good quality of life and welfare.

Oral Presentation

Dolphin training applied to scientific projects in cognition and bioacoustics

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Training is a key component to an animal's wellbeing and should be considered as an essential part of good animal care. During a period of two years, apart from the daily veterinary care and educational training, we trained our dolphins several essential behaviours that were used on five research projects in bioacoustics and cognition. For bioacoustics, we trained four dolphins on eyecups, stationing on a biteplate, discrimination tasks in cylinder wall thickness as well as material discrimination using spheres. For cognition we trained five dolphins for the concepts of "wait and go", "repeat" and "mark". We always trained extra dolphins even when not necessary by the experimenters as this type of training was enriching for the animals.

With our presentation we would like to share the knowledge that the dolphin team have acquired during the training of our dolphins for those scientific projects. The implementation of research training in the team was also important for the personal development of the trainers. They felt a great increase of their bond and trust with their animals as well as a personal fulfilment because their experience and expertise were serving to help develop global strategies to protect the animals in the wild.

Oral Presentation

Voluntary Corneal Debridement in a California Sea Lion

Pamela Midwood (1)

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The treatment 20 year old California sea lion with reoccurring eye ulcers with no obvious or consistant trigger. No correlation between water quality, exposure to direct sunlight or duration of treatment with eye drops and the development of eye ulcers could be found. Eventually a temporary tare in the upper corneal layer was observed and debridement of the cornea was prescribed. Through extensive generalization of eye care in the animals training history this was able to be done with minimal specific debridement training and was successfully completed when an ulcer next occurred. This treatment has been completely successful as it took place in January 2023 and the individual has had no ulcers or eye problems since – though extra eye care is still undertaken daily as a precaution.

Short Talk Presentation

Training for voluntary biopsy in a Californian sea lion (*Zalophus Californians*)

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In recent years much emphasis has been placed on preventive medicine in Loro Parque's sea lions since through medical behaviours. We can take care of the animal's health and welfare in a non-aggressive and non-stressful way which also reinforces trust between caregivers and animals. As part of this practice, every morning the animals are checked and eyes, teeth, body and flippers are examined carefully.

Magy, a 22-years-old Californiano sea lion (*Zalophus californianus*), was found to have a small lump on the left side of her body behind the pectoral fin. A round mass was detected with a nucleus that only could be identified performing a biopsy.

That puncture required a concise training by the sea lion team to be able to do it voluntarily, reducing the stress produced by a restrain and / or general anaesthesia. The training procedure consisted of placing her in a prone position with her flippers open, and introducing a piece of wood next to her, to serve as protection for the veterinary team. After several successful training sessions, the veterinary team was able to perform the puncture of the lump voluntarily while the animal remained calm at all times.

Short Talk Presentation

The ophthalmologist is coming! Training our pinnipeds for a full day consult with the specialist.

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Mundomar is a zoological park located in Benidorm, Spain, with emphasis on marine mammal species. The pinnipeds department hosts ten individuals of three different species of pinnipeds, including 2.0 harbor seals (*Phoca vitulina*), 3.3 Californian sealions (*Zalophus californianus*) and 1.1 Patagonian sealion (*Otaria byronia*). Pinnipeds under human care tend to develop ophthalmic diseases, influenced by their environment. A veterinary ophthalmologist specialized in pinnipeds was visiting to complete ophthalmologic assessment of every individual, including photos, videos, infrared light, slit lamp evaluation in a dark room, and eye ultrasounds. To fulfill these exams in a single day for our ten specimens, we create a training plan that we are presenting today.

Short Talk Presentation

Training voluntary gastroscopy in orcas (*Orcinus orca).*

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Voluntary gastroscopy of the orcas is the most recent and also most important husbandry training at Loro Parque. Being able to see the stomach of such a big animal provides new and valuable information about their health.

Before achieving the complete behaviour of tubing an orca for at least five minutes, many previous training sessions had to be done separately. Obviously, this was needed to be done by the trainers who had the best relationship with the animals to make them feel confident during the whole process. First step of all, was to teach them to keep their mouth open for a period, so for this we used a tube that was previously desensitized and that they had to bite to keep their mouth open. Next step was to desensitize the endoscope, it took a lot of small approximations before introducing it. Finally, the black tube and endoscope was done all together and just had to make the session longer. Also, we asked our vet to come to get the animals used to see him and all the devices needed for the gastroscopy around.

Once first gastroscopy was successfully performed a monthly scheduled was set in place.

Round Table

Peer-Review Publishing – Process How-To's & Recommendations

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This round table session is designed to review the peer-review process from manuscript and cover letter submission to reviewer responses and communicating with a journal editor. Scientists focus on hypothesis generation and data collection, analyses, and presentation, but navigating the path toward publication is also important to conducting science and can be equally challenging to that process. This round table will present suggestions for a strong and informative cover letter, responding to reviewer comments, and communicating with a journal's editor. It will allow for questions from the audience and general discussion on topics related to Open Access, supplemental material, and identifying and selecting the most appropriate journal for your material. The session will be videotaped to allow for placement on the EAAM and Aquatic Mammals' websites for future reference. The round table organizer will be joined by one to two colleagues with previous experience as journal editors to share experience(s). While scientific writing is not the same as creative writing, peer-reviewed publications offer a valuable outlet for our stories and science.

Oral Presentation

Presence of bottlenose dolphins (*Tursiops truncatus*) in the coast of Marina Baixa (Alicante, Spain) and associated environmental factors

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It is known that some bottlenose dolphins (*Tursiops truncatus*) are present in the coast of Alicante, Spain. The protected area of Sierra Helada, in the region of Marina Baixa, seems to be especially important for the species. This littoral suffers a high level of human pressure not only because of the great number of tourist activities developed there, but also because of the existence of several fish farms, even inside the protected area. Furthermore, there is no scientific studies either about the abundance of dolphins or the factors that affect their presence. Because of that, the aim of this study was to try and answer these questions. To achieve it, daily observations were carried out both from a boat and from the coast along the littoral of Marina Baixa between March and August 2022. Bottlenose dolphins were detected up to 30 times, inside (66,67%) and outside (33,33%) the protected area. 26,6% and 56,6% of the sightings happened closer to 1 and 2 nautical miles from a fish farm respectively. Moreover, the presence and quantity of dolphins showed significant correlation with tide, temperature, wind direction and speed, the time of the day, the day of the week and the fishing activity.

Oral Presentation

25 years of research: a marine protected area for cetaceans and marine turtles in the strait of Gibraltar

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This comprehensive 25-year study in the Strait of Gibraltar, a key ecological corridor between the Atlantic Ocean and the Mediterranean Sea, focuses on the conservation of a wide array of marine species. These include bottlenose dolphins, common dolphins, striped dolphins, long-finned pilot whales, sperm whales, fin whales, killer whales, and notably, the loggerhead sea turtle. During the extensive research period, the team covered 47382.23 kilometres in their campaigns, employing a variety of methodologies such as linear and aleatoric transects, distance measurements, and the analytical depth of Generalized Additive Models (GAM), to intricately estimate the abundance and distribution of these species. The findings from this study emphasize the urgent need for establishing a marine protected area. This designated area would greatly benefit from the integration of marine mammal observers and the implementation of wide-reaching educational programs. These initiatives are crucial for enhancing public awareness and understanding of these species. Moreover, the study highlights the importance of effective management strategies in the conservation of these significant resident and migratory species. It underscores their ecological importance and the urgency of protecting their habitat, ensuring their preservation for future generations.

Video Presentation

Interactions between orcas and sailboats in the Strait of Gibraltar

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In this video, we explore the interactions between orcas and sailboats in the Strait of Gibraltar, focusing on various crucial aspects. The introduction highlights the growing concern about these encounters, emphasizing the need for a multidisciplinary approach backed by scientific evidence. The dynamics of interactions are addressed, emphasizing the intelligence and adaptability of orcas, which tend to target the rudders of vessels, regardless of their type, possibly motivated by play, curiosity, and social learning.

Risk and speed emerge as key themes, with the primary recommendation being to maintain a constant speed to reduce interaction time and, consequently, the risk of damage. The section on rudder design underscores the importance of balancing strength and flexibility. Deterrents, such as 'pingers' and sounds of pilot whales, are examined, but it is concluded that they do not offer effective solutions and may have unintended consequences. Satellite and video camera tags are used to study their behaviour and position.

