



European
Association for
Aquatic Mammals

2022

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EUROPEAN ASSOCIATION FOR AQUATIC MAMMALS (EAAM)

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



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30th March – 3rd April

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Unlike what we were all hoping for, once again 2021 was a year of challenges and uncertainty. I want to thank the team at Oceanographic for working extremely hard in the attempt to organize our 2022 annual Symposium and host it in Valencia.

I like to also acknowledge the incredible work of our scientific community that supported those in the front line of the discussion and institutional collaboration with the French Government during the development of the new animal welfare law, that included many changes for the part related to marine mammals.

In May 2021 the EAAM signed a rebuttal letter for the French Government that provided science-based corrections to a long list of inaccurate statements on marine mammals used as a base to draft new marine mammals' standards and guidelines in the proposed French welfare new law. Thanks to the many people that collaborated to create such a document that was instrumental to educate many politicians and help them have a correct understanding of great work done by Zoos and Aquariums taking care of marine mammals in France and around the world.

Omicron and a new wave of last minute restrictions in early 2022 forced our organization to move the Symposium to a virtual format for the second year.

I would like to thank our President Elect, Guillermo, who once again was able to coordinate with very little time notice an amazing scientific program rich in content and with great keynote speakers. Thanks to all the committee members and volunteers that did support the organization of our virtual Symposium. The committees did a great work this year even if one more time they could not meet in person at the Symposium. Again, it was a moment of resilience in our organization's history. We all were hoping to celebrate the amazing milestone of 50 years of the EAAM in a different way. That time will come soon, and I am sure we all are looking forward to celebrating together.

I like to congratulate the new Board and welcome the new members as well thanks Javier and Camino that have served for these years in our organization. It is time for Guillermo to step in as new EAAM President and while it was an honor and a privilege to represent you all for these past year and half as President, I look forward to serving you for the rest of the term as Past President.

Renato Lenzi
President EAAM



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Dear Participants, colleagues and friends,

WE ARE BACK!

Despite the unanimous desire for an “on-site” event, the global sanitary situation made us take the decision of organizing a virtual meeting again. The experience generated in 2021 gave us a good base to put together a better and improved virtual edition of the EAAM Annual Conference. This edition is taking place from March 30th till April 3rd, meaning that we have a more complete program ready for all of you.

During the course of these 5 thematic days we will have interactive round tables, international Keynote Speakers, multiple relevant guests, and of course Scientific Sessions. These sessions will include both Oral and Video Presentations.

The outstanding quality of the abstracts submitted has facilitated the Scientific Committee’s job in selecting the finest topics. Training, conservation, reproduction, neonatology, animal welfare, research, and veterinary cases are included in the programme. Both the Best Training Presentation and the Best Student Presentation will be awarded this year too! EAAM Training and Student Committees would not miss it!

Last year the conference was called “A conference for the future”, today I can say EAAM 2.0 is now a reality. I hope you enjoy the virtual event as much as I have enjoyed organizing it.

Thank you for participating in this EAAM 2022 Virtual Conference, “We are back”.

Guillermo J. Sánchez Contreras
President-Elect EAAM



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MARCH		EAAAM 2022 VIRTUAL CONFERENCE - FULL PROGRAM			
		APRIL			
30	31	1	2	3	
WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY	
15:30	15:30	15:30	11:00	11:00	Scientific Session 6 (5m)
Welcome Ceremony (20m)			Round Table "RESEARCH & WELFARE 2.0"		
			Welfare Committee MMRN and guests (120m)		
15:50	15:50	15:50		11:05	Silvia Becerra Actions and Specialized Training in the Association of Marine Mammals Specialists (20m)
Round Table "The Secret Life of the M/Then Contingency: Six Hidden Ways the 4 Quadrants are Impacting your Training"	Round Table "Let's talk about the FUTURE"	Round Table "EEP Updates"			
Barbara Heidenreich (100m)	Laura van der Meer Martin Boye Isabella Clegg Ana Daniela Soares Ferreira Francis De Rugy (120m)	Robert Gojceta Sonia Mattas Izabela Krause (120m)			
17:30	17:30	17:30		11:25	Guillermo J. Sánchez- Contreras Acute Lead Intoxication in 5 Bottlenose Dolphins (<i>Tursiops truncatus</i>): Clinical Management (20m)
BREAK	BREAK	BREAK	BREAK		
18:00	18:00	18:00	15:00	11:45	Barbara Biancani Lead Intoxication in Bottlenose Dolphins (<i>Tursiops truncatus</i>): Pathological Findings (20m)
Scientific Session 1 (5m)	Scientific Session 2 (5m)	Scientific Session 3 (5m)	Scientific Session 4 (5m)		
			KEY NOTE (45m)		
18:05	18:05	18:05	15:05	12:05	Teresa Álvaro-Álvarez Suspected immune-mediated hemolytic anemia in a female bottlenose dolphin (<i>Tursiops truncatus</i>) (20m)
KEY NOTE (45m)	KEY NOTE (45m)	KEY NOTE (45m)	Lisa Yon Development and validation of a novel behavioural welfare assessment tool (and android App) for use with elephants		
Ken Ramirez Training, Welfare, and Husbandry - The Secret to Excellent Animal Care	Rendall Wells Lessons from Sarasota's dolphins	Claire Simeone Healthy Animals, Healthy Conservation: How Health Factors into the Impact We Make		12:25	Umberto Romani-Cromeschi Cutaneous lesions associated with a <i>Staphylococcus delphini</i> infection in an aged bottlenose dolphin (<i>Tursiops truncatus</i>) under human care (20m)
18:50	18:50	18:50	15:50	12:45	CLOSING CEREMONY (10- 15m)
Sponsor Talk (10m) ST LAURANT	Sponsor Talk (10m) SEAFOODIA	Sponsor Talk (10m) NEXUS INTEGRA	Sponsor Talk (10m) ANIMAL NECESSITY		
			Daniela Rickert Saliva and Blood Cortisol Measurement in Bottlenose Dolphins (<i>Tursiops truncatus</i>): Methodology, Application, and Limitations (20m)		
19:00	19:00	19:00	16:00		
Perrilla Mosesson Similarities and differences of using Operant Conditioning in marine mammals versus other animal species in zoological settings (40m)	Isabella Clegg What might the public display of cetaceans look like in the future? (20m)	Maria Carmen Fuentes- Albero Proteomic study in <i>Tursiops truncatus</i> semen (10m)	Ana Pascaud Implementation of a protocol for animal welfare assessment in the bottlenose dolphin (<i>Tursiops truncatus</i>) in a zoological park (Planète Sauvage) (20m)		
19:40	19:20	19:10	16:40		
Adrián González-Quintero Desensitization of MRI scanner in bottlenose dolphins (10m)	Roberto Sánchez-Okrucky 2000-2021 Efforts towards Research and Conservation of Manatees in Mexico and South America (20m)	Carmen M. Arjja Fertility and reproductive success (RS) in orcas (<i>Orcinus orca</i>) in human care (20m)	Aviva Charles First assessment of temperament in Antillean manatees under human care and potential implication for their welfare (20m)		
19:50	19:40	19:30	17:00		
Giuseppe Ganassin Management of a stereotyped behavior (regurgitation) in a California sea lion (<i>Zalophus californianus</i>) under human care (20m)	Graysen Boehning Abundance, Site Fidelity, and Association Patterns of Coastal Bottlenose Dolphins (<i>Tursiops truncatus</i>) off the Coast of Southeast Florida (20m)	Guillermo J. Sánchez- Contreras Physiological Parameters Monitored on Bottlenose Dolphin Neonates (<i>Tursiops truncatus</i>) over the First 30 Days of Life (20m)	Sabrina Brandt Environmental Enrichment Devices Reduce Stereotypical Behaviors and Habituation During Rehabilitation of Wild California Sea Lions and Northern Elephant Seals (20m)		
20:10	20:00	19:50	17:20		
Manuel Garduño Conditioning Olfactory sd's with sea lions (20m)	Vicente Marco-Cebedo New insights of lethal interaction between dolphin species on Western Mediterranean (20m)	Agustín Rebolledo-Merino Neonatal hypoxic-ischemic encephalopathy in a bottlenose dolphin (<i>Tursiops truncatus</i>): Cause or consequence of a meconium-aspiration syndrome? (20m)	BREAK		
20:30	20:20	20:10	18:00		
José Luis Bernabeu Separate but together (10m)	Kimon Koemtzopoulos Operation of a Mediterranean monk seal (<i>Monachus monachus</i>) Rescue and Information Network during 2021 in Greece (20m)	Carmen M. Arjja Evaluation of the vocalizations between two bottlenose dolphin mother-calf dyads during the first 10 days of life. (20m)	Scientific Session 5 (5m)		
20:40	20:40	20:30	18:05		
Cynthia Henderson Control training methods for bottlenose dolphins (<i>Tursiops truncatus</i>) during the pandemic period in order not to decrease animal welfare despite the necessary reduction of training staff at the Lisbon Zoo. (20m)	Manuel Garcia-Hartmann Report on the 2022 stranding of a Cuvier's beaked whale near the harbor of Athens, Greece (20m)	Maria Renée Arreola Management of a chronic degenerative lesion in the lumbo sacral region in a bottlenose dolphin (<i>Tursiops truncatus</i>) (20m)	Carlos Rojo-Solis Ultrasensitive Cardiac Troponin I Levels in Marine Mammals Under Human Care: Establishing Baseline Values in a Healthy Multi-Species Population (20m)		
21:00	21:00	20:50	18:25		
Paola Righetti To discriminate or not to discriminate? What our dolphins learned during Covid pandemic (10m)	Closing session	Jorge Carlos Ortiz-Ortiz Use of cannabidiol (CBD) as an alternative to manage the pain in South American Sea Lions (<i>Otaria flavescens</i>) kept in human care: Our experience. (20m)	Marta Muñoz-Baquero Diagnosing <i>Brucella</i> in cetaceans: thoughts from the field (20m)		
21:10		21:10	18:45		
Myriam Weiss How we trained creative thinking in killer whales (<i>Orcinus orca</i>) (20m)		Closing session	Simone Segura-Götthlin The validation of a non- invasive skin sampling device for detecting cetacean poxvirus (20m)		
21:30			19:05		
Closing session			Jordi Agulló-Gisbert SARS-CoV-2 detection methods for cetacean and pinnipeds otarid species (20m)		
			19:25		
			Ignacio Vargas-Castro Herpesvirus screening and evaluation in Western Mediterranean Cetaceans (20m)		
			19:45		
			Closing session		

Oral Presentations last 20 minutes in total. 15 minutes presentation, 5 minutes questions.

