"A Conference For The Future"

MARCH 11th - 13th

EUROPEAN ASSOCIATION FOR AQUATIC MAMMALS (EAAM)
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"A Conference For The Future"

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"A Conference For The Future"

These last 12 months have been extremely different from what we have experienced in the history of our organization. In 2020 we had to postpone twice our annual symposium and for many colleagues and friends it was a period of great challenge.

It was toward the end of the year that the board was faced with the decision on what to do with the 2021 Symposium and how even plan for it. Following other likeminded association experience under the leadership of our President Elect, the Board launched a quest to organize an online new format annual Scientific Meeting. 2020 was a year filled with unusual events and uncertainty but despite the many challenges many colleagues managed to continue providing excellent care for their animals as well as collecting valuable data. So when the announcement was made that our new meeting was going to be an Online event, "2021 a Conference for the Future", the Board was extremely pleased to see how many outstanding and value filled works were submitted for this new presentation format. We sure missed some of the fun and traditional moments of our annual meeting, but I am happy to have been part of a unique an new format that allowed us to be virtually together, share valuable experiences and even sit at virtual round tables filled with great contents and discussions.

Finally I would like to thank and acknowledge the many people that helped organizing this event including our sponsors and volunteers. A special thank and recognition to our President Elect, Guillermo, who captained this special task force with enthusiasm and many late nights. A new page in EAAM history has been written, and this was a true moment of resilience in face of such unprecedented world challenge. I like to think that our "Conference for the future" will remain as an added value to our ways to foster the EAAM mission, I like to applaud every single people involved with this new meeting format, since it was easier to give in and step back, than face the challenge. The end result was a great success and a true testimony of the power of team work, in this case even when working from remote locations.

Dear colleagues and friends more than ever we need to support each other over the near future, foster our shared passion for science and animal welfare with new tools and experiences. I wish you all a healthy and successful year and I hope to see you all at our next year meeting.

Renato Lenzi
President EAAM

"A Conference For The Future"

Dear Participants, colleagues and friends,

Let me start by saying that it is an honor for me to be part of the EAAM Board. I have to thank you all for the nomination and believing in me. I hope that in these years we can, together, continue developing the association and bring it to the next level.

This year EAAM is presenting a special conference. This edition is taking place as an "online" meeting from March 11th till March 13th. We would have loved to host an "on-site" event, as it was planned for 2020 in Valencia, but we had to adapt to the current pandemic situation.

During the course of these three days we will have International Key Speakers and, of course, Scientific Sessions that will include two new formats: Round Tables and Video Presentations. We have compiled a dynamic and participative programme that we hope you will enjoy.

The Scientific Committee´s role in selecting top quality topics has been outstanding. These topics will demonstrate the importance of combining the knowledge generated by research in the wild and under professional care.

I cannot finish without saying that despite the sleepless nights and the amount of meetings, the experience of organizing this Online Event was great and I hope it will bring us closer, even though virtually.

Thank you for participating in this EAAM 2021 Conference, "A conference for the future".

Guillermo J. Sánchez Contreras
President-Elect EAAM

2021 MARCH 11th – 13th Join Us! Conference

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"A Conference For The Future"

THURSDAY 11TH MARCH 2021

17:30	**Opening**		
		Title	Author
17:50	**Key speaker**	"From nature to zoos and back: how field and captive initiatives can contribute to conserve Amazon´s aquatic mammals"	Miriam Marmontel
18:30	**Sponsor talk**	SEAFOODIA	David Sussmann
18:35	**Scientific session: WELFARE**		
18:40	Oral Presentation	Dolphin-WET (Welfare Evaluation Tool): a protocol for the evaluation of bottlenose dolphins (*Tursiops truncatus*) welfare - part 1	Katrin Baumgartner
18:55	Oral Presentation	Dolphin-WET (Welfare Evaluation Tool): a protocol for the evaluation of bottlenose dolphins (*Tursiops truncatus*) welfare - part 2	
19:10	Oral Presentation	Dolphin Welfare Assessment under Professional Care: 'Willingness to Participate', an Indicator Significantly Associated with Six Potential 'Alerting Factors'	Tania Monreal-Pawlowsky Ruta Vaicekauskaite Fabienne Delfour
19:25	Oral Presentation	First evidence that environmental enrichment could help assessing dolphins' welfare	Ruta Vaicekauskaite
19:40	Oral Presentation	Do marine mammal trainers perceive a bond with the cetaceans they care for?	Thomas Welsh
19:55	Oral Presentation	Visitor attachment to dolphins during an interaction programme: are there behavioural implications?	
20:10	Oral Presentation	D-Track – a method to quantify animal movements. What can it say about the welfare of dolphins?	Patricia Rachinas-Lopes
20:25	**Closing session**		
20:30	Bye		