A proposal for an Anti-Rudder-Breakage System, in collaboration between the Loro Parque Foundation and the University of Cádiz, aims to minimize the impact of these interactions. Citizen participation, facilitated by a Telegram group, has been essential for managing interactions, leading to changes in maritime routes and reduced encounters. The conclusion highlights the complexity of the phenomenon and the need for a comprehensive, evidence-based approach to achieve harmonious coexistence.

Oral Presentation

Public Aquarium, Engineering, Science, Funding: a case study on reducing marine mammal by-catch

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Whales become entangled in gillnets and subsequently drown. Successful by-catch reduction requires the cooperation of many players. The acoustic warning device PAL (pat.) was developed to enhance the acoustic 'visibility' of gillnets. Its effectiveness was tested step by step in an aquarium, in the field and then with professional fishermen in the Baltic, North and Black Seas.

In the Western Baltic, 19 porpoises were bycaught in 4,350 km of nets during 752 trials: 16 in standard nets but only 3 in PAL- nets, a by-catch reduction of 80%. With an adapted signal, fishers around Iceland reported 100% bycatch reduction and in the Black Sea, Bulgarian fishers achieved a reduction of 73%. Trials by the FAO (United Nations) are ongoing.

Since 2017, the Baltic Sea Infocenter (OIC) in Eckernförde, a local public aquarium, is handling a deployment project to provide around 100 local fishermen with 2,000 PAL, free of charge. in Sept. 2023, 1.4 Mio. € were approved to continue this validation study, funded by the European Union as well as from the government of Schleswig-Holstein. This enables the OIC to finance PAL, servicing, personnel, and equipment including a survey boat to coach and monitor fishers.

Short Talk Presentation

Whale-PAL to successfully prevent orca-attacks on pleasure craft

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The acoustic warning device “Whale-PAL” (Pat.) was deployed by over 200 pleasure-craft navigators sailing the orca alley off the Iberian Peninsula since 2021. It emits a specific alerting signal and complies with EU regulations (2020/967). Feedback from 150 users shows that the probability of orca encounters and damage is significantly reduced by comparison to sailing without protective measures (literature data). 107 of 109 passages with the Whale-PAL went without any problem, a ratio of 98% as opposed to 89% without. The chance of not seeing any orcas at all was increased from 75% without protective measures to 88% when sailing with whale-PAL. 13 sailors deploying a whale-PAL reported an orca interaction: there were 11 sightings and furthermore 2 damaged vessels. The whale-PAL increases the chance of sailing away from an interaction unscathed from 50% to 85%. All differences are statistically significant (binomial test, $p<0.05$). The evaluation of the feedback shows that the Whale-PAL, with its specific towing rigg and its unique acoustic signal, measurably reduces the risk of an orca attack. This is the first report on the effectiveness of a protective and minimally invasive orca-countermeasure for sailors sailing the orca alley.

Oral Presentation

Unexpected context-dependent vocal use in bottlenose dolphins (*Tursiops truncatus*) under human care

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Dolphins elicit large scientific interest for their complex acoustic communication. However, the difficulty in identifying the exact behavior performed while vocalizing leave the investigation of their vocal use underexplored. Dolphins are known to produce three main vocal types: clicks for scanning the environment, whistles during positive social interactions and burst pulses in competitive contexts. Also, they can produce sequences of those sounds, whose functions still remain unclear. We conducted underwater observations on a group of bottlenose dolphins (Tursiops truncatus) where the vocal emission was matched with the associated behavior (N = 479). Our results showed unexpected non-random associations between vocalizations and behavioral contexts. Clicks were related to affiliative interactions, pinpointing towards a complementary communicative function. Whistles were used in non-social contexts, suggesting a broader function as general contact call. Highly arousal contexts (agonistic and social play) were preferentially punctuated by burst pulses, highlighting their role as “emotively charged” signals. Mixed vocal emissions were associated to sexual (bust pulse-whistle-click), affiliative (click-whistle) and solitary play (burst pulse-whistle), suggesting a potential role of those sounds in modifying, confirming or refining the functions of simple vocal counterparts. Taken together, these findings open new scenarios in the study of dolphins’ flexible vocal use.

Oral Presentation

Developing an AI-based acoustic and video system to study Orca communication and welfare

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The welfare of marine mammals is a subject of keen interest for managers, legislators, and scientists. However, assessing welfare is a complex task, involving diverse information sources, models, and parameters. In response, we propose a modular automatic system currently under development for deployment at Orca Ocean in Loro Parque. It focuses on the estimation of animal activity in the pools by continuously monitoring their vocal activity and location. This necessitates the recording of substantial amounts of acoustic and video data, which then must be reduced to extract relevant information about animal behaviour. The infrastructure is comprised of a set of acoustic and video devices, each of which provides a data stream. These streams must be integrated to derive global status information over time. The advent of AI makes it feasible to address multiple engineering challenges of such a system, like automatic visual and acoustic detection and identification. The system can generate information on vocal activity as well as the cinematic and dynamic behaviour of the animals. Ultimately, this information contributes to an improved understanding of animal communication and facilitates the development of welfare indicators.

Oral Presentation

Monitoring sound in marine mammal facilities: a practical approach

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We all are exposed to sound, even animals in free range and under human care. However, in some cases sound can be considered unpleasant or unwanted and considered noise. When this noise exceeds certain intensity or it occurs constantly, it is when it might affect well-being. The negative effects of underwater noise have been widely described in wild cetaceans, and its effect on aquatic mammals in zoological settings is a common subject for speculation among the anti-captivity groups. The European Association for Aquatic Mammals has considered underwater noise as one of the potential stressors for aquatic mammals under human care and, consequently it has established the need to implement a noise monitoring program on its member facilities. But monitoring underwater sound is not a simple task, and the interpretation of the results can be challenging. The goal of this communication is to present the monitoring program implemented at Loro Parque using hydrophones installed in marine mammal facilities Orcas (*Orcinus orca*), Californian sea lions (*Zalophus californianus*) and common bottlenose dolphins (*Tursiops truncatus*), as a model of a simple monitoring program that could be easily adapted to other facilities improving the welfare of the aquatic mammals in zoos.

Video Presentation

Overcoming the Challenges of Blow-Sampling with UAS in Small Cetaceans

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Quantifying the health of marine mammals is an important task in understanding how these animals behave and react to anthropogenic stimuli. It is possible that by examining the hormone cortisol, the relative stress level of small cetaceans can be discerned. This hormone can be analysed from examination of the breath and/or mucus of the mammal. It is known that the mucus found in the flow generated from dolphins' blowholes can be tested for hormones that help understand the current health status of the dolphin. Recently, attempts have been made to capture this hormone in the breath of both whales and dolphins with multi-rotor uncrewed air systems (UAS) by flying through the "blow" of the mammal as it breaks the water surface to breath. We announce the development of a first of its kind blow collection UAS platform designed to mitigate the limitations of UAS platforms in the study of small cetacean health, through the use of acoustic and visual stealth advancements specifically built around the sensory capabilities and blow physics of small toothed whales. We will highlight device development, capability, advantages, and operation along with blow-field modelling and high-speed particle image velocimetry data granting a better estimation of successful blow collections. We will also preview new imagining technology and the implications for AI guided flight and approach.

Oral Presentation

Mechanical water disinfection – a new way to keep aquariums clean and healty

Norbert Fleck* (1)

(1.) See "corresponding author"

Originally developed for industrial applications, the mechanical disinfection and microfiltration (as fine as 0,05 µm absolute) of water-based fluids turns out to be an extraordinary cheap and sustainable method of controlling the water quality of small to mid-sized aquariums while reliably taking parasites, fungi, algae, bacteria and even viruses out of the circuit.