Video Presentations (in red, and yellow) last 10 minutes in total. Questions will be allowed at the discretion of the host/chaire of the session.

Key Notes have 45 minutes in total. 40 minutes presentation, 5 minutes questions.

Round Tables consist of a block of presentations or discussions lead by a moderator. Questions will be allowed at the end of the session.

****IMPORTANT: Program is based on Central European Time (CET)**



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Key Speakers



Ken Ramirez

“Training, Welfare, and Husbandry - The Secret to Excellent Animal Care”

Wednesday 30th March 2022 - 18:05h

BIO: Ken Ramirez is the EVP and Chief Training Officer for Karen Pryor Clicker Training. Previously, Ken served as EVP of animal care and training at Chicago's Shedd Aquarium. A nearly 50-year veteran of animal care, Ramirez is a biologist and behavior specialist who continues to work and consult with many zoological organizations and dog programs throughout the world. He authored the book ANIMAL TRAINING: Successful Animal Management through Positive Reinforcement in 1999. He taught a graduate course on animal training at Western Illinois University for 20 years and currently co-teaches animal training for AZA.



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Dr. Randall Wells

“Lessons from Sarasota's dolphins”

Thursday 31st March 2022 – 18:05h

BIO: Randall Wells is a co-founder and directs the Chicago Zoological Society's Sarasota Dolphin Research Program, which conducts the world's longest-running study of a wild dolphin population. He began studying bottlenose dolphins in Sarasota Bay, Florida, as a high school volunteer at Mote Marine Laboratory in 1970. Wells received his Bachelor's degree in Zoology from the University of South Florida in 1975, his Masters in Zoology from the University of Florida in 1978, his PhD in Biology from the University of California, Santa Cruz in 1986, and he was awarded a post-doctoral fellowship with Woods Hole Oceanographic Institution in 1987. Wells' current research program uses a collaborative approach to examine the behavior, social structure, life history, ecology, health, and population biology of bottlenose dolphins along the central west coast of Florida, with studies focusing on up to five concurrent generations of a locally resident ~170-member dolphin community. Recent research topics include the effects of human activities on coastal dolphins, such as boat traffic, fishing activities, human feeding of wild dolphins, and environmental contaminants, and the impacts of other environmental disturbances such as red tides; he and his team are called upon to lead rescues of entangled dolphins. He has conducted research on a variety of marine mammals including spinner, Atlantic spotted, franciscana and other dolphin species, vaquita porpoises, bowhead, humpback, blue, and gray whales, and manatees. Wells has authored or co-authored 4 books and more than 290 peer-reviewed journal articles and book chapters. He has been presenter or co-author of more than 725 presentations at professional meetings or invited public or university lectures. Wells also serves on the Committee of Scientific Advisors on Marine Mammals for the U.S. Marine Mammal Commission, on the NOAA/USFWS Atlantic Scientific Review Group, and he is past-chair of the NOAA/USFWS Working Group on Marine Mammal Unusual Mortality Events. Wells serves on IUCN's Cetacean Specialist Group, and on the Steering Group for the national Animal Telemetry Network.



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Dr. Claire Simeone

“Healthy Animals, Healthy Conservation: How Health Factors into the Impact We Make”

Friday 1st April 2022 – 18:05h

BIO: Dr. Claire Simeone is a marine mammal veterinarian, and founder of Sea Change Health, dedicated to improving health for all who rely on the sea. A graduate of the Virginia-Maryland College of Veterinary Medicine, Claire completed post-graduate training with the National Marine Mammal Foundation and SeaWorld San Diego. Claire has worked with the National Marine Fisheries Service investigating unusual mortality events, and worked for seven years at The Marine Mammal Center as a conservation medicine veterinarian. Prior to founding Sea Change Health, Claire was most recently the director of The Marine Mammal Center's Hawaiian Monk Seal Conservation Program. In 2018 Claire was the first veterinarian ever selected as a TED Fellow. She is currently based in Valencia, Spain, and is a research associate with Oceanogràfic.

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Dr. Lisa Yon

“Development and validation of a novel behavioural welfare assessment tool (and android App) for use with elephants”

Saturday 2nd April 2022 – 15:05h

BIO: Dr. Lisa Yon is an Associate Professor in Zoo & Wildlife Medicine in the School of Veterinary Medicine and Science at the University of Nottingham (UK); she joined the University of Nottingham in 2007. She is a veterinarian (Cornell University) with a PhD in elephant endocrine physiology (UC Davis). Her research focuses on the health and welfare of captive wildlife (with an emphasis on captive elephant welfare). For the past ten years, she has served on the government advisory committee, the Elephant Welfare Group, leading a series of projects exploring ways to improve captive elephant welfare. She also led a project to develop and validate a behavioural welfare assessment tool designed for routine and regular use by elephant keepers, to enable them to monitor their elephants’ welfare over time. Her research has had direct and substantial impact on government policy in both the UK and Ireland, and she helped lead the development of guidelines for a newly developing Association of elephant-back safari operators (Zimbabwe, Zambia and South Africa) to improve elephant welfare. She is routinely asked by colleagues across Europe, North America, Southeast Asia and Africa to consult on issues relating to captive elephant welfare. She has also served as consultant for the Catalan government on captive elephant welfare, and delivered international workshops for stakeholders (owners, veterinarians, handlers, managers) on ways to improve captive elephant welfare in Thailand, and in southern Africa (in South Africa, Zambia and Zimbabwe). Dr. Yon has engaged in extensive knowledge exchange and outreach activities relating to her research. This has included dissemination through radio and video interviews, through invited talks at Schools and Universities, and invited public presentations. She has also organised and taken part in numerous seminars and workshops for people in the captive elephant industry in the UK, in Africa and in Asia.



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ROUND TABLES



Barbara Heidenreich

“The Secret Life of the If/Then Contingency: Six Hidden Ways the 4
Quadrants are Impacting your Training”

Wednesday 30th March 2022 – 15:30h

BIO: Barbara Heidenreich is an animal training consultant specializing in exotic animals. She consults worldwide working with zoos, universities, veterinary professionals, and conservation projects. She has worked onsite with over 80 facilities in 27 countries. She is an adjunct instructor at Texas A&M University. She has produced 7 DVDs, authored 2 books, and contributed to 4 veterinary textbooks. She is a co-author of two Fear Free® Avian Certification Courses. Much of her work focuses on training exotic species to cooperate in medical care. She operates the online education program AnimalTrainingFundamentals.com. This virtual learning program features award winning courses, tracks to guide professional development, verifiable badges to share and prove course completion, community, and more. Barbara is an advisor for the Animal Training Working Group and the Parrot Taxon Advisory Group for EAZA. She has provided her expertise to conservation projects The Kakapo Recovery Program and The Borneo Orangutan Survival Foundation. She has a Bachelor of Science in zoology and has begun the journey towards a Master of Science in applied behavior analysis. Her goal is to leave behind a legacy of kindness to animals by sharing her expertise.



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Laura van der Meer

“Let's talk about the FUTURE”

Thursday 31st March 2022 – 15:30h

BIO: Laura van der Meer is a U.S. qualified lawyer specializing in the field of international environmental law, policy and advocacy. Among other areas of practice, since 1994, Laura has been actively advising and representing clients with interests in multilateral environmental agreements (MEAs) including the Convention on International Trade in Endangered Species (CITES), the Convention on Biodiversity, and the Convention on Migratory Species. She has served as International Counsel to the Alliance of Marine Mammal Parks and Aquariums (AMMPA) since 2007 and has worked closely with the European Association for Aquatic Mammals since 2008, including through representation of EAAM members at the European institutions in Brussels, Belgium. After more than twenty years living and working in Europe, Laura and her family have recently relocated to the U.S. where she continues consulting work via MEA Strategies, LLC.

GUEST SPEAKERS: *Martin Boye, Isabella Clegg, Ana Daniela Soares Ferreira, and Francois De Ruyg.*



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EAZA - EEP COORDINATORS

Robert Gojceta - *Tursiops truncatus* EEP

Sonia Matias - *Zalophus californianus* EEP

Izabella Krause - *Otaria flavescens* EEP

Lorenzo von Fersen - *Trichechus manatus* EEP

“EEP Updates”

Friday 1st April 2022 - 15:30h



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WELFARE & RESEARCH 2.0

Friday 2nd April 2022 - 11:00h

Katrin Baumgartner - EAAM Welfare Committee

Ruta Vaicekauskaite - Welfare Researcher

Audra Ames - Marine Mammal Research Network

GUEST SPEAKERS: *Dr. Fabienne Delfour, Prof. Peter Tyack, and Carmen Arija.*



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Abstracts

Similarities and differences of using Operant Conditioning in marine mammals versus other animal species in zoological settings

Pernilla Mosesson^{1*}

¹ *Animal & Human Inspirations, Klumpstugevägen 17, 618 92 Kolmården, Sweden.*
animalandhumaninspirations@gmail.com

Using Operant Conditioning is an effective, safe and reliable tool to improve the welfare of the animals in our care. That is no news, but similarities and differences become evident between the different species when we make an inventory of the animal's needs in daily routines, of their prevention/husbandry behaviors and their physical and mental health requirements. What do we need to have in mind when we structure a training plan in all those amazingly different species? What challenges, conditions and qualifications do we need to deal with in order to get the results that we want? We can get amazing results using Operant Conditioning no matter what species we use it for, as long as we are open-minded, curious and willing to read and feel every single individual. The key to success is responsive behaviors from all levels of staff involved in the operation towards all animals in our care.