"A Conference For The Future"

FRIDAY 12TH MARCH 2021

		Title	Moderator
15:00	Round table	**What´s new in dolphin welfare science?** This round table would like to present and discuss various aspects of recent developments in dolphin welfare science, including the following topics: 1. Dolphin-WET by the EAAM welfare committee 2. Recent developments in animal welfare 3. How do/can facilities assess their welfare?	Manuel Garcia Hartmann Katrin Baumgartner
15:00	Round table	**Role of Marine Mammal Parks in advancing research** Studies on marine mammals under human care and on wild populations are critical to understanding these animals' cognition, communication, physiology, and general biology. Both approaches provide valuable information about conservation challenges. This workshop includes an introduction of different published studies on both managed and wild animals. The focus of this round table is to provide insights into experimental science used in managed and wild animals to show how the approaches can complement and inform each other, given the limitations of working with either group. For example, research on managed animals give a level of data independence and experimental control you cannot find in the wild; studies on wild populations, can give an opportunity to evaluate the effect of natural ecology on the biology and behavior of vulnerable groups. Semi-captive work can bridge some differences between the two kinds of study. We aim to bring together a discussion how to transform the vision and philosophy of marine parks that may help provide important information for scientific problems related to conservation, disease, pathology, and welfare; as well as to our basic understanding of animal biology, physiology, behavior, cognition and communication, and recommendations for the best approaches to solve specific conservation issues.	Andreas Fahlman Peter Tyack
17:00	**BREAK**		
17:30	**Welcome to the Session**		

		Title	Author
17:35	**Key speaker**	Synergy between studies in wild and managed populations of marine mammals.	Peter Tyack
18:15	**Sponsor talk**	ANIMAL NECESSITY	Johanna Mejia-Fava

18:20	**Scientific session: SCIENCE / RESEARCH**		
18:25	Short Talk	Why do they yawn? Potential functions of spontaneous yawning in *Otaria flavescens*	Clara Llamazares
18:30	Short Talk	Optimization of a Platelet-Rich Plasma Centrifugation Isolation Protocol for South American sea lions (*Otaria byronia*).	Pablo Morón-Elorza

Continue next page →

→ Continuation

18:35	Short Talk	An Investigation into the Use of Signature Whistles in the Local Subpopulation of Short Beaked Common Dolphins (*Delphinus delphis*) in the Eastern Aegean Sea, Greece	Luana Clementino
18:40	Oral Presentation	Trajectories of Vocal Repertoire Development in Beluga (*Delphinapterus leucas*) Calves: Insights from Studies a Decade Apart	Audra E. Ames
18:55	Short Talk	*Xenobalanus globicipitis* as an indicator of cetacean hydrodynamics: spatial distribution and aggregation on the flukes of striped dolphins, *Stenella coeruleoalba*	Sofía Ten
19:00	Short Talk	Phagocytosis functional assays in the Sarasota Dolphin Health Assessment Project.	Mar Felipo Benavent
19:05	Oral Presentation	Lessons learned from necropsies done on harbour porpoises (*Phocoena phocoena*), live stranded on the Dutch and adjacent coasts from 2003 to 2016	Niels van Elk
19:20	Oral Presentation	Statistically refusing the popular belief that orcas under human care live less than their wild counterpart	Carmen M. Arija
19:35	Oral Presentation	Towards a new conservation strategy for the Franciscana Dolphin (*Pontoporia blainvillei*)	Lorenzo von Fersen
19:50	Short Talk	Non-invasive monitoring of the reproduction in *Tursiops truncatus* males: evaluation of testosterone levels and reproductive behavior in "males only" and in a mixed male-female group	Chiara Trucco
19:55	Video-Presentation	Collection, refrigeration, and subsequent evaluation of dolphin (*Tursiops truncatus*) spermatozoa	María Carmen Fuentes-Albero

20:05	**Closing session**
20:30	Bye

SATURDAY 13TH MARCH 2021

		Title	Moderator
11:00	Round table	**Marine Mammal Nutrition: from the sea to the mouth** Description: As marine mammal caretakers, we need to provide the best food we can to the animals we have under our care. Adequate nutrition is one of the parameters of the animal well-being. The main objective of the nutrition is the health of the animals, to avoid nutritional diseases which can be frequent in zoo animals. Other important considerations when making diets, is the respect of a budget, and the respect of the oceans or long- term stock management. Taken these constraints all together, nutrition is one of the big challenges that our community will have to overcome within the next years. Sitting all around a table and sharing our experiences, our difficulties and our successes, is a first step towards this common goal.	Isabelle Brasseur Theo Alario
13:00	**Break**		
15:00	**Welcome to the Session**		