Short Talk Presentation

Studying Animal Behavior with Artificial Intelligence – A Case Study on Polar Bears

Zuerl, Matthias* (1), Almunia, Javier (2), Eskofier, Bjoern (1)

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Animal observation performed by biologists, ecologists and veterinarians can be divided into three stages. **First, localising the animals** in their habitat. **Second, identifying individual animals**. This step is crucial, allowing biologists and animal keepers to tailor possible measures or treatments to individual needs. **Third, recognition of behaviour**. We have automated each of these stages through close collaboration between biologists and computer scientists. The AI-driven algorithms we have developed enable continuous monitoring of individual behaviour. We can determine activity/inactivity patterns, stereotypical behaviour, group interactions, enclosure usage, and distance travelled, among others. It is also possible to train the AI models on very complex ethograms. For polar bears, we were able to distinguish over 170 different behaviours. Our proposed methods are species-agnostic and can be transferred to other animal species and environments. In collaboration with Loro Parque, we trained one model to identify the four kept orcas. This stage allows for detecting individual changes in behaviour at an early stage. In addition to the countless applications in zoological institutions, the methods we have developed are also relevant for research in the field. We apply our models to wildlife video data for tracking individuals and their behaviour over time.

European Association for Aquatic Mammals

52nd Annual Symposium European Association for Aquatic Mammals
EAAM
TENERIFE 11-15 MARCH 2024

2024 POSTERS

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European Association for Aquatic Mammals
Rue de la Science 14b, 1000 Brussels – Belgium – information@eaam.com

Poster

Intestinal volvulus as cause of death in two killer whales kept under human care.

Alonso-Almorox, P.* (1), Arbelo, M (1), Sierra, E. (1), Fiorito, C. (1), Suárez Santana, C. (1), Rivero, MA. (1), Câmara, N. (1, 2, 3), Consoli, F. (1), Alcaraz-Rico, L (1), Suárez González, Z. (1), Molpeceres-Diego, I. (1), Navarro, J. (12), Marrero, L. (1), Castro, A. (11), Colom Rivero, A. (1), Felipe, I. (1), Grande, F. (2), Bernaldo de Quirós, Y. (1), Fernández, A. (1)

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Two female killer whales (ages 3 and 17) died in 2021 at Loro Parque Zoo. Necropsies were carried out within a 24-hour post-mortem period, and a detailed pathological study, as well as complementary laboratory analyses were performed. These two animals were determined to have died due to intestinal volvulus.

Intestinal volvulus has been recognized throughout the literature as the cause of death in 18 cetacean individuals. All cases originated from under human care institutions (11 facilities) and included both captive bred (n = 9) and free-ranging (n = 9) animals. When clinical history was available (*n* = 9), animals consistently demonstrated acute dullness 1 to 5 days prior to death. In 3 of these animals (33%), there was a history of chronic gastrointestinal illness. The pathological findings were like those described in other animal species and humans and consistent with intestinal volvulus. Potential predisposing causes were recognized in most cases (13 of 18, 72%) but were variable.

Through the present poster, we will examine and deeply analyse these two intestinal volvulus cases in killer whales, adding valuable information to the discussion around an important cause of death in cetaceans kept in zoological facilities

Posters included in this section are the poster PDFs from the conference that were provided to us.

Intestinal volvulus as cause of death in two killer whales kept under human care : a pathological study

Alonso-Almorox, P.* (1)., Arbelo, M (1)., Sierra, E. (1)., Fiorito, C. (1)., Suárez Santana, C. (1)., Rivero, MA. (1)., Câmara, N. (1)(2)(3)., Consoli, F. (1)., Alcaraz-Rico, L (1)., Suárez González, Z.(1)., Molpeceres-Diego, I. (1).,Navarro, J. (12)., Marrero, L. (1)., Castro, A. (1)., Colom Rivero, A. (1)., Felipe, I. (1)., Grande, F. (2)., Bernaldo de Quirós, Y. (1)., Fernández, A. (1)

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CASE STUDY BACKGROUND

Two female killer whales died in 2021 at Loro Parque Zoo. Necropsies were carried out within a 24-hour post-mortem period, and a detailed pathological study, as well as complementary laboratory analyses were performed. Through the present poster, we will examine and deeply analyse these two intestinal volvulus cases in killer whales, adding valuable information to the discussion around an important cause of death in cetaceans kept in zoological facilities.

PATHOLOGICAL STUDY

CASE 1 :
Orcinus Orca; Female; 3 y/o.
Progressive weight loss, chronic gastrointestinal history

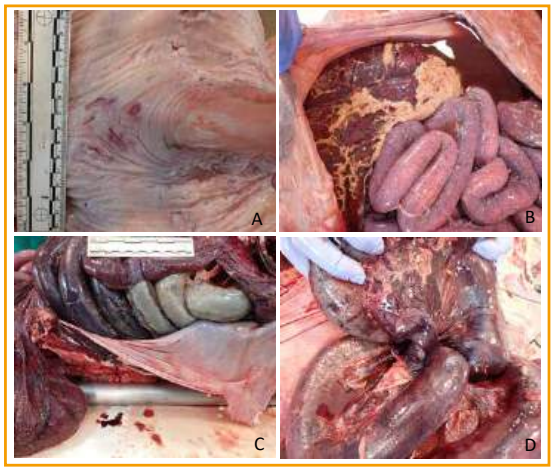

Figure 1. (A) Ulcerative pharyngitis; (B) Opening of the abdominal cavity: hemoabdomen; fibrinous peritonitis and serositis with petechia; (C) Marked dilation, congestion and hemorrhages in intestine; (D) Complete mesenteric torsion in distal intestine (9 loops), severe haemorrhages and venous congestion.

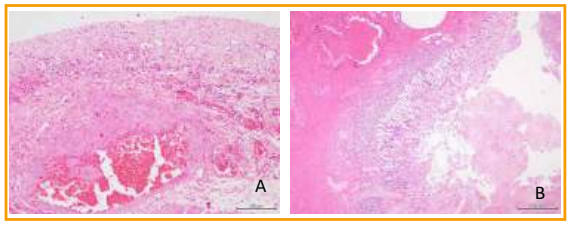

Figure 2. (A) Active-chronic serositis with thrombosis (H-E) 20x; (B) Haemorrhages and congestion in intestinal mucosa (H-E) 4x.

Microbiological cultures and antibiogram :
Multidrug-Resistant *Escherichia coli* in intestine, lung, kidney, liver, blood, etc.

Morphological diagnosis :
Intestine: Severe intestinal volvulus with marked venous infarction and ischemic necrosis in the distal intestine associated with the diffuse presence of Gram-negative bacteria (identified as Multidrug-Resistant *E.coli*).
Other findings: Severe fibrinonecrotic transmural multifocal enteritis. Moderate chronic-active serositis with severe congestion and thrombosis. Moderate multifocal calcinosis of the intestinal mucosa; Esophagus: moderate chronic lymphocitic esophagitis with multifocal hyperkeratosis.; Stomachal compartiments: moderate chronic-active serositis, moderate chronic lymphocytic gastritis with irregular hyperplasia of the mucosa.
Cause of death: Severe intestinal volvulus, enterotoxic shock

CASE 2 :
Orcinus Orca; Female; 17 y/o.
Good body condition, subacute process.

Figure 3. (A) Necrosis and venous infarction of intestinal loops ; (B) Congestion, haemorrhages and distension of the distal intestine; (C) Complete mesenteric torsion; (D) severe haemorrhages in mesenterium and mesenteric lymph nodes.

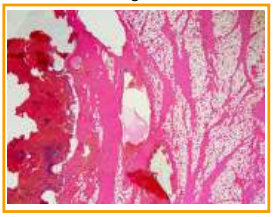

Figure 5. Intestine. Severe hemorrages in serosa, venous infarction with transmural hemorrhages and trombosis (H-E).4x.

Morphological diagnosis and cause of death:
Severe intestinal volvulus with vanous infarction and massive ischemic necrosis in middle section of the intestinal, enterotoxic shock.

DISCUSSION

Intestinal volvulus has been recognized as the cause of death in 18 cetacean individuals, both captive and free-ranging (Begeman., 2012). When clinical history was available (n = 9), animals consistently showed acute dullness prior to death. In 33% of cases, there was a history of chronic gastrointestinal illness. The pathological findings were similar to those described in other species and humans and consistent with intestinal volvulus. Potential predisposing causes were recognized in most cases a 72% of cases but were variable.

In the present study, both individuals presented acute dullness prior to death, but only 1 of the individuals presented chronic previous gastrointestinal illness, a potential predisposing factor for intestinal volvulus. No predisposing factors for intestinal volvulus were identified for the other animal.