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Desensitization of MRI scanner in bottlenose dolphins

González Quintero, A^{1*},

¹ *Oceanogràfic. Ciudad de las Artes y las Ciencias. C/ Eduardo Primo Yúfera (Científic), 1B. 46013 Valencia. Spain.*
agonzalez@oceanografic.org

The desensitization of situations and objects in bottlenose dolphins (*Tursiops truncatus*) can allow us to have access to knowledge about this species through research projects. Neptuno, the first dolphin born at Oceanogràfic, has a natural ability to accept different types of situations. In fact, he has already participated in numerous research projects carried out at Oceanogràfic using different elements such as D tags, suction cups, hydrophones... In this case, he was desensitized for a CT scan introducing visual, tactile and sound stimuli. Visual desensitization consisted in showing all the elements that we would use during the scan, from a stretcher to the structure of a scanner, antennas, suction cups and speakers. For tactile desensitization, we involved all these elements in routine sessions, including them as part of the day to day. Once we had shown everything to Neptuno, in a beaching position, we introduced him into a structure similar to the scanner. Finally, we conditioned Neptuno to perform apneas in a beaching position of about 2 minutes in order to prepare him for all the necessary cycles of the scanner. Thanks to this process, we can perform brain scans within the ideal values of animal welfare.



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Management of a stereotyped behavior (regurgitation) in a California sea lion (*Zalophus californianus*) under human care

Authors: Ganassin, G.^{1*}; Dekker Cabrera, C.¹; Anguis Montiel, D.¹; Jinariu, V.¹; Sanchez Martinez, J.¹; Criado Maestre, J.¹

¹ Pinnipeds Department, Mundomar, Calle Sierra Helada s/n, 03503, Benidorm, lmarios@mundomar.es

Malù, a female California sea lion (*Zalophus californianus*), developed, since her first years of life, a stereotyped behavior that would continue and consolidate for the next 16 years, with medical and social repercussions. At the end of each feeding, she regurgitates the food multiple times until reaching the texture of digested food. This behavior conditioned her husbandry and well being for years. Several approaches were attempted in the past without consistent successful results. In July 2020, a plan integrating new husbandry and training approaches was developed with the main goal of improving her welfare and minimizing, hopefully eliminating, this detrimental behavior. To do so, Malù's life history was reconstructed and a possible origin of her stereotypia was found in the incorrect husbandry of the animal during rearing and weaning (isolation, lack of space and stimuli, and reduced feeding frequency). Different methods were proposed and scientifically evaluated in order to overcome the regurgitation: multi-stimuli sessions, specifically food-based enrichment, and the presentation of fully-blended food. The triturated food method had a positive outcome and was promptly implemented in her daily husbandry. At the time of the abstract submission, the stereotyped behavior has been under control and extinguished for over a year now. The sea lion has gained weight, is integrated in a social group with other females, and participates voluntarily in medical and non-medical behaviors. Introducing solid fish in her diet is one of the principal goals in the near future.



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Conditioning Olfactory sd's with sea lions

Edgar Urbina¹, Manuel Garduño¹, Alex Mata¹, Mario Andrade¹, Rosa M° Cerrillos^{1*}, Shelley Wood¹

¹ *The Dolphin Company, Banco Chinchorro 87, 13, 77504 Cancún, Quintana Roo, Mexico.*
mgarduno@thedolphinco.com

Dolphin Discovery is home to 36 California and Patagonian sea lions. The sea lions participate in interactive and educational programs and as such have an extensive repertoire of show and husbandry behaviors. Dolphin Discovery training philosophy considers mental stimulation training to be important amongst the entire population, both juvenile and advanced age animals and it is this reason that the animal training department began an experimental enrichment project. The goal of this project is to use olfactory Sd's on selected previously conditioned behaviors with sea lions. Sea lions have an acute sense of smell but to what extent this sense can be used in training scenarios was unknown. All sea lions have previously been conditioned using three types of Discriminative Stimuli (Sd's), tactile, visual, and audible. 15 sea lions were selected for the olfactory training. The type of smell selected for conditioning was species specific and individually based. Both Classical and Operant Conditioning techniques were applied resulting in 20 behaviors under olfactory stimulus control utilizing distinct types of smells. Nine olfactory Sd's have been successfully conditioned at the time of this paper. The olfactory Sd's are extremely useful in both educational and interactive programs and have proven to be a highly motivating tool especially with the advanced aged sea lions. Sea lions had a high response rate using olfactory Sd's as compared to visual Sd's.



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Separate but together

Luis Bernabeu, J.^{1*}; Moñino Rodríguez, L.^{1*}

¹ *Oceanogràfic. Ciudad de las Artes y las Ciencias. C/ Eduardo Primo Yúfera (Científic), 1B. 46013 Valencia. Spain.*
luis.bernabeu@gmail.com & loretomoninorodriguez@gmail.com

Team Building is one of the most important aspects of our work. Although it is not a topic that is usually directly related to animal management, it is a fundamental part in ensuring the welfare of animals in human care. Maintaining a good relationship among team members, establishing different communication channels, and setting clear objectives are some of the tools that ensure the successful development of teams. In this video, we want to show some team building strategies that we used during the Covid-19 lockdown, when we had to split the team into two groups without contact between them. We will demonstrate how we managed to turn a potentially damaging situation to team cohesion into the opposite: A situation which strengthened team bonding.



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Control training methods for bottlenose dolphins (*Tursiops truncatus*) during the pandemic period in order not to decrease animal welfare despite the necessary reduction of training staff at the Lisbon Zoo.

Henderson, C.^{1*}

¹ LISBON ZOO, Estrada de Benfica 158-160, 1549-004 Lisbon, Portugal. CHenderson@zoo.pt

Due to Covid-19, companies faced several strategic and operational risks such as delayed or interrupted supply of raw materials, problems protecting the health and safety of employees, or insufficient workers. At a time when countries were struggling with the pandemic, the role played by trainers was fundamental. As key players in animal welfare, the trainers' responsibility consisted in carrying out good management of the training, ensuring the safety, mental and physical health of the animals based on proper organization with methods that allowed a small group of trainers to handle situations and dynamics that were normally managed by a larger team. We describe the challenges and adaptation of training at Lisbon Zoo, the implementation of a plan to ensure the safety for the workers, in which the dolphinarium went from 9 trainers to 6, while following a contingency protocol (only 3 trainers working per day), and how training problems were solved forcing a single trainer to conduct group training in pursuit of individual goals for each animal. It was concluded that the control techniques implemented allowed the staff to assure the level of animal welfare despite the number and schedules of trainers involved in the process.



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To discriminate or not to discriminate? What our dolphins learned during Covid pandemic

Paola Righetti^{1*}; Rita Celli¹; Stefano Furlati¹

¹ *Oltremare, via Ascoli Piceno 6, 47838 Riccione (RN). paolriq@libero.it*

During the covid-19 pandemic several institutions were forced to close to the public and resources were limited. Our animals were also affected by a lack of variability due to a loss of interaction with the public. In order to improve the variability and enrichment of their daily activities a new discriminating training behavior was introduced at Oltremare. The aim was to understand if dolphins could discriminate between a specific symbol when shown multiple symbols. Pieces of laminated paper showing symbols (i.e. star, square, triangle, circle etc.) were prepared and presented to 2 males and one female. A specific symbol was assigned to each dolphin. The animals were first trained to touch their assigned symbol with their rostrum, which was reinforced. Afterwards dolphins were then presented with two symbols and rewarded when they touched their assigned symbol. The number of symbols presented to the animal increased over time until 10 were presented, although a maximum of 2 symbols were shown during a single trial. At no point was a hand signal used to help the dolphins: prior to each trial, the assigned symbol was shown to the dolphin, then followed by a trial where 2 laminated papers were presented. The animal had to discriminate against the assigned one. Within approximately 3 months the animals learned to identify the assigned symbol and, although the work is not concluded, it is presented here that the three dolphins are able to identify a symbol from among others and also that they enjoy participating in these training sessions.

Acknowledgment

We want to thank our Veterinarian Dr. Barbara Biancani for encouraging us to present our work and for her constant support.



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30th March – 3rd April

How we trained creative thinking in killer whales

Myriam D. Weiss^{1*}, Isabelle Brasseur¹, Kathleen M. Dudzinski², Heather Manitzas Hill³

¹ Marineland Côte D'Azur, 306 Av. Mozart, 06600 Antibes, France. weiss.myriam87@gmail.com

² Dolphin Communication Project, Port St. Lucie, Florida, USA

³ St. Mary's University, San Antonio, Texas, USA

Many species' behavioral repertoires include foraging, mating, defending territory or mates, resting, and socializing actions. Yet, despite these universal components, individuals and species often innovate behavior in response to peers and the changing environment. The capacity to create new behaviors is assumed for many species including the killer whale (*Orcinus orca*). For this research project investigating creativity, we trained innovative thinking in our killer whales (KW) while under stimulus control. First, the KW needed to understand the concept of being creative. We trained a creativity SD, which was different from the usual “one SD-one behavior” concept they knew. One final training goal was for each animal to present a new behavior in response to the creativity SD. All sessions were recorded for scientific coding. In the subsequent sessions, appropriate reinforcement played a big role. The more “creative” the performed behavior was, the more it got reinforced. This reinforcement schedule impacted KW's motivation. From August 2020, four animals performed 12 test-sessions. A variable reinforcement schedule was installed: As long as the KWs were motivated to participate, the test-session went on. Our six-year-old male even performed a 30-minute session. In conclusion, we learned that KW are capable of creative thinking. The younger KW was more creative during this research. Several remarkable changes in behavior occurred, for instance the KWs would sometimes stop eating during test-sessions while still showing great motivation to continue. Our group of four KWs invented 38 new behaviors in only one month of formal testing and even more during training. They continue to perform these behaviors on a daily basis, even today. This research project clearly enriched the animals' repertoires of behaviors and hopefully their way of thinking.



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30th March – 3rd April

What might the public display of cetaceans look like in the future?