		Title	Author
15:05	**Key speaker**	Advancements in Diagnostic Ultrasound for Cetacean Medicine, Conservation, and Research	Cynthia R. Smith
15:45	**Sponsor talk**	ST. LAURENT	Carole Juin

15:50 Scientific session: VET SESSION

15:55	Oral Presentation	Preliminary validation of radiographic assessment of skeletal ossification in pectoral fins to estimate chronological age in a wild bottlenose dolphin (*Tursiops truncatus*) population.	Daniel García-Párraga
16:10	Oral Presentation	The use of posaconazole delayed-release tablets and serum drug monitoring in the clinical approach of respiratory mucormycosis in a bottlenose dolphin (*Tursiops truncatus*) calf	Gonçalo Nogueira Marques
16:25	Oral Presentation	Long term ovarian ultrasonography study in killer whales: potential secondary effect of Regumate®	Geraldine Lacave
16:40	Oral Presentation	Lymphoblastic leukemia in a South American sea lion (*Otaria flavescens*)	Carlos Rojo-Solís
16:55	Oral Presentation	Successful treatment through a temporary nictitating membrane flap in Patagonian sea lion (*Otaria flavescens*) with a bullous keratopathy.	Mónica Valls-Torres

Continue next page →

→ Continuation

	17:10	Oral Presentation	Contributions of osteopathy to veterinary medicine through a case of paralysis in a California sea lion (*Zalophus californianus*).	Corinne Godet Geraldine Lacave

17:25	**Closing session**
17:30	**Break**
18:30	**Welcome to the Session**

18:35 Scientific session: TRAINING & VET SESSION

18:40	Video-Presentation	The Sealion Rescue Project	Amy Walton
18:50	Oral Presentation	The beauty of communication between training and research: a chance for understanding energy expenditure while diving in the pacific walrus.	Alicia Borque Diana Ferrero Fernandez
19:05	Video-Presentation	Need a needle?	Antonio Martinez-Sanchez
19:15	Oral Presentation	To go or not to go: Training cognitive tasks across different modalities in bottlenose dolphins (*Tursiops truncatus*)	Tim Huettner
19:30	Video-Presentation	The challenging training process for assessing the energetic cost of whistling in Bottlenose dolphin (*Tursiops truncatus*)	Julieta Arenarez-Lozano
19:40	Short Talk	New diagnostic approaches to detect cetacean Poxvirus: Non-invasive skin sampling procedure and molecular detection of Poxvirus in bottlenose dolphins (*Tursiops truncatus*) under human care.	Simone Segura-Göthlin
19:45	Short Talk	Is gammaherpesvirus a new virus to study in the central nervous system of dolphins?	Ignacio Vargas-Castro
19:50	Video-Presentation	Using spirometry to study respiratory physiology and detect lung disease in the bottlenose dolphin (Tursiops truncatus)	Alicia Borque
20:00	Short Talk	Lungworm communities in cetaceans from the Mediterranean Sea	Rachel Pool

Continue next page →

→ Continuation

20:05	Short Talk	Epibiotic macrofauna of Antarctic minke whale, *Balaenoptera bonaerensis* Burmeister, 1867, in the Southern Ocean	Sofia Ten
20:10	Short Talk	First steps towards a stranding response network in Cantabria, Spain.	Manena Fayos-Martinez
20:15	Short Talk	Characterization of Microbial Composition of Different Body Sites of Two Groups of Bottlenose Dolphins (*Tursiops truncatus*) and their controlled environment	Guillermo J. Sanchez-Contreras
20:20	Short Talk	Updates on the case of a Hepatocellular carcinoma in a South American sea lion (*Otaria flavescens*)	
20:25	Short Talk	First report of striped dolphin (*Stenella Coeruleoalba*) strandings due to dolphin morbillivirus In Greece	Manuel Garcia-Hartmann
20:35	**Closing Ceremony**		
21:00	Bye		

Oral Presentation	12 minutes talk + 3 minutes questions
Short Talk	5 minutes
Video Presentation	10 minutes video
Key Speaker	40 minutes
Round Tables	2 hours

*Note: Sessions will be recorded and will be available on demand for a limited time after the conference.