CONCLUSIONS

- Both individuals in the present study were determined to have died due to intestinal volvulus and entero-toxic septicaemia (ETS Shock), and the pathological findings were similar to those described previously in cetaceans (both wild and under human care), other animals and humans.
- Intestinal volvulus in cetaceans has been previously described throughout the literature, all being fatal. Further studies are imperative to identify more predisposing factors to prevent the occurrence and improve early diagnosis and handling of the process.

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Poster

Parameters associated with the behaviour of a harbour seal (*Phoca vitulina*) showing aerophagia

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Aerophagia could be considered a type of oral stereotypy related to stressful or boring situations, influencing animal welfare. Because of that, it is very important to identify the factors that increase this behaviour in order to take them into consideration in daily planning.

The present study focuses on an adult male harbour seal (Phoca vitulina) with aerophagia. This animal lives in Mundomar Benidorm with another male of the same species, and several California and South American sea lions (Zalophus californianus and Otaria byronia, respectively). To identify the situations that motivated the aerophagia, ethograms were conducted during May and June 2023.

The results showed that the mentioned aerophagia was affected by the hour of the day, being significantly higher during the afternoon ($W=5,6$, $p=0,018$). Equally, the rate of aerophagia was statistically correlated with diving frequency ($\rho=-0,65$, $p=0,029$). Furthermore, there were several factors related to daily activities that influence other behaviour rates: the presence of maintenance people, the door noise, the specific holding where the seal was or the animals that shared it with him.

These results will help us plan ways to reduce the frequency of aerophagia in this seal.

52nd Annual Symposium
European Association for Aquatic Mammals
Tenerife 12-15 March 2024

Parameters associated with the behaviour of a harbour seal (*Phoca vitulina*) showing aerophagia

Alba Segura, Carmen M. Arija & Juan Criado

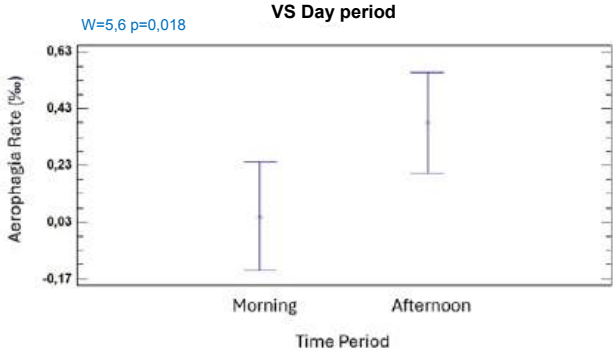
INTRODUCTION

Aerophagia is the medical term used to describe excessive and repetitive swallowing of air. It could be considered a type of oral stereotypy related to stressful or boring situations, which influence animal welfare. Therefore, it is very important to identify the factors that increase the display of this behaviour in order to take them into consideration in the daily planning.

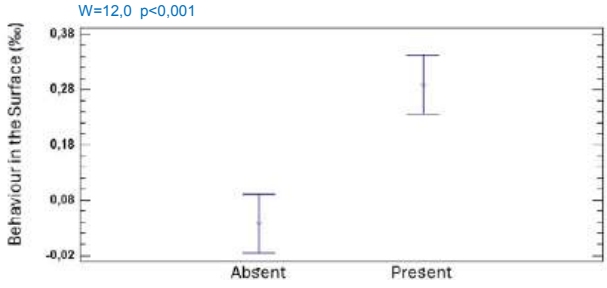
MATERIAL AND METHODS

The present study focuses on an adult male harbour seal (*Phoca vitulina*) with aerophagia. This animal lives in Mundomar Benidorm (Spain) with another male of the same species, and several California and South American sea lions (*Zalophus californianus* and *Otaria byronia*, respectively). To identify the situations that promote the abnormal swallowing of air, ethograms were performed during May and June 2023. Statistical analysis (ANOVA, Mood's Median and correlation tests) were used.

Aerophagia behavior VS Day period



Behaviour in the Surface VS Door noises



RESULTS

Aerophagia

The results showed that the rate of aerophagia was affected by:

- The hour of the day, being significantly higher during the afternoon than in the morning (W=5,6 p=0,018).
- The diving frequency, existing a negative correlation between both variables (ρ=-0,65 p=0,029). The rate of aerophagia increases significantly when diving frequency decreases.

Other Behaviour

Furthermore, there were several factors related to the daily activities that had an effect on other behaviour rates:

- The presence of a particular member of the staff (maintenance) increases search behaviour (W=5,6 p=0,0179)
- The existence of door noises increases the amount of time the animal spends in the surface (W=12,0 p<0,001), reducing the diving behaviour and affecting negatively the aerophagia behaviour.
- The specific holding where the seal stays conditions the type of locomotion (slow or fast swimming) used by the animal. The increase in the space available involves a significant increase in the frequency of fast swimming.
- The animals that shared the holding with the seal have an effect on his behaviour. Specifically, a female of California sea lion whose presence in the holding produces a significant reduction of the time the seal spends swimming fast (F=10,71 p=0,0084).

DISCUSSION AND CONCLUSION

The frequency of aerophagia in this seal correlates to the reduction in the activities occurring during the afternoon. Since the rate of aerophagia is directly affected by the frequency of diving, the use of environmental enrichment devices that promote underwater behavior could be a good option to reduce the factors that trigger aerophagia especially in the afternoon. Due to the short observation period, more data would be needed to clarify if any other factors could have some type of influence in the presentation of the aerophagia too.

Poster

Bottlenose dolphin pyometra: A Case Report

Canales, R.* (1), Saviano, P. (2), Diez de Velasco, A. (1), Morcillo, E. (3), Sampayo, J. (4), Robeck, T. (5)

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An estimate 50-year-old female bottlenose dolphin (Tursiops truncatus) under anti conceptive treatment with altrenogest and nursing a juvenile calf presented leukocytosis, four times her normal white blood cell counts, during routine monthly health assessment, with no clinical symptoms.

Abdominal ultrasonography revealed enlarged uterus with echogenic content suggestive of pyometra.

Altrenogest was discontinued and initial treatment consisted of oral antibiotics, estrogens and misoprostol with no changes observed in white blood cell counts.

By ultrasonography uterine contractions and content movement could be observed withing uterine horns and uterine body, but pyometra remain closed with no discharge.

Two hysteroscopies were performed to collect samples (culture, cytology, and biopsy) and uterus was flushed via endoscope. Pus culture was suggestive of Pseudomonas aeruginosa growth, and antibiotic therapy was prescribed according to sensitivity results.

We present clinical and pathological data, diagnostic and treatment and discuss the important role medical imagen plays in diagnosis and treatment in dolphins' reproductive pathologies.

Bottlenose dolphin Pyometra: A Case Report

Rocio Canales [1*], Pietro Saviano [2], Angela Diez de Velasco [1], Esther Morcillo [3], José Sampayo [4], Todd Robeck [5]

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INTRODUCTION

An estimate 50-year-old female bottlenose dolphin (*Tursiops truncatus*) under anti conceptive treatment with altrenogest and nursing a juvenile calf presented leukocytosis, four times her normal white blood cell counts, during routine monthly health assessment, with no clinical symptoms. Closed pyometra was diagnosed. Altrenogest was discontinued and initial treatment consisted of oral antibiotics, estrogens and misoprostol with no changes observed in white blood cell counts.

By ultrasonography uterine contractions and content movement could be observed withing uterine horns and uterine body, but pyometra remain closed with no discharge.

Two hysteroscopies were performed to collect samples (culture, cytology, and biopsy) and uterus was flushed via endoscope. Pus culture was suggestive of *Pseudomonas aeruginosa* growth, and antibiotic therapy was prescribed according to sensitivity results.

In Figure 1 blood results are presented a long with most significant events during treatment. Note the dramatic wbcc decrease after uterine was opened and flushed during hysteroscopy.

TREATMENT PLAN

REGIME FOR OPENING CERVIX

- Estradiol 3 mg PO BID x 15 days.
- Misoprostol PO TID as follows: Day 1: 50mcg, Day 2: 75 mcg, Day 3: 100 mcg x 5 days, 150 mcg x 8 days. Total 15 days.
- Second round of Misoprostol was started 5 days before the second hystersopy: Day 1: 75 mcg SID, day 2: 100 mcg BID, then 3 days 150 mcg and 5 more days at 150 mcg TID. Total 10 days.