I. L. K. Clegg^{1*}

¹ *24 St Marys Road, Birmingham, B67 5DQ*

To continue its business sustainably, any industry that uses animals must largely align their ethical position with that of the general public: 'the mainstream social ethic'. While zoos are transitioning from entertainment venues to conservation actors, many cetacean (whale and dolphin) facilities present the animals in unnatural-looking enclosures and entertainment-driven contexts. But what is the 'mainstream social ethic' regarding cetacean facilities, and what might it mean for the industry's future? The evidence is first reviewed on cetacean welfare and the stated purposes for publicly displaying cetaceans, from the past to the present. The mainstream social ethic is then defined, suggesting we may be at a crossroads for this industry. Welfare has improved in the last decades but could be further enhanced through providing more choice and control in cetaceans' environments, particularly in enrichment, training and social groupings. Sanctuary settings provide a potential environment with more choice and control, but are still in the very initial stages of development. Fundamental, structural changes to the mission, presentation of the cetaceans and business model seem to be needed to realign the public display of cetaceans with the mainstream social ethic of the times.



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30th March – 3rd April

2000-2021 Efforts towards Research and Conservation of Manatees in México and South America

Sánchez-Okrucky, R.^{1*}; Arreola, M.R.²; López-Romahn, C.¹; Sánchez-Contreras, G.J.¹

¹ *The Dolphin Company, Banco Chinchorro 87, 13, 77504 Cancún, Q.R., Mexico. okrucky@thedolphinco.com*

² *Dolphin Discovery, Plano 1 Plaza Comercial Marina, Bahía Xcacel Mz 23, 77400 Puerto Aventuras, Q.R., Mexico*

The Mexican Government made a special commitment for the conservation of various aquatic and terrestrial species, which are called "Priority Species Program". The Caribbean manatee *Trichechus manatus manatus* is part of this group. Together with the Government and academic institutions, private initiatives have carried out research projects and actively participated in the attention to contingencies, the rehabilitation of orphaned calves, clinical assessments of wild populations, demographic studies, and the designation of protected areas. For more than 20 years, The Dolphin Company has been very proactive in the conservation of this endangered and charismatic species. More than 25 researchers have been studying the population of manatees in our care. Furthermore, we have been in charge of the veterinarian clinical assessment of the wild population in the Yucatan Peninsula and played an important role in the 2018 environmental contingency, supporting diagnosis, necropsies, treatments and assisting with pathology, bacteriology, and hematology studies. Our veterinarians have been involved in the rehabilitation of several animals both in Mexico and Latin-America. The present work will list all the actions in which The Dolphin Company has participated, highlighting the importance of the zoological institutions in ex-situ studies to promote conservation of the species in their natural habitats.



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Abundance, Site Fidelity, and Association Patterns of Coastal Bottlenose Dolphins (*Tursiops truncatus*) off the Coast of Southeast Florida

Graysen D. Boehning^{1,2*}; Barbara Brunnick¹; Stefan Harzen¹; Lauren Nadler²; Amy Hirons²

¹ Taras Oceanographic Foundation, Jupiter, Florida. boehning@taras.org

² Halmos College of Arts and Sciences, Nova Southeastern University, Dania Beach, FL, United States

The coastal ecotype of Atlantic bottlenose dolphins is one of the most thoroughly studied marine mammals, however a majority of these studies have occurred in protected habitats such as bays and estuaries. Very little work has been done on the population structure or site-fidelity in open-ocean habitats, and none has been done on the narrow, open-ocean sandbanks of South Florida, USA. Using photo-identification techniques, we analyzed the site-fidelity and association patterns of coastal bottlenose dolphins encountered along the coast of Palm Beach County, Florida. Between 2014 and 2019, we conducted 293 boat-surveys in the region, resulting in 226 encounters with coastal bottlenose dolphins. In total, we identified 727 unique individuals. Based on resighting ratios, 53 of these individuals were determined to be residential, using the study area year-round, 97 were regular or seasonal visitors, and 577 were sporadic visitors. These findings suggest that a small, permanent population exists within the study area, which shares the habitat with transitory individuals from other nearby populations. Preliminary association analysis indicates that there is a high level of social interaction between residential animals and visitors, making genetic or informational transfer likely. Many residential animals were also observed with neonates and calves, suggesting that the study area may serve as a nursing ground.



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30th March – 3rd April

New insights of lethal interaction between dolphin species on Western Mediterranean.

Marco-Cabedo, V.^{1*}; Crespo-Picazo, J.L.¹; Jiménez-Martínez, M.A.²; Aznar Avedaño, F.J.³; Sánchez-Vizcaíno, J.M.⁴; Gozalbes, P.³; Muñoz-Baquero, M.¹; Escribano, F.⁵; García-Párraga, D.¹

¹ *Fundación Oceanogràfic de la Comunitat Valenciana, 46005, València, Spain. mmunoz@oceanografic.org, jlcrespo@oceanografic.org, vmarco@oceanografic.org, dgarcia@oceanografic.org*

² *Department of Animal Medicine and Surgery, Veterinary Faculty, Complutense University of Madrid, 28040 Madrid, Spain. mariadji@vet.ucm.es*

³ *Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, 46071 València, Spain. patricia.gozalbes@uv.es, Francisco.Aznar@uv.es*

⁴ *VISAVET Center and Animal Health Department, Veterinary School, Complutense University of Madrid, 28040 Madrid, Spain. jmvizcaino@ucm.es*

⁵ *Centro de Recuperación de Fauna Silvestre El Valle. Dirección General de Medio Natural, Región de Murcia. 30150. Murcia, Spain. fescribano@orthem.com*

Here we present an update on interspecific aggressions from bottlenose dolphins (*Tursiops truncatus*) towards other dolphin species in Western Mediterranean as the cause of death, mainly in striped dolphins (*Stenella coeruleoalba*). The cause of death was established based on complete necropsies and specific findings such as rake marks, severe internal lesions with traumatic origin, including skull and ribs fractures, brain hemorrhage, haemothorax or hemoabdomen. Diagnosis was reinforced by comprehensive complementary analysis of collected samples. From the first description of these events, more cases appeared in our study area and other adjacent locations. Aggressive interactions are considered common behaviors between cetacean species. Many hypotheses have been proposed to explain these behaviors, but the reason is still unclear. Territorial behavior related to human activities, such as aquaculture facilities, has been suggested as a possible reason to explain those events. The current research summarizes all cases detected in the Western Mediterranean and provides new insights of these aggressive interactions as an increasing mortality cause in the area.



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30th March – 3rd April

Operation of a Mediterranean monk seal (*Monachus monachus*) Rescue and Information Network during 2021 in Greece.

Koemtzopoulos, K.^{1*}; Adamantopoulou, S.¹; Paxinos, O.¹; Dendrinou, P.¹; Karamanlidis, A.¹

¹ MOm/Hellenic Society for the Study and Protection of the Monk Seal, Solomou Street 18, 10682, Athens, Greece.
info@mom.gr

The Rescue and Information Network (RINT), established in 1991, has yielded over 7500 sighting reports of Mediterranean monk seals by non-specialists to date. It has proved to be an invaluable conservation tool in two major aspects: 1) the collection of information (spatiotemporal, biological, behavioral) subsequently analyzed and applied in the protection of the species at a local and national level; 2) the immediate response in cases where either dead animals requiring a necropsy or live animals in need of treatment are located. The year 2021 is presented as a case study of RINT's operation. Throughout 2021 a total of 350 reports of monk seal sightings were collected. Of these 305 concerned live animals and 45 dead. 36 sightings concerned newborn pups. Of the 45 animals found dead, a cause of death was determined for 18: 6 were determined as natural deaths, 5 accidental deaths related to entanglement in fishing gear and 7 deliberate killings. In 10 cases of live animals (6 pups, 4 non-pups), an immediate rescue response was required. The data highlight an increased occurrence of animals requiring rescue compared to previous years, most likely related to the population increase reported for the species in the last decade.



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30th March – 3rd April

Report on the 2022 stranding of a Cuvier's beaked whale near the harbor of Athens, Greece

Anastasia Komnenou^{1,2}; Aimilia Drougas²; Pantelis Sarris²; Nikitas Vogiatzis²; Evangelia Kofidou^{1,2,3}; Dimitra Psalla¹; Alexander Costidis⁴; Manuel Garcia Hartmann^{5*}

¹ School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, AUTH, Thessaloniki, Greece. natakomn@vet.auth.gr

² ARION Cetacean Research, Rescue and Rehabilitation Center, M. Botsari 110, 54453, Thessaloniki, Greece

³ Department of Biological Applications & Technology, University of Ioannina, 45110 Ioannina, Greece

⁴ Virginia Aquarium Stranding Response Program

⁵ MarLab, France, hartmann@zoo-vet.de

With 15.147 kilometers length, the coastline of Greece is the largest of the Mediterranean basin and includes the mainland and island shores of the Ionian Sea, the Aegean Sea and the Levantine basin. The Greek coastline, second in length in Europe and ninth in the world, has been under great pressure in recent decades from both anthropogenic activities and climate change. Eight species of cetaceans are described as “residents” of Greek waters, including the striped dolphins, common bottlenose dolphins, short-beaked common dolphins, harbor porpoises, Cuvier's beaked whales, sperm whales, Risso's dolphins and fin whales. Several more species are “occasional visitors”. The immense distances of the Greek coastline with more than 6000 islands makes any intervention difficult when live cetacean strandings are reported. The Greek Cetacean Stranding Veterinary Network is centrally coordinated by the first author AK through the NGO Arion and the School of Veterinary Medicine of the Aristotle University of Thessaloniki. More than 150 trained veterinarians based all over the Greek coasts act both as local stranding coordinators and first aid. In this talk we present the work of the Greek Cetacean Stranding Veterinary Network in January 2022 with a live Cuvier's beaked whale *Ziphius cavirostris* which was located in very shallow waters, only few meters from the coast, at few kilometers distance of the entrance of the main Harbor of Piraeus, Athens, Greece. The animal was a female of 4,80 m length and an estimated weight of 800 kg. The animal was very weak and almost motionless. The approach of the Greek Stranding Veterinary Network to live cetacean strandings -which is half “rescue” and half “rehabilitation”- is explained based on the example of this beaked whale.



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30th March – 3rd April

Proteomic study in *Tursiops truncatus* semen

Fuentes-Albero, M.C.^{1,2*}; González-Brusi, L.³; Cots, P.³; Luongo, C.²; Abril-Sánchez, S.²; Ros-Santaella, J.L.⁴; Pintus, E.⁴; Ruiz-Díaz, S.⁵; Barros-García, C.¹; Sánchez-Calabuig, M.J.^{5,6}; García-Párraga, D.^{1,7}; Avilés, M.³; Izquierdo Rico, M.J.³; García-Vázquez, F.A.²

¹ Department of Biology, Avanqua-Oceanogràfic S.L, Valencia, Spain. mcarmenfuentesalbero@gmail.com

² Department of Physiology, Faculty of Veterinary Science, University of Murcia, Murcia, Spain

³ Department of Cell Biology and Histology, Faculty of Medicine, University of Murcia, Murcia, Spain

⁴ Department of Veterinary Sciences, Faculty of Agrobiological, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czechia

⁵ Department of Animal Reproduction, National Agricultural and Food Research and Technology Institute (INIA), Madrid, Spain

⁶ Department of Medicine and Surgery, Faculty of Veterinary Science, Madrid, Spain

⁷ Research Department, Fundación Oceanogràfic, Valencia, Spain

Tursiops truncatus is the most common species of dolphin in aquariums, due to its adaptive capacity to captivity. Thanks to this, several procedures have been carried out to know specific aspects of this species, such as obtaining seminal samples and developing reproductive techniques. All this has established this species as a model organism of the cetaceans. The objective of this study has been to identify, describe and classify proteins present in sperm and seminal plasma of bottlenose dolphins. For this, four semen samples from two male dolphins located at the Oceanogràfic València, were analyzed. The proteomic profile was analyzed using an LC-MS / MS, and a list with the gene symbols corresponding to each protein was submitted to the DAVID database. A total of 730 different proteins were identified, 423 in sperm and 307 in seminal plasma, 111 being common to both groups. The dolphin protein database was compared with the proteomic profiles of other mammalian species, and some similarities were found. This allows identifying biomarkers of quality and fertility of semen, which are essential to detect and select the highest quality samples and resistance to preservation processes, being able to generate in the future gene banks more efficiently.



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30th March – 3rd April

Fertility and reproductive success (RS) in orcas (*Orcinus orca*) in human care

Arija, C.M.^{1*}

¹ SeaWolves, Urb. Pinar de Garaita 86, 03530 La Nucía, Spain. arija@seawolves.es

There exists a great amount of information about the reproduction of orcas in human care, although aspects like fertility and lifetime reproductive success (LRS) are still poorly developed. Longevity and their late sexual maturity make these variables hardly measurable, but not impossible. To do it, a database with the historical and global ex-situ population of orcas was generated using the Marine Mammal Inventory Report and information shared by zoological facilities regarding births and deceased. Although several valid mathematical methods to calculate LRS can be used, the lifetime retrospective method was utilized in the present study to avoid biases and get a real measure of lifetime reproductive success instead of an estimation. This system involves defining the age as to which offspring survival is measured. Both 10 and 15 years were used as minimum calves age to establish a comparison between them. Considering this criteria, 17 and 9 mothers and their calves were included in the two samples. LRS ranged between 0-6 and 0-4 calves/mother and averaged $1,35 \pm 1,77$ and $1,11 \pm 1,45$ calves/mother, respectively. The results for both criteria, 10 and 15 years as minimum offspring age, were similar but the amount of females was considerably different.



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Physiological Parameters Monitored on Bottlenose Dolphin Neonates (*Tursiops truncatus*) over the First 30 Days of Life

Sánchez-Contreras, G.J.^{1*}; Furlati, S.²; Benaglia, F.^{1,3}; Gili, C.⁴; Arija, C.M.⁵; Biancani, B.²

¹ Mediterraneo Marine Park, Coast Rd. NXR9038 Bahar ic-Caghaq, Malta. gscvetscience@gmail.com

² Costa Edutainment S.p.A., Via Ascoli Piceno 6, 47838 Riccione, Italy

³ AniCura Kleintierspezialisten Augsburg, Max-Josef-Metzger-Straße 9, 86157 Augsburg, Germany

⁴ Stazione Zoologica Anton Dohrn, 80122 Naples Italy

⁵ Sea Wolves, Gorrión 64, 28019 Madrid, Spain

Bottlenose dolphins (*Tursiops truncatus*) have been bred under human care for more than 60 years. Calves up to 30 days of life have presented the highest mortality rate, although comparable data for free-ranging neonates within this age group is not available. Husbandry measures to increase survivability have therefore been constantly improved. This work shows the results of a structured veterinary program that established the procedures to collect relevant physiological parameters on 13 calves during their first 30 days of life. Standardized observation protocols facilitated statistical analysis of the respiratory pattern, nursing, morphometric measurements and bloodwork. These allow early detection of health issues. Healthy neonates had longer apnea duration, despite the shape and size of the facility. The nursing pattern showed that successful calves started nursing 3 to 18 h postpartum. Although with different individual patterns, a steady increase in suckling time occurs during the first 24 h of life. The healthy neonates grew 0.428 ± 0.102 kg per day and reference blood values were determined, including for the first time values of cholesterol, triglycerides, α -amylase, lipase, magnesium and cortisol for such young calves. A list of indications for prompt intervention will be included.



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Neonatal hypoxic-ischemic encephalopathy in a bottlenose dolphin (*Tursiops truncatus*): Cause or consequence of a meconium-aspiration syndrome?

Rebollada-Merino, A.^{1,2*}; Romani-Cremaschi, U.³; Rodríguez-Bertos, A.^{1,2}; Canales-Merino, R.³

¹ VISAVET Health Surveillance Centre, Complutense University of Madrid, 28040 Madrid, Spain. agusrebo@ucm.es

² Department of Internal Medicine and Animal Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, 28040 Madrid, Spain

³ Veterinary Department, Mundomar, Calle Sierra Helada s/n, 03503 Benidorm, Spain

Meconium-aspiration syndrome (MAS) is a life-threatening condition in many newborn mammals. After a physiological delivery, a newborn, female, bottlenose dolphin (*Tursiops truncatus*) calf showed progressive dyspnea and sudden death 4 minutes after birth. The cadaver, placenta and umbilical cord were recovered, and a complete postmortem examination was performed. Samples of the main organs and tissues were collected in parallel for microbiological, histopathological and immunohistochemical studies. Histological findings were consistent with MAS. Central nervous system (CNS) histopathological examination displayed central chromatolysis and necrosis in neurons, as well as gliosis. Immunohistochemical expression of caspase 3 and nitric oxide synthase was assessed. The arterioles displayed hyaline degeneration and, surrounding blood vessels, there were vasogenic edema and occasional hemorrhages in the gray matter of cerebrum. *Escherichia coli* was isolated from placenta, lungs, and liver but there was no histological evidence of septicemia. This report describes hypoxic-ischemic CNS lesions associated with MAS in a newborn dolphin. The lesions resemble those observed in human neonates affected by this syndrome and demonstrated the involvement of the CNS in the pathogenesis. Based on these observations, further research on MAS in newborn cetaceans is needed and prenatal risk assessment protocols for intrauterine and perinatal hypoxia should be improved.



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30th March – 3rd April

Evaluation of the vocalizations between two bottlenose dolphin mother-calf dyads during the first 10 days of life.

Arija, C.M.^{1*}; Sánchez-Contreras, G.J.²

¹ SeaWolves, Urb. Pinar de Garaita 86, 03530 La Nucía, Spain. arija@seawolves.es

² Mediterraneo Marine Park, Coast Rd. NXR9038 Bahar ic-Caghaq, Malta

Vocalizations between bottlenose dolphin mothers and calves have been previously studied but the information regarding the relationship among this behavior and other activities carried out by the dyads is scarce. Two mother-calf pairs kept in human care have been studied. During the first 30 days of calves' lives, lots of behavioral data were recorded as part of the calf management protocol. From this data, vocalizations were considered for the first 10 days of the calves' life and compared to different behavioral and environmental variables. The results show that there is an evolution with significant differences in the number of vocalizations produced by the mothers and the calves along the days considered for the study. Although these differences are specimen-dependent too, finding one calf that vocally behaves like his mother and the other one developing his own pattern. There are also correlations between vocalizations and other types of behavior such as those related to nutrition, exploration, protection of the calf, and bonding. However, there is no significance when comparing the vocalizations to the circadian rhythm or specific times of the day. Understanding how vocalizations and other behavioral variables interact can improve the knowledge regarding the early development of the mother-calf relationship.



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Management of a chronic degenerative lesion in the lumbo sacral region in a bottlenose dolphin (*Tursiops truncatus*)

Arreola, M.R.^{1*}; López-Romahn, C.^{1,2}; Sánchez-Okrucky, R.^{1,2}; Sánchez-Contreras, G.J.^{1,2}

¹ Dolphin Discovery, Plano 1 Plaza Comercial Marina, Bahía Xcacel Mz 23, 77400 Puerto Aventuras, Q.R., Mexico.

marreola@dolphindiscovery.com

² The Dolphin Company, Banco Chinchorro 87, 13, 77504 Cancún, Q.R., Mexico.

A 25 year old male bottlenose dolphin started showing slow swimming and an increase in buoyancy behavior. Although the animal presented initially normal appetite and no evident signs of pain, there was a clear lethargy. No significant results came out of the blood work but his behavior was worrying, he either remained buoyant or down at the bottom of the sea pen for long periods. He was very uncomfortable when asked any behavior that would imply actively keeping his position vertical, including that for hydration. Radiographic study of the peduncle revealed a vertebral compression at the level of the lumbar spine. Analgesia and stomach protectors were administered. Massages, ultrasounds, electrostimulation and dry needle therapy were also implemented together with specific physiotherapy exercises. Improvement on the behavior and mobility of the spine were evident during the first days after this therapy started. Analgesia was removed without problems. Blood work has shown no relevant changes throughout the process. Although this is a chronic problem, the animal still enjoys a good quality of life, and is able to participate in all the activities he was always part of. He follows a daily plan of exercises and periodically receives his physiotherapy sessions.