ANTIOBIOTHERAPY

- Levofloxacin 1 g PO BID + Clindamycin 1,5 g PO BID x 2 weeks.

Chaged after culture resuls to:

- Amikacin 2.2 g IM SID + Ceftriaxone 4 g IM SID x 3 weeks.
- After second uterine hysteroscopy and flush, uterine content culture resulted positive *to Proteus spp* sensitive to Levofloxacine so a new course of Levofloxacin 1 g PO BID was given. Clyndamicyn was added after increase on wbcc was observed.

PREVENTIVE AND SUPPORT

During all treatment the following was given preventlvely:

- Fluconazol 400 mg PO BID.
- Hydration 2 l PO BID; TID during Amikacin+Ceftriaxone treatment
- Oral probiotics

Figure 1. Orange line=Average wbcc for this individual since arrival at Mundomar.

ULTRASONOGRAPHY

Figs 2-5. 2) uterine body, 3-4) uterine horns, 5) spleen.

The appearance of reproductive tract was typical of the pyometra (Figs 2-4). The ovaries did not show any presence of cysts/follicles or CL. Myometrium presented a maximum average thickness of 1.26cm sonographically homogeneous with no evidence of cysts or mass present inside the wall layer. The lumen was filled with heterogeneous suspension of echoic liquid with fine hyperechoic spots.

After 7 days of treatment with estrogens and misoprostol cervix was expected to open and the frequency of ultrasound sessions were intensified to confirm. A total of 9 ultrasound exams have been performed in the following 48h to evaluate if there was any evidence of uterine content being discharged. Although it was possible to observe the movement of this liquid inside the lumen, pyometra remained closed. The dilatation and the contraction of the uterine horns resulted in moments where the liquid filled more the body of the uterus vs the horns and vice versa, however the volume of the liquid did not show a significant decrease or change in the echogenicity. The average dilatation of the left and right uterine horns observed the 12th of September was significantly reduced at the 13th and 14th of September meanwhile the dilatation of the uterine body resulted increased. The cervix and pseudocervix were easily visualized but it was never possible to see liquid passing through them.
The only other remarkable ultrasound finding was enlarged spleen (Fig. 5), compatible with reported findings of enlarged spleen related to chronic infectious diseases.

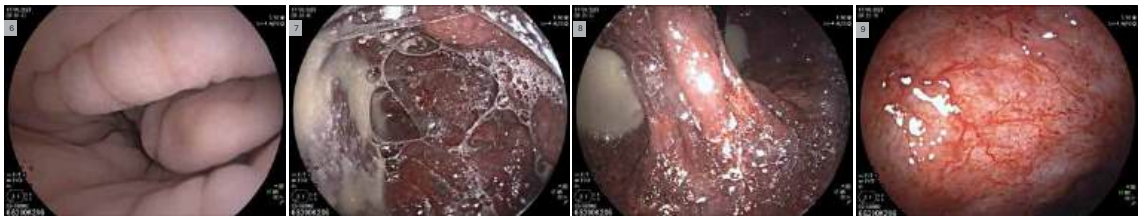
After this 48 h of intense monitorization by ultrasound, misoprostol dose was increased to 150 microgram PO TID and a hysteroscopy was planned for opening the cervix, uterine evaluation and flush, keeping estrogens and misoprostol treatment for a total of 15 days.

ENDOSCOPY

Two hysteroscopies were done 3 weeks apart.

Transvaginal hysteroscopy was performed using "ultraslim" gastroscope (EG-580NW2, Fujifilm Vet) 5.9 mm and 2.4 mm working channel.

Access through the vaginal vestibule was completed avoiding air insufflation to prevent any pressure on the organ with the consequent pain and risk of sepsis.



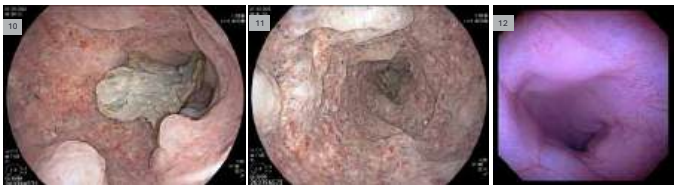
Figs 6-9. Images from the first hysteroscopy

The vaginal mucosa had a pale pink color, smooth surface, and no content inside (Fig.6). When reaching the cervix, it appeared closed. Apposition was made with the tip of the endoscope and access was achieved by applying pressure on it. It was not necessary to catheterize the cervix with guides or catheters to achieve intubation.

Once inside the uterus body, thick purulent content was found (Fig.7), and multiple flush and aspiration were needed to clear that content. It was possible to visibly identify the bifurcation of the right and left uterine horns (Fig.8). Generalized, the uterine mucosa was edematous, hyperemic, with increased submucosal vascularization and vesicles along the entire surface (Fig.9). Samples were taken for culture of the uterine contents and biopsies for histopathology of the uterine mucosa. A total of 1 l of sterile 0.9 % saline solution was needed in each procedure, by flushing and aspirating bolus of 60 ml each time. Final flushes were done with 2% gentamicin solution.

During the first hysteroscopy, body uterus and both horns were full of thick white exudate, compared with the second procedure where uterus body was clean, and only some light whitish fluid was coming from the tips of the horns.

Second hysteroscopy showed a less edematous and hyperemic uterine mucosa, but remnants of necrotic tissue could be observed in the uterine lumen (Figs. 10-11). Imagen from a normal uterine lining has been included for a better comparison with our female´s findings (Fig. 12).



Figs 10-11. Images from the second hysteroscopy

Fig 12. Imagen from a normal uterus

HISTOPATHOLOGY

Endometrium consisted of fibrovascular tissue with few glands covered by simple or cylindrical epithelium, with plugs of amorphous eosinophilic material inside. Mild globular deposits of hemosiderin were seen in the stroma. The appearance of both biopsies may be within normal limits for an endometrium from an elderly, multiparous female bottlenose dolphin.

CONCLUSIONS

To the best of our knowledge, this is the first report of pyometra in bottlenose dolphin, *Tursiops truncatus.*

The use of ultrasonography and endoscopy have been fundamental to confirm the diagnosis and to treat the pathology. It is important to highlight that, as in this case, microscopic results do not always correspond to the macroscopic findings.

This 50 years old female arrived at Mundomar on January 2021 and was under altregostest treatment since 2018. Age and long term progestagen contraception may have contributed to the development of pyometra in the present case report.

The routinary check up of the female reproductive system is mandatory and age, reproductive history and the medical treatments enhance the level of attention required.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Angela Sastre from Penta Laboratorio, in Altea, Spain and Mundomar dolphin trainers team for their incredible work and support during this case.

Poster

Comprehensive Underwater Observations of Object Play in Bottlenose Dolphins *(Tursiops truncatus)*

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Bottlenose dolphin object play was examined at the Roatan Institute for Marine Sciences. Eighteen dolphins (8.10) were observed during 32 30-min. sessions. Eleven adults, 2 subadults, 3 juveniles, and 2 calves were observed. Play bouts ranged between 1-77 s. Events were categorized by body parts utilized, type (natural or manmade), and solo or mutual play. The rostrum was used for 96% of events. A general linear mixed model analysis indicated there were significant differences in object play by age, with the longest bouts observed for subadults, and the shortest by calves. Subadults played with natural items longer than other age groups while calves and juveniles played with trash longer than other types. Contingency tables showed that adults played with natural objects and subadults played with manmade objects more frequently than expected. There was a significant interaction for age and duration of solo/mutual play; however, there was no difference in bout frequency by age. There was no significant difference between sexes for play duration, even though females played longer, and males showed more play bouts. Males also played more with manmade objects and females more with natural. Investigating object play in dolphins may be a potential indicator of animal well-being.

Comprehensive Underwater Observations of Object Play in Bottlenose Dolphins (*Tursiops Truncatus*)

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Introduction

- Object play is used as an indicator of cognitive ability in many species (Greene et al., 2011).
- Studying object play is important to understand how different species learn and develop skills that may assist with survival, such as problem solving (Kuczaj et al., 2006).
- Dolphins participating in object play exhibit more creative and varied play behaviors than when engaging in social play (Greene et al., 2011).
- Juvenile dolphins play more often than older animals as play declines into adulthood (Cappiello et al., 2018).

Study objectives

- Explore object play in dolphins looking at:
  - Frequency of play vs. Age & Sex
  - Play duration vs. Age & Sex
  - Play duration vs. Object type

Methods

- 32 underwater video sessions (30-min) collected by DCP at RIMS in October 2019, March 2020, and June 2021 were used.
- 18 dolphins engaged in object play in the selected sample: 11 adults (A), 2 sub-adults (S), 3 juveniles (J), and 2 calves (C).
- Play bouts were coded with BORIS (Friard & Gamba, 2016) and analyzed in SPSS statistical software.
- Play events were coded for object type, object, posture, body parts utilized, and solo or mutual object play. See Table 1.

Table 1. List of modifiers used for each category to describe a play event.

Categories	Modifiers list
Object type	Man-made, natural
Object	Fins, seaweed, seagrass, camera, trash, net/gate
Posture	Horizontal dorsal side up, Horizontal ventral side up, Vertical head up, Vertical head down, On left side, On right side
Body part	See Figure 1.
Solo/Mutual	Solo = 1 dolphin playing with an object (Figure 2, a) Mutual = 2 or more dolphins playing with the same object (Figure 2, b)

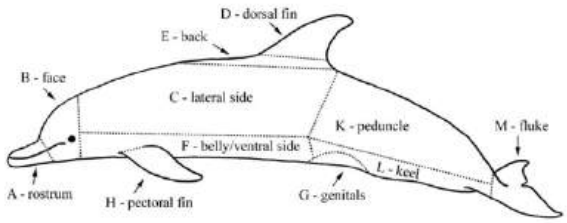

Figure 1. Illustration of the 11 body parts categories. From Dudzinski et al. (2009).

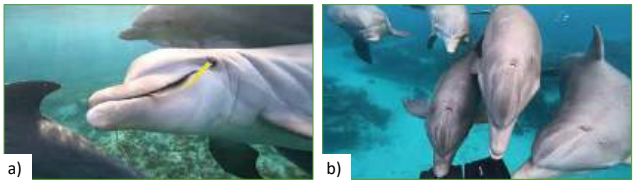

Figure 2. Examples of a) solo play with natural object and b) mutual play with researcher fins (man-made object). Both examples include the dolphins' rostrums.

Results

- 96% of object play events involved the rostrum.
- No significant difference were found for play duration between sexes.
- Males played more frequently than females, but female play bouts were longer than males'.
- Significant differences in bout durations were found based on age, with sub-adults having longest bouts. See Figure 3.
- No significant differences were found in bout frequency across age classes.
- Significant differences were found in play duration based on age and object type, with sub-adults playing longer with natural items. See Figure 4.
- Significant differences were found in play duration of solo or mutual play based on age, with sub-adults engaging more in mutual play. See Figure 5.

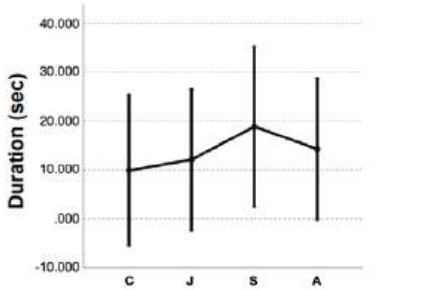

Figure 3. Mean duration of object play by age (GLMM, $F = (3, 670) = 5.15$, $p = .002$). Sub-adult played in longer bouts ($M = 18.90$, $SE = 8.35$). Calves played in shorter bouts ($M = 9.94$, $SE = 7.87$). Sidak pairwise comparisons: $p < 0.05$.

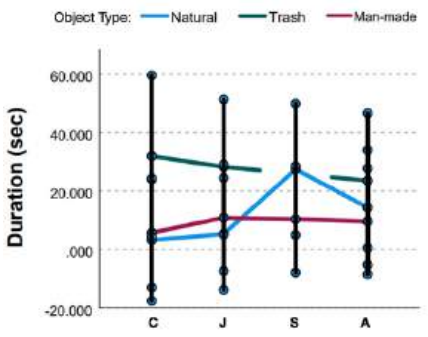

Figure 4. Mean duration for interaction between age and object type ($F = (4, 670) = 4.46$, $p = .001$). Sub-adults played with natural items longer ($M = 27.45$, $SE = 11.46$) than other age groups. Calves ($M = 32.01$, $SE = 14.07$) and juveniles ($M = 28.26$, $SE = 11.78$) played with trash longer than other types.

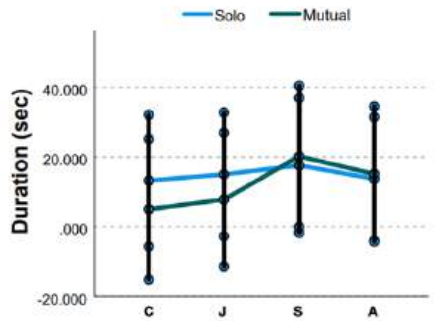

Figure 5. Mean duration for interaction between age and solo or mutual play with objects ($F = (3, 670) = 2.65$, $p = .048$).

Discussion

- Sub-adults played longer and more with natural items. This suggests play can extend and evolve as the animal ages, and does not inevitably decline into adulthood (Cappiello et al., 2018).
- A better understanding of how dolphins play with objects and how play evolves with age can give us a good tool to assess animal welfare, including during senescence.
- Sex's differences in play frequency and duration could come from a difference in opportunities to engage in play due to each sex social pressure (Mann & Smuts, 1999; Hill et al., 2008).
- Details about lateralization, vocalization, and number of social partners observed in an object play bout will be added to future analyses.
- Future studies of spontaneous object play in dolphins may be used to assess creativity (i.e., Greene et al., 2011).

Acknowledgements
We thank the Roatan Institute for Marine Sciences and Anthony's Key Resort for providing access to study the dolphins. We also thank all the students who helped coding the data.

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Poster

Bottlenose Dolphin (*Tursiops truncatus*) Immortalized Fibroblasts on Novel 3D *in Vitro* Collagen-Free Scaffolds

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In vitro three-dimensional (3D) cell culture systems, compared to two-dimensional once, better mimic the condition of cells *in vivo*. However, to date no such models have been developed with cetacean cells, partly due to the lack of fresh dolphins' tissues for the isolation of cell lines. In this study, different 3D systems of bottlenose dolphin (Tursiops truncatus) skin fibroblasts have been analyzed. Particularly, novel scaffolds based on hyaluronic acid and ionic-complementary self-assembling peptides such as RGD-EAbuK and EAbuK-IKVAV have been compared to Matrigel. Histological and fluorescent staining, electron microscopy (TEM) analyses, viability assays and RT-PCR have been performed. Results showed that Matrigel induced cells to form aggregates with lower viability and no ECM production compared to the novel scaffolds. Indeed, scaffolds allowed cells to produce a collagenous ECM containing collagen1a1, laminin B1 and elastin. Moreover, the HA-EAbuK-IKVAV scaffold resulted in the most suitable 3D model in terms of cell quantity and viability. These new 3D *in vitro* models can be considered important tools to study the specific pathogenesis and effects of different stressors both in wild and under human care (UHC) cetaceans

BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) IMMORTALIZED FIBROBLASTS ON NOVEL 3D *IN VITRO* COLLAGEN-FREE SCAFFOLDS

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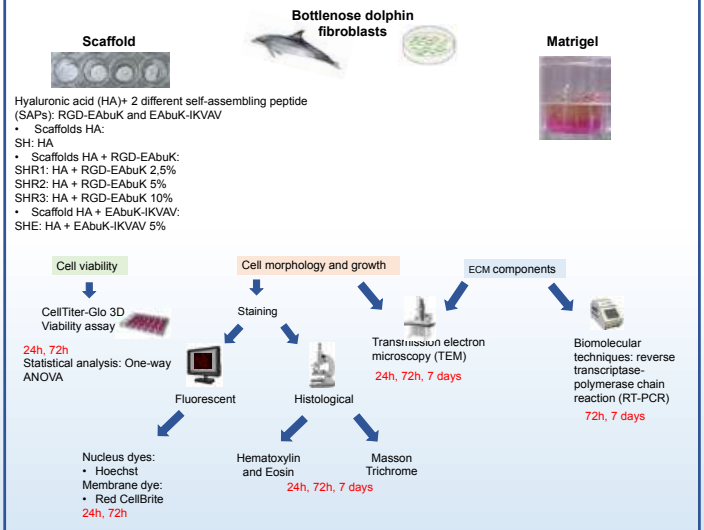
Introduction

In vitro 3D cultures, in which cells grow in complex interactions with the extracellular matrix (ECM), compared to two-dimensional once, better mimic the condition of cells *in vivo* and are considered a potential bridge between 2D cultures and *in vivo* animal models (Vanderburgh et al., 2017).[1]
In the past years different 3D models have been developed in human and veterinary medicine, however, to date no such models have been developed with cetacean cells, partly due to the lack of fresh tissues for the isolation of cell lines.
The aim of this study was to develop and assess 3D systems of bottlenose dolphin (*Tursiops truncatus*) skin fibroblasts. Particularly, novel scaffolds based on hyaluronic acid and ionic-complementary self-assembling peptides such as RGD-EAbuK and EAbuK-IKVAV have been compared to Matrigel.