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30th March – 3rd April

Use of cannabidiol (CBD) as an alternative to manage the pain in South American Sea Lions (*Otaria flavescens*) kept in human care: Our experience.

Ortíz-Ortíz, J.C.^{1*}; López-Romahn, C.²; Sánchez-Okrucky, R.²; Sánchez-Contreras, G.J.²

1 Dolphin Discovery Nuevo Vallarta, Carretera a Tepic Km. 155 S/N, 63735 Nuevo Vallarta, Bahía de Banderas, Nayarit, México. vetspv@dolphindiscovery.com

2 The Dolphin Company, Banco Chinchorro 87, 13, 77504 Cancún, Q.R., Mexico.

The use of cannabidiol (CBD) to manage pain has been described both in human and veterinary medicine. However, this use has not been described in marine mammals yet. Corneal lesions and musculoskeletal affections that imply a limitation in mobility are frequent in South American sea lions (*Otaria flavescens*) both associated with intense pain. In this regard, a 22 years old female and a 24 years old male, both members of this species and kept in human care, presented with a corneal ulcer and a dysfunction in the mobility of the hindquarters respectively. The initial treatment with conventional analgesia and different combinations of painkillers had little to no evident effects on the relief of pain in these two cases. Thus, it was decided to start administering oral CBD drops. The use of adjusted doses of CBD resulted in significant changes in the clinical manifestations related to pain (ie. less blefarospasm and less lethargy or reduced activity). This pain disappeared eventually, improving the wellbeing of the animals. More studies are needed to demonstrate the therapeutic potential of CBD in South American sea lions and other marine mammal species, but our experience seems promising.



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30th March – 3rd April

Saliva and Blood Cortisol Measurement in Bottlenose Dolphins (*Tursiops truncatus*): Methodology, Application, and Limitations.

Rickert, D.^{1*}; Baumgartner, K.¹

¹ Nuremberg Zoo, 90480 Nürnberg, Germany. daniela.rickert@amberg-mail.de

To physiologically validate welfare observations, glucocorticoid levels are usually assessed. The measurement of health biomarkers (especially cortisol, which can be measured in saliva), have become the focus of cetacean stress research. However, there are two problems associated with saliva measurements in cetaceans: saliva might either be diluted with pool water or be contaminated by fodder fish, as frozen fish usually contains high levels of cortisol. In our study, we investigated how saliva cortisol levels are connected to blood cortisol levels and how saliva cortisol can be influenced by fodder fish. We examined saliva and blood samples in eleven bottlenose dolphins (*Tursiops truncatus*) kept in an outdoor and indoor facility. Furthermore, we assessed the cortisol levels of different kinds of fodder fish. Our data show that, although saliva cortisol values are elevated under stress and arousal, they seem not to be correlated with blood cortisol values. We also show that, after feeding, saliva cortisol values are increased up to 100-fold. Our results suggest that saliva cortisol measurements in dolphins have to be conducted and considered with care, as they can easily be contaminated. Moreover, it is important to use the right laboratory method in order to specifically detect cortisol.



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30th March – 3rd April

Implementation of a protocol for animal welfare assessment in the bottlenose dolphin (*Tursiops truncatus*) in a zoological park (Planète Sauvage)

Pascaud, A.^{1*}; Böye, M.²; Durand, C.²; Guintard, C.¹

¹ ONIRIS - Ecole Nationale Vétérinaire de Nantes, 101 Route de Gachet, 44307 Nantes, France.

pascaud.ana@gmail.com & claud.guintard@oniris-nantes.fr

² Planète Sauvage, La chevalerie, 44710 Port-Saint-Père, France. martin.boy@planetesauvage.com & candice.durand@planetesauvage.com

Animal welfare is one of the zoological institution's aims. Its definition evolved over time and its evaluation is now carried out based on the most recent scientifically based models on a growing number of species. In this context, a cooperation between Oniris veterinarian school and Planète Sauvage Safari Park in the context of a veterinary thesis led to the creation of a bottlenose dolphins (*Tursiops truncatus*) welfare evaluation software. This tool is meant to assist the animal keepers in their daily decisions regarding the group of dolphins in their care in order to optimize their well-being. It is based on several parameters selected for their scientific relevance related to the animals such as food intake, willingness to participate in daily activities, behavior or social network dynamic. Resource-based criteria are, in parallel, monitored and formally identified as means of actions to optimize animal-based criteria. This tool has been now successfully used for more than one year and led to a formal database suited to further formal and scientific analysis in the field of welfare and animal care. Developmental steps and chosen orientations will be here listed and discussed as well as our software effectiveness and its future developments.



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30th March – 3rd April

First assessment of temperament in Antillean manatees under human care and potential implication for their welfare.

Charles, A.^{1,2*}; Saint-Jalme, M.¹; Mulot, B.²; Henaut, Y.³; Lecu, A.⁴; Delfour, F.⁵

¹ *Centre d'Ecologie et des Sciences de la Conservation (CESCO, UMR 7204, CNRS, MNHN, Sorbonne-Université, MNHN). aviva.charles@mnhn.fr*

² *ZooParc de Beauval & Beauval Nature, 41110, Saint Aignan, France*

³ *Laboratorio de Conducta Animal, GAIA-BIO, El Colegio de la Frontera Sur (ECOSUR). Av. del Centenario Km. 5.5, C.P. 77014, Chetumal, Quintana Roo, Mexico*

⁴ *Parc Zoologique de Paris, 53 avenue de Saint Maurice, Paris, France*

⁵ *Ecole Pratique des Hautes Etudes, 75005 Paris, France*

Growing scientific evidence shows that assessing temperament, i.e. consistent inter-individual differences in behavior, of captive animals could be an effective tool to monitor and improve their welfare. Especially, positions along the bold-shy axis are linked to how an individual copes with environmental conditions and may reflect its response to stress. We assessed the level of boldness in 16 captive Antillean manatees (*Trichechus manatus manatus*) housed in 2 European zoological parks. During novelty tests, we recorded behaviors related to exploration as well as social interactions (i.e. physical proximity, social contacts). We assessed manatees' reactivity to humans through a participation score during hand-feeding sessions. Finally, we rated keepers' subjective impression about individuals' temperament through a questionnaire. Our results showed that boldness score was significantly associated with physical proximity with conspecifics, as well as with a high participation score to hand-feeding sessions in one zoo. Finally, surveys completed by keepers were reliable and positive associations were found between rated items and behavioral observations. More investigations on a larger sample size are needed to explore boldness-shyness continuum in manatees as well as other potential traits that could be useful to improve the management and ultimately the welfare of this species in and ex situ.



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30th March – 3rd April

Environmental Enrichment Devices Reduce Stereotypical Behaviors and Habituation During Rehabilitation of Wild California Sea Lions and Northern Elephant Seals

Kirsten Donald¹; Amanda Walters¹; Vinícius Donisete Lima Rodrigues Goulart²; Hendrik Nollens³; Alissa Deming¹; Grey Stafford⁴; Sabrina Brando^{5*}

¹ Department of Conservation Medicine and Science, Pacific Marine Mammal Center, Laguna Beach, California, 92651, USA. kdonald@pacificmmc.org

² Transportation Research and Environmental Modeling Lab (TREM Lab), Programa de Pós-Graduação em Análise e Modelagem de Sistemas Ambientais, Instituto de Geociências, Universidade Federal de Minas Gerais – UFMG, Brazil. viniciusdonisete@gmail.com

³ San Diego Zoo Wildlife Alliance, San Diego, CA, 92101, USA. hnollens@sdzwa.org

⁴ KongZoo, McKinney, TX 75069, USA. grey@ireinforce.com

⁵ AnimalConcepts, P.O. Box 378, 03725 Teulad, Alicante, Spain. sbrando@animalconcepts.eu

The value of animal enrichment programs in reducing stereotypical behaviours, reducing stress, and encouraging natural behaviours in managed care settings has been demonstrated in several species, including marine mammals. However, there is limited information from marine mammal wildlife rehabilitation facilities. In 2021 the Pacific Marine Mammal Centre incorporated and assessed an environmental enrichment program with stranded California sea lion (*Californianus zalophus*) pups (n=7) and Northern elephant seal (*Mirounga angustirostrus*) weanlings (n=8) to determine the efficacy of environmental enrichment devices (EEDs) in reducing stereotypical behaviour and decrease risk of habituation. Three enrichment devices were compared (Horse Kong, Wubba Kong, artificial kelp) as well as a control session (no enrichment). The most significant result was when enrichment items were present, sea lion pups and elephant seal weanlings spent significantly less time looking at caretakers, and animals that displayed stereotypical behaviours had a significant reduction in these behaviours. Our findings support that EEDs are safe and effective tools for decreasing stress and stereotypical behaviour in young sea lion and elephant seal patients during their time in rehabilitation. Incorporating enrichment programs into marine mammal rehabilitation settings may not only increase animal welfare, but also could contribute to improved success upon reintroduction to the wild.



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30th March – 3rd April

Renewable energy – a deadly threat for aquatic animals

Norbert Fleck^{1*}

¹ Stefan-Flötzl-Str. 26, D-83342 Tacherting, Germany. fleck.norbert@idv-engineering.de

Everyone is talking about "Green deals", Climate protection and Energy transition.

Engineers (especially when engaged in civil protection) talk about service security and projectable power generation. The unprojected failure of several nuclear power stations in France and the non substituted shutdown of power stations in Germany point a spotlight on the emerging threat of a Europe-wide blackout. While countries like Austria run national campaigns to get their people prepared, other countries try to avoid a public discussion about this issue. But physics does not care about public discussions and as the German transmission network operators went from 5 - 7 emergency interventions per year (2010) to more than 1200(!) last December alone, the threat of a sudden and long lasting blackout is getting immediate. The author will go through the effects of a blackout on life support systems in zoos and the possibilities you might have in order to bring your animals through such a disaster. The author is an Electronic Engineer and is a member of a Civil-Military-Cooperation team for disaster control in Germany. This is to show that through training it is possible to achieve goals that promote animal welfare.