Materials and methods



Results

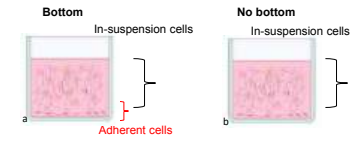


Figure 1. Two different collecting conditions were tested for cell viability assay: bottom condition (a) in which both cells in-suspension within the matrix and cells adherent to the bottom of the well were mechanically scraped and collected for the assay; no bottom condition (b) in which only cells in-suspension within the matrix were measured.

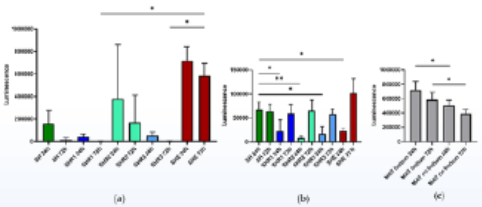


Figure 2. CellTiter-Glo® 3D Cell Viability Assay at 24h and 72h from cell seeding on tested scaffolds in bottom condition (a) and in no bottom condition (b) and on Matrigel in both conditions (c). In scaffolds bottom condition the viability was higher than that measured in the no bottom condition (Fig 1a and Fig 1b), but it decreased after 24h. In the no bottom condition, the viability of the in-suspension cells increased from 24h to 72h in SHE, SHR1, SHR2, and SHR4 but not in SH scaffold (Fig 1b). The SHE scaffold showed the greatest viability in both conditions. in Matrigel the viability of the cells in the bottom condition was higher than that found in the no bottom condition, however, in both cases it decreased after 24h (Fig 1c).

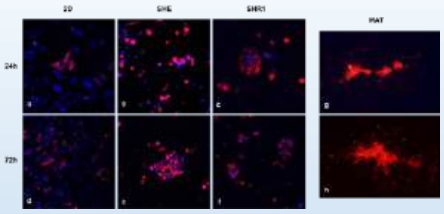


Figure 3. Confocal microscopy of culture grown on 2D system (a, d), SHE scaffold (b, e), SHR1 scaffold (c, f) and on Matrigel (g, h) at 24h and 72h after seeding. All the figures show a merge of cellBrite and Hoechst staining, 20xair magnification. The cells in-suspension within the different 3D matrices had a round or polygonal shape (Fig 2b, 2c, 2e, 2f, 2g, 2h) whereas in the 2D culture they appeared more elongated (Fig 2a, 2d).

Results

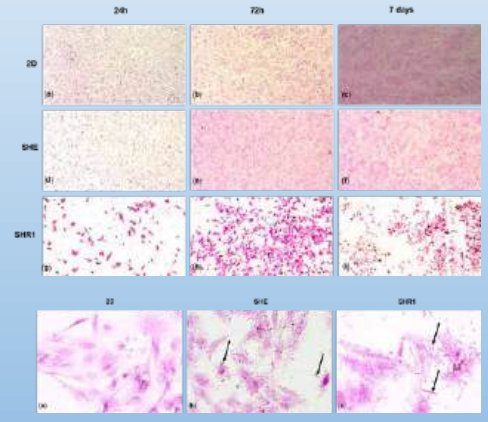


Figure 4. HE images of the adherent cells in the 2D (a, b, c) and 3D scaffold systems (d, e, f, g, h, i) at different post-seeding times (24h, 72h and 7 days), 5x magnification. Cells within SHE reach 60% confluence at 24h (d) and a 100% confluence at 72h (e). Cells within SHR1 reach a confluence of 50% at 72h (h), then the cellularity decreases at 7 days (i). 2D culture show a higher cellularity in each time point compared to scaffolds (a, b, c).

Figure 5. HE images of the adherent cells in the 2D (a) and 3D scaffold systems (b, c) at 72h, 40x magnification (a, c), 20x magnification (b). In both HA-RGD-EAbuK and HA-EAbuK-IKVAV scaffolds, extracellular filaments are visible mostly around cells (black arrow). No filaments were found in the 2D cultures (a).

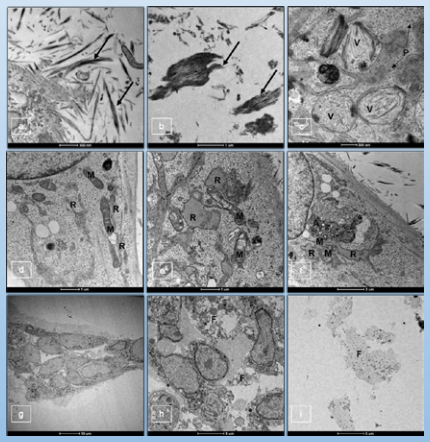
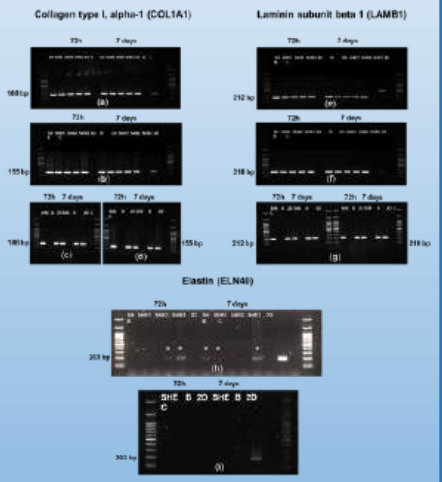


Figure 6. Details of TEM images of adherent cells seeded within SHR1 (a, b), SHE (c, e) at 72h from the seeding, adherent cells within SHR2 (d) at 24h and within SHR3 (f) at 7 days. In g, h and i images, details of adherent cells within Matrigel at 24h, 72h and 7 days. In a, b and c images, presence of collagen microfibrils around the cells (black arrows), within intracytoplasmic vesicles (V) and in the surrounding area (black arrow) is visible. In the c image it is also possible to observe polyribosomes (P). Well-developed cisternae of RER (R) and intact mitochondria (M) are shown in image d. Dilated cisternae of RER (R), damaged mitochondria with broken or disrupted cristae (M), and autophagic bodies (A) are visible in e and f images. In the g image, Matrigel cellular aggregate of morphologically intact cells at 24h is shown, while in the h image presence of cellular fragments (F) within the aggregates at 72h can be observed. In the i image only cellular fragments are visible at 7 days.



Figiure 7. Use of RT-PCR for the detection of collagen, laminin and elastin RNA expression in seeded and unconditioned scaffold (blank) and in cells with their medium (2D) at 72 h and 7 days from seeding. Collagen and laminin transcripts were present in all the scaffolds matrices and in the 2D samples (a, b, c, d, e, f, g). Elastin transcripts were found in the 2D samples in both time points and in SHR3 at 72h, and in SH and SHR1 at 7 days (h, i). C: (control) RNA from dolphin skin. B: (blank) RNA from unconditioned scaffolds.