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30th March – 3rd April

Ultrasensitive Cardiac Troponin I Levels in Marine Mammals Under Human Care: Establishing Baseline Values in a Healthy Multi-Species Population

Rojo-Solís, C.^{1*}; Álvaro Álvarez, T.¹; Valls Torres, M.¹; Barros García, C.¹; Crespo-Picazo, J.L.²; Marco-Cabedo, V.²; Muñoz-Baquero, M.²; García-Párraga, D.^{1,2}

¹ *Veterinary and Laboratory Department, Oceanogràfic, Valencia, 46013, Spain. crojo@oceanografic.org*

² *Fundación Oceanogràfic, Valencia, 46013, Spain*

Troponin is an intracellular protein involved in muscle contraction and its isoform 3 is only present in cardiac muscle and frequently referred to as cardiac Troponin I (cTnI). cTnI presence in serum is used as a biomarker of acute myocardial infarction in human medicine, where its quantification serves as the reference indicator in emergency medicine triage.[1–2] cTnI has widely been used in veterinary medicine as a marker of heart disease in domestic and wild species, including some marine mammals.[3–9] Although previously determined in cetaceans, reference ranges are not very precise due to technique limitations and a low number of individuals tested.[10–11] High sensitivity troponin I assay allows a more accurate level of quantification, measuring concentrations as low as 2.3 pg/mL in humans. A total of 84 samples from 29 individuals were used for cTnI quantification, of which 69 were from cetaceans (*Tursiops truncatus* and *Delphinapterus leucas*) and 15 from pinnipeds (*Otaria flavescens* and *Odobenus rosmarus*). Mean cTnI concentration in cetaceans was 1.44 ± 1.86 pg/ml and significantly higher in pinnipeds 11.84 ± 7.99 pg/ml. Values obtained in cetaceans are considerably lower than previously reported intervals (0–25.6 pg/ml) and may serve as a better baseline value for animals under human care.



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30th March – 3rd April

Diagnosing Brucella in cetaceans: thoughts from the field

Muñoz-Baquero, M.^{1*}; Vargas-Castro, I.⁴; Marco-Cabedo, V.¹; Crespo-Picazo, J.L.¹; Muñoz, P.M.²; De Miguel, M.J.²; Jiménez-Martínez, M.A.³; Sánchez-Vizcaíno, J.M.⁴; Fayos, M.^{5,6}; Serdio, A.^{5,6}; Andrés-Barranco, S.²; Varas, J.^{5,6}; García-Párraga, D.¹

¹ *Fundación Oceanogràfic de la Comunitat Valenciana, 46005, Valencia, Spain. mmunoz@oceanografic.org, jlcrespo@oceanografic.org, vmarco@oceanografic.org*

² *Instituto Agroalimentario de Aragón (IA2), Departamento de Ciencia Animal del Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)-Universidad de Zaragoza, Zaragoza, Spain. pmmunoz@cita-aragon.es, mjmiguel@aragon.es, sandres@aragon.es*

³ *Department of Animal Medicine and Surgery, Veterinary Faculty, Complutense University of Madrid, 28040 Madrid, Spain. mariadji@vet.ucm.es*

⁴ *VISAVET Center and Animal Health Department, Veterinary School, Complutense University of Madrid, 28040 Madrid, Spain. ignavarg@ucm.es, jmvizcaino@ucm.es*

⁵ *Centro de Recuperación de Fauna Silvestre de Cantabria, 39690, Cantabria, Spain. externo.fayos_m@cantabria.es*

⁶ *Dirección General de Biodiversidad, Medio Ambiente y Cambio Climático, 39011, Cantabria, Spain. externo.fayos_m@cantabria.es*

Despite Brucellosis in cetacean has been reported worldwide (including Spain) along the last decades, specific research is needed to understand aspects like prevalence, transmission, species susceptibility, associated pathology or zoonotic potential. A proper diagnosis routine is critical to detect and characterize infected animals and unravel the significance of this disease in cetaceans. In brucellosis, the bacteria isolation is the only unquestionable proof of infection. However, culturing is cumbersome and its sensitivity limited, so additional diagnostic techniques are needed to support presumptive diagnosis. The aim of this work is to present and discuss data from 48 stranded dolphins, belonging to four different species: striped dolphin (*Stenella coeruleoalba*), Risso's dolphin (*Grampus griseus*), bottlenose dolphin (*Tursiops truncatus*) and short-beaked common dolphin (*Delphinus delphis*), where *Brucella* spp. was suspected of being involved. The Rose Bengal serological test, post-mortem examination, histopathology and PCR on tissue samples are compared with culture results. We isolated *Brucella* from 16 animals (mainly from brain, spleen, cerebrospinal fluid and lung lymphnode). Histopathological and direct PCR results also suggested *Brucella* infections in dolphins involved in a mass stranding event. Field diagnostic strategies and the potential zoonotic implications of *Brucella* spp. in marine cetaceans need to be considered.



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30th March – 3rd April

The validation of a non-invasive skin sampling device for detecting cetacean poxvirus

Segura-Göthlin, S.^{1,2*}; Fernández, A.¹; Arbelo, M.¹; Felipe-Jiménez, I.¹; Colom-Rivero, A.¹; Almunia, J.²; Sierra, E.¹

¹ *Animal Health and Food Safety Institute (IUSA). Veterinary School. University of Las Palmas de Gran Canaria. Campus Universitario de Arucas, Carretera de Trasmontaña, s/n; 35416 – Arucas, Las Palmas; (+34 928 45 97 11). direccion_iusa@ulpgc.es*

² *Health and Welfare Program. Loro Parque Foundation, Av. Loro Parque, 38400 Punta Brava, Santa Cruz de Tenerife (+34 922 373 841 (ext.: 281). lpf@loroparque-fundacion.org*

Poxvirus-like lesions are characterized by very distinctive irregular, slightly in relief, hyperpigmented pinhole and stippled skin patterns which are potentially used as health indicators in cetaceans. Due to their distinguishable form, most studies have identified those lesions by visual appraisals, lacking in diagnostic methods to corroborate the presence of the virus and, consequently, correctly confirm Tattoo Skin Disease (TSD). Aiming to address skin biopsy intrusiveness, in this preliminary study we compared both suitability and reliability between cytology cell samples (CSS) and skin biopsies as sampling methods to molecularly detect cetacean poxvirus (CePV-1) by performing two different DNA extraction procedures of 12 tattoo-like-lesions from two free-ranging stranded cetaceans in the Canary Islands. Thus, genomic extraction was accomplished through DNA Tissue Kit STM (QuickGene, Kurabo, Japan) and DNeasy™ Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Molecular detection of CePV-1 was performed through a real-time PCR. Results of this work will be presented and discussed to ascertain the suitability for CCS to collect and detect CePV-1 in a non-invasive manner and to consider this method as a promising procedure in cetaceans under human care.



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30th March – 3rd April

SARS-CoV-2 detection methods for cetacean and pinnipeds otariid species

Rubio-Guerri, C.¹; Aguiló-Gisbert, J.^{1*}; Canales-Merino, R.²; Romani-Cremaschi, U.²; Maiques, E.¹; Padilla-Blanco, M.¹; Pérez, R.¹; Ruíz, H.³; Lacasta, D.³; Fernández, A.³; Villanueva-Saz, S.³.

¹ *Universidad Cardenal Herrera-CEU, Valencia, Spain. consuelo.rubio@uchceu.es; jordi.aguilo@uchceu.es;*

² *Veterinary Department, Mundomar, Calle Sierra Helada s/n, 03503 Benidorm, Spain. veterinario@mundomar.es*

³ *Laboratorio de Inmunopatología Clínica, Facultad de Veterinaria, Universidad de Zaragoza, Spain. svs@unizar.es*

SARS-CoV-2 virus is the causal agent of the COVID-19 pandemic that has been affecting the world since January 2020. It is considered a pathogen of animal origin that has crossed the species barrier to affect humans. Since the beginning of the pandemic, several species have been naturally or experimentally infected by the virus, including pet, zoo and wild animals. Moreover, SARS-COV-2 persists in residual water and can contaminate the marine ecosystem, eventually threatening marine mammal health. Here we present different detection methods used to identify the virus or the host immune response (antibodies) in cetaceans and pinnipeds, including RT-PCR, human or animal antigen tests and serology. Each of these tests has been subjected to fine tuning in our labs to be adapted to these species, including the sampling method and laboratory methodology, whether using commercial or in-house methods. We present also the preliminary results of a study on the detection of SARS-CoV-2 infection in a captive population of *Tursiops truncatus*, *Otaria byronia*, *Zalophus californianus* and *Phoca vitulina*.



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30th March – 3rd April

Herpesvirus screening and evaluation in Western Mediterranean Cetaceans

Vargas-Castro, I.^{1*}; Melero, M.^{1,2}; Crespo-Picazo, J.L.³; Jiménez, M.A.⁴; Sierra, E.⁵; Rubio-Guerri, C.⁶; Arbelo, M.⁵; Fernández, A.⁵; García-Párraga, D.³; Sánchez-Vizcaíno, J.M.¹

¹ VISAVET Health Surveillance Centre and Animal Health Department, Veterinary School, Complutense University of Madrid, 28040 Madrid, Spain. ignavara@ucm.es

² External Health Service, Government Delegation in the Community of Madrid, Ministry of Territorial Policy, 28071 Madrid, Spain

³ Research Department, Fundación Oceanogràfic de la Comunitat Valenciana, 46013 Valencia, Spain

⁴ Department of Animal Medicine and Surgery, Veterinary Faculty, Complutense University of Madrid, 28040 Madrid, Spain

⁵ Division of Veterinary Histology and Pathology, Institute for Animal Health, Veterinary School, University of Las Palmas de Gran Canaria, 35416 Canary Islands, Spain

⁶ Department of Pharmacy, Facultad de CC de la Salud, UCH-CEU University, 46113 Valencia, Spain

Herpesvirus has been described in several cetaceans species, associated with lesions or with asymptomatic infections. In this study, the prevalence of herpesvirus (HV) was evaluated in stranded cetaceans in the Valencian Community, Spain. In addition, tissue tropism, the effect of age and sex, and the presence of lesions associated with the infection were studied. Finally, a molecular epidemiological study of the detected sequences was carried out. 966 tissue samples from 47 cetaceans stranded in the Valencian Community between 2010 and 2013 were analyzed by nested-PCR. A prevalence of 80.85% was obtained, the highest that has been reported so far in cetaceans. The highest proportion of HV-positive tissue samples were found in the reproductive system, nervous system, and skin. The infection was more frequent in females, juveniles and calves. The lesions observed were similar to those caused by HV in other species, mainly on the skin, mucosa of the upper digestive tract, genitalia and central nervous system. Other lesions were nonspecific or compatible with potential concomitant etiologies. Six novel sequences were detected. This work provides us with valuable information about HV infection in cetaceans, but it also raises interesting research questions for future research.