Conclusions

Has previously demonstrated, fibroblasts produce their own pericellular matrix consisting of a complex architecture of structural, fibrous proteins such as fibronectin, collagen, and laminin embedded in a highly hydrated gel-like material of glycosaminoglycans, proteoglycans, and glycoproteins (Lee 2008).[2] In these novel scaffolds we reported deposition of ECM around the cells similarly to the *in vivo* fibroblasts. The scaffold made exclusively of HA, was found to be the least suitable matrix for fibroblasts growth, emphasizing the importance of the SAPs within the scaffold systems. Matrigel was poorly suited to the growth of fibroblasts as well. It is consistent with fact that Matrigel is used as a reconstituted basement membrane gel, however, fibroblasts *in vivo* are predominantly found in the connective tissue stroma and not in the thin basement membrane that separates the epithelium from the connective tissue. Others have also found that fibroblasts do not grow well in the presence of Matrigel (Hakkinen et al., 2011).[3] On the other hand, the HA-EAbuK-IKVAV scaffold resulted the best 3D model in terms of cell viability, growth and ECM components rich in collagen and laminin. Besides some limitations, the approach and the main findings reported in this study should facilitate the development of new 3D *in vitro* models which can be considered important tools to study the specific pathogenesis and effects of different stressors both in wild and under human care (UHC) protected species as cetaceans.

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Poster

Aortic flow traces in bottlenose dolphins (*Tursiops truncatus*) under human care. A new acoustic window

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Cetaceans show hemodynamic and morphological adaptations of the anatomy and cardiovascular architecture due to the physiology of diving, compared to terrestrial mammals. The acoustic windows used for echocardiographic scans in dolphins are difficult to use in clinical practice due to the position of the heart in the thorax. However, cardiac pathologies in dolphins are underestimated. The goal of the study was to identify an alternative acoustic window that allows the measurement of aortic flow through a reliable Doppler trace comparable to the standard scans already described. Ultrasound examination was carried out (GE Versana active, 3.5 MHZ convex probe) in 12 bottlenose dolphins (Tursiops truncatus), 5 males and 7 females, 5 to 50y. Aortic flow traces obtained from the two echocardiographic scans are comparable in terms of aortic peak velocity and arctic flow integral (VTI Ao). This technique show a greater ease of execution and high repeatability compared to the previous ones. However, further studies are necessary on a larger number of subjects and with different probes to assess a methodology that allow to evaluate any congenital or acquired myocardial alterations and to check the cardiac health status.

Poster

Ultrasonographic findings of pregnancy and embryo organogenesis: the case report of a Jungle Park's sea lion (*Zalophus californianus*)

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Ultrasonography is widely used in veterinary medicine and can be used to monitor estrous cycle, abnormal pregnancies, embryonic resorption, or fetal abortion. Ultrasonography plays an important role in modern-day marine mammal preventative medicine because it is a non-invasive technique, it is safe for both patient and operator, and it can be performed routinely using trained responses that enable medical procedures. The aim of this study is to provide additional relevant data on feto-maternal ultrasonographic monitoring in sea lion (Zalophus californianus) species. This study describes since the ovulation to the birth, the sonographic findings of the sea lion pregnancy and the embryo organogenesis.

Poster

The first insights into the horizontal movements of rehabilitated Grey seal pups (*Halichoerus grypus*) in the Baltic Sea.

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Weak and/or sick Baltic Grey seal pups (Halichoerus grypus) are brought by currents and found on the Lithuanian coast every year. Whereas these marine mammals are included in the Red list of IUCN, stranded seals have been rehabilitated in Lithuanian Sea Museum since 1987. The intention is to release animals which survive and display appropriate behaviours, therefore post-release monitoring is crucial for future decisions on rescue, rehabilitation, and release protocols.

The aim of this investigation was to assess spatial usage of rehabilitated Grey seal pups, involving trip characteristics' (trip length and duration, covered distance, haul-out duration) analysis. For this aim, eighteen rehabilitated seals were released back to the Baltic sea with the Argos SPLASH10-297 transmitters (Wildlife Computers, Redmond, WA) glued to their fur. It was found that pups dispersed one by one and occupied southern as well as southwestern parts of the Baltic. Tracking duration covered an average of 140 days, with a total cumulative distance of 4597 km. The individuals covered a wide range of distances from 1 to 130 km/day, with an average of 30,9 km/day, and haul-out duration of 6,98 h/haul-out). In general, 21% of the deployment period seals spent hauled-out, 79 % - in the trips.

Poster

Human face recognition in beluga whales (*Delphinapterus leucas*) from static images

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Facial and emotional recognition are essential qualities for social adaptation, but they are understudied among cetaceans. This study proposes a new paradigm to prove whether beluga whales (Delphinapterus leucas) can discriminate between familiar and unfamiliar human faces, as well as their facial expressions.

We evaluated spontaneous responses of two beluga specimens from the Oceanographic (Valencia) to different images. Picture pairs used showed known versus unknown humans (phase 1) and smiling versus neutral expression (phase 2). We conducted 18 fifteen-minute sessions per phase. Every session was filmed and analysed blindly to the experimental conditions. We collected data on the type, duration, and frequency of interactions with the images.

We used a single-case design and binomial statistics to analyse the data. Results were inconclusive for phase 1, but differences were found at individual level, while we observed a general preference to smiling faces in phase 2 (Z interaction duration = 3.807). These results could be explained through their behavioural traits and individual experiences.

In conclusion, belugas can probably distinguish between known and unknown people, but a larger sample is needed. Moreover, this study provides new perspectives on heterospecific emotional recognition, but further studies are needed to assess belugas' emotional recognition and categorization.

Poster

A methodological approach to develop a Five Domains Model framework to assess the welfare of a captive group of Yangtze Finless Porpoises (*Neophocaena asiaeorientalis asiaeorientalis*), part 2

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As a complement to our oral presentation, we would like to present in this poster additional data to the symposium participants.

A Five Domains Model (FDM) framework was developed to assess the welfare of a captive group of Yangtze Finless porpoises (*Neophocaena asiaeorientalis asiaeorientalis*) held at the Baiji Dolphinarium (Chinese Academy of Sciences, Wuhan, P.R. China). The following steps were considered to develop the FDM framework: 1) the literature review of potential welfare indicators for the considered species; 2) the review from the panel of experts to validate the selected indicators; 3) the determination of the framework to structure the welfare assessment tool; 4) the development of a scoring system; 5) the evaluation of the validity, practicality, and reliability of the tool, (6) the final development and 7) the implementation. The steps 1 to 5 have already been completed and will be presented.

Poster

Voluntary urine collection via bladder catheterization in a female bottlenose dolphin with renal problems

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Urinalysis is a test that can document abnormalities in urine that reflect various types of renal, hormonal, or metabolic diseases, which could help to properly monitor the health of dolphins under human carea. For collecting sterile urine samples by voluntary bladder catheterization to determine baseline values of biochemical and microscopic variables in urine, a 22-year-old female *Tursiops truncatus* dolphin with renal disorders was trained. The urine collection behaviour by bladder catheterization included the train steps: giving the stimulus discriminative for the ventral lay out position, animal keeping the genital area above the water level, the desensitization and opening of the genital slit area as well as the introduction of the catheter into the bladder entrance and the extraction of the sample. The procedure take 3 minutes maximum, we condition the animal to hold her breath for longer than the time to take the sample. This procedure was performed by using operant conditioning. Samples were successfully collected on a completely voluntary basis via the medical behavioural train. This behaviour can be used as a baseline for monitoring dolphin health in dolphinaria and for monitoring renal condition and function in dolphins in rehabilitation or under human care.

Poster

Toxoplasmosis in striped dolphins stranded in the Canary Islands

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We present, for the first time, *Toxoplasma gondii* infection in striped dolphins (Stenella coeruleoalba) stranded in the Canary Islands. Between 2000 and 2020, 1183 cetaceans were found stranded in the Canary coasts, 217 of them belonging to the species Stenella coeruleoalba, of which 167 were subjected to a complete anatomopathological study, two of them being relevant in our study. Both animals showed poor body condition and lesions consistent with multiorgan necrosis and lymphohistiocytic inflammation with intralesional protozoa; specifically in the central nervous system, heart, rete mirabile, adrenal glands, pancreas, liver, lung, spleen and pituitary gland. Despite the presence of other findings, the main lesions were compatible with systemic toxoplasmosis, as the cause of death. The identification of T. gondii was confirmed by molecular methods. However, morbillivirus nucleic acid was not detected in the tissues of any of the dolphins, supporting the hypothesis that this protozoan played a primary etiologic role in the development of severe lesions in cetaceans. This study therefore describes in detail the histopathological findings associated with T. gondii parasitosis in two striped dolphins and report severe extensive coagulative necrosis in the pancreas and the pituitary gland, two lesions not previously reported in cetaceans' toxoplasmosis.

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