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30th March – 3rd April

Actions and Specialized Training in the Association of Marine Mammals Specialist

Becerra, S.^{1*}; Urbina, E.¹; Garduño, M.¹; Camarena, C.¹; Capote, T.¹; Martínez J.¹

¹ *Asociación Mexicana de Especialistas en Mamíferos Marinos (ADEMM)* ademmsocialm@gmail.com

In the Association of marine mammal's specialist training improves the efficiency of specialists so that they contribute to increasing animal welfare in a unified manner. The training integrates basic and organized elements in a planned way, which makes it easier to be clear about the training of the specialists that today they need to acquire for personal development and to guarantee the good care and handling of the animals. In accordance with the above, the successive independent but interrelated phases of how specialized training in marine mammals was formed, such as: planning, organization, dissemination and results, will be mentioned. In addition to the professional growth of the specialists, the development of the goals of the association is carried out through different actions or activities such as the creation of campaigns, participation in social networks that contain topics aimed at the public. Both the actions and the training offered to specialists contribute to giving a positive value to the profession of specialists in marine mammals.



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30th March – 3rd April

Acute Lead Intoxication in 5 Bottlenose Dolphins (*Tursiops truncatus*): Clinical Management.

Sánchez-Contreras, G.J.^{1*}; Biancani, B.²; Abarca, J.¹; Gavazza, A.³; Galossi, L.³; Rossi, G.³

¹ Mediterraneo Marine Park, Coast Rd. NXR9038 Bahar ic-Caghaq, Malta gscvetscience@gmail.com

² Costa Edutainment S.p.A., Via Ascoli Piceno 6, 47838 Riccione, Italy

³ School of Biosciences and Veterinary Medicine, University of Camerino, 62024 Matelica, Italy

Acute lead intoxication was diagnosed in 5 bottlenose dolphins (2 adult nursing females, their respective male calves, and a juvenile female). A nursing female and the juvenile dolphin showed sudden anorexia and gastrointestinal discomfort. Complete blood screening was performed. Due to the icteric color of the serum, lead poisoning was considered in the differential diagnosis. Results of the CBC were unremarkable and blood chemistry showed minor alterations in liver parameters and a decrease in the ALKP. Heavy metals panel revealed a high concentration of lead in all animals. Supportive treatment was implemented, and further diagnostics showed the presence of Lead pellets in the first and second stomach. One of the calves had no pellets in the stomach but the highest concentration of lead in his blood. He was considered to have received the lead indirectly through his mother's milk. Chelators were implemented to treat the intoxication among the medical plans. The management of the situation required up to 5 maneuvers a day per animal. Despite this, the three females died within the first month. At this point the calves seem to be thriving. The long term effects of the lead intoxication remain unclear and will need to be monitored.



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30th March – 3rd April

Lead Intoxication in Bottlenose Dolphins (*Tursiops truncatus*): Pathological Findings.

Biancani, B.^{1*}; Sánchez-Contreras, G.J.²; Abarca, J.²; Gavazza, A.¹; Galossi, L.¹; Rossi, G.¹

¹ School of Biosciences and Veterinary Medicine, University of Camerino, 62024 Matelica, Italy.
b.biancani@gmail.com

² Mediterraneo Marine Park, Coast Rd. NXR9038 Bahar ic-Caghaq, Malta. gscvetscience@gmail.com

Five bottlenose dolphins (3 females, 2 males) were diagnosed with an acute lead intoxication and despite clinical efforts, the 3 females died within 10 to 30 days after diagnosis. Necropsies, immediately performed, revealed severe icteric discoloration of sub-cutis, blubber, and internal organs; almost complete absence of blood in the aorta and organs; collection of fluids in the lungs, cerebral edema, signs of gastritis with different grades of ulcerations, splenic hypoplasia, and hyperplasia of adrenal cortex with a reduction of the medulla. Histologically, the most relevant alterations were the increase of pale striations in the myocardium in absence of Zenker's necrosis with unaltered coronary vessels; injured areas with the presence of lead in the liver and kidneys, characterized by the presence of inclusion bodies (IBs). The lungs exhibited focal thickening of the interstitial connective tissue. A Total depletion of the red marrow with severe myelophthisis was observed. In CNS, the most prominent degenerative changes were related to Purkinje cells, associated with small hemorrhages, edema, gliosis, swelling/proliferation of capillary endothelia, interspersed perivascular cuffing, and cerebral neuronal degeneration. Histologic tissue changes can be of considerable diagnostic value in suspected cases, and IBs within renal and hepatic epithelia may be considered diagnostic in dolphins.



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30th March – 3rd April

Suspected immune-mediated hemolytic anemia in a female bottlenose dolphin (*Tursiops truncatus*)

Alvaro, T.^{1*}; Valls, M.¹; Rojo-Solís, C.¹; Barros, C.¹; Garcia-Párraga, D.¹

¹ *Avanqua Oceanogràfic, SL. Ciudad de las Artes y las Ciencias. C/ Eduardo Primo Yúfera (Científic), 1B. 46013 Valencia. Spain. talvaro@oceanografic.org*

Immune-mediated haemolytic anemia (IMHA) is the most common immune-mediated disease in dogs associated with considerable morbidity and mortality. Haemostatic disorders appear to be uncommon in marine mammals, but AIHA and thrombocytopenia were suspected in bottlenose dolphins based on macroscopic agglutination. A 15-years-old female captive-born bottlenose dolphin (*Tursiops truncatus*) was presented in November 2021 with anemia (Hb:10.6mg/dl-Htc:31%) and leukocytosis 12.500cells/ul on a routine blood analysis. Gastrointestinal bleeding was diagnosed, and treatment established with oral antibiotics and antihemorrhagics. Five days later, WBC raised to 30.000cells/ul and hemoglobin dropped to 5.4mg/dl. Reticulocytes, bilirubin, LDH, fibrinogen and ESR increased as well and spherocytes and atypical lymphocytes were detected. Haemoglobinuria and bilirubinuria were presented in urine samples and a slide agglutination test was done with a positive result. Based on these findings IMHA was suspected, and immunosuppressive treatment with prednisolone was started at 1mg/kg OID. A second immunosuppressive drug, cyclosporine 1mg/kg BID, was added to decrease glucocorticoid dosage required. Supportive treatment with omeprazole, antibiotics, antihemorrhagics and liver protectants were also included. No other causes of anemia were identified. Ten days after starting treatment WBC count decreased to 12.000cell/ul and hemoglobin and haematocrit raised to 6.5mg/dl and 19%. Since then, the clinical condition and laboratory tests have been improving, reaching 11.5mg/dl of hemoglobin and 36% of haematocrit.



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Cutaneous lesions associated with a *Staphylococcus delphini* infection in an aged bottlenose dolphin (*Tursiops truncatus*) under human care.

Romani-Cremaschi, U.^{1*}; Rebollada-Merino, A.^{2,3}; Canales-Merino, R.¹; Vargas, I.^{2, 4}; Pérez-Sancho, M.^{2,4}; Sánchez-Vizcaíno, J.M.^{2,4}; Domínguez, L.^{2,4}; Rodríguez-Bertos, A.^{2,3}

¹ *Veterinary Department, Mundomar, Calle Sierra Helada s/n, 03503 Benidorm, Spain. veterinario@mundomar.es*

² *VISAVET Health Surveillance Centre, Complutense University of Madrid, 28040 Madrid, Spain*

³ *Department of Internal Medicine and Animal Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, 28040 Madrid, Spain*

⁴ *Department of Animal Health, Faculty of Veterinary Medicine, Complutense University of Madrid, 28040 Madrid, Spain*

Staphylococcus spp. are widespread bacteria that cause superficial and invasive infections in several species. A 47 years-old, female bottlenose dolphin (*Tursiops truncatus*) displayed recurrent severe focally extensive papular and ulcerative lesions in the skin around the blowhole. Sputum samples were collected for microbiology whereas an ulcerative lesion was collected for viral detection by PCR and histopathology. Bacteriological samples were cultured on agar plates, and all isolates were subjected to a protein–peptide extraction protocol to obtain MALDI-TOF MS profiles. A complete antibiogram was performed on isolates. *Staphylococcus delphini* was identified using MALDI Biotyper software and an antibiogram revealed penicillin G resistance. Fungal culture was positive for *Candida albicans*, deemed as an opportunistic pathogen. Samples were negative for herpesvirus, papillomavirus, and poxvirus. Histopathological findings revealed moderate suppurative dermatitis characterized by neutrophilic infiltration along the basal lamina and multifocally in the epidermis. Immunohistochemistry using a rabbit polyclonal anti-*Staphylococcus* antibody demonstrated immunoreaction in neutrophils and multifocally in epithelial cells. This report describes the gross, histopathological and immunohistochemical findings associated with *S. delphini* cutaneous infection in dolphins. These results may contribute to the understanding of *S. delphini* pathogenesis aside from highlighting the need to monitor antimicrobial resistance to successfully manage these cases.



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SPECIAL THANKS TO: AVELINO MARTÍNEZ, BEA PÉREZ, MAX PARANCHYCH,
AND JUAN LUCES.



European Association for Aquatic Mammals

About the EAAM

The European Association for Aquatic Mammals (EAAM) was founded in 1972. The mission of the EAAM is the welfare and protection of marine mammals through research, good veterinary practice, training, education, conservation, management and all associated activities. EAAM members include veterinarians, biologists, zoo and marine park directors and managers, ethologists and animal scientists, students and others who are dedicated to the welfare and in situ and ex situ conservation of marine mammals.