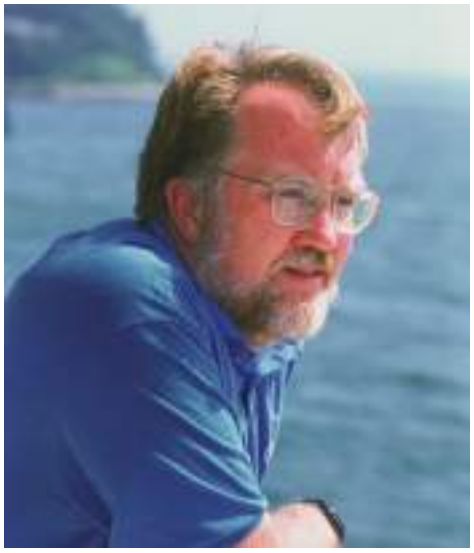
Key Speakers

Dr. Miriam Marmontel

"From nature to zoos and back: how field and captive initiatives can contribute to conserve Amazon´s aquatic mammals"

Thursday 11th March 2021 – 17:50h

BIO: Dr. Marmontel is a Brazilian oceanographer who left the saltwater to dedicate her life to freshwater mammals in the Amazon. She got a MSc from University of Miami and a PhD from University of Florida, working with manatees. Upon completing her studies she moved to Tefé in the Brazilian Western Amazon, where she leads a research group on all five genera of aquatic mammals from the region (manatees, dolphins and otters).



Prof. Dr. Peter Tyack

"Synergy between studies in wild and managed populations of marine mammals"

Friday 12th March 2021 – 17:35h

BIO: Professor of Marine Mammal Biology, Sea Mammal Research Unit, Scottish Oceans Institute, School of Biology, University of St Andrews. Prof. Tyack is a behavioral ecologist who studies acoustic communication and social behavior in marine mammals in the wild and in managed settings. Tyack worked at the Woods Hole Oceanographic Institution until 2011 when he moved to the University of St Andrews in Scotland. Tyack has studied the role of vocal learning in individually distinctive contact calls in dolphins both in the wild and under managed care and in reproductive advertisement displays (songs) of wild baleen whales. Working with marine mammal bioacoustics at sea, it was always obvious to him that anthropogenic sound was ubiquitous. Observations of the prevalence of anthropogenic sound in the oceans, coupled with his appreciation of how important sound is for marine mammals led him to be concerned about the impact of anthropogenic sound on marine mammals. Tyack has served on the US Federal Advisory Committee on Acoustic Impacts on Marine Mammals, has advised international, EU and US government agencies on this topic, and has testified to the US Congress on the Marine Mammal Protection Act.

Dr. Cynthia Smith

"Advancements in Diagnostic Ultrasound for Cetacean Medicine, Conservation, and Research"

Friday 13th March 2021 – 15:05h

BIO: Dr. Smith, DVM, is the Executive Director of the National Marine Mammal Foundation (NMMF), a nonprofit organization based in San Diego, California, USA. The NMMF's mission is to improve and protect marine mammal, human, and ocean health through science, service, and education. She also serves as Chief Medical Officer, providing her veterinary expertise for high-priority marine mammal projects, specifically for at-risk, threatened and critically endangered marine mammals. She has more than 20 years of veterinary and research experience, resulting in over 100 peer-reviewed manuscripts and published proceedings. She contributed as a lead veterinarian for NOAA's Natural Resource Damage Assessment of the Deepwater Horizon (DWH) oil spill's impact on marine mammals. She also served as the General Program Manager for the Consortium of Vaquita Conservation, Recovery, and Protection (VaquitaCPR), an effort aimed at rescuing the endangered vaquita porpoise from extinction. Additionally, her team provides veterinary, research, and animal care support to the US Navy's Marine Mammal Program. She understands the importance of giving back locally and globally through medical expertise and high-quality research to improve conservation efforts worldwide.

ROUND TABLES

What´s new in dolphin welfare science?

This round table would like to present and discuss various aspects of recent developments in dolphin welfare science, including the following topics:

1. Dolphin-WET by the EAAM welfare committee
2. Recent developments in animal welfare
3. How do/can facilities assess their welfare?

Moderator	Info	Photo	Contact
Manuel García Hartmann	Certified specialist in zoo and wild animal medicine (German title, not de facto) President of Marlab, a non-profit laboratory for research aimed to promote conservation Director of Platypus, a laboratory only for wild, zoo and exotic animals Independant consultant on wild and zoo animals.		manuel@zoo-vet.de
Katrin Baumgartner	DVM by the University of Bologna. Veterinarian and curator of Nuremberg Zoo since 1996. She is specialized in zoo and wild animal medicine and animal welfare. She is the TAG Vet advisor for manatees and African buffalos. She is the chair of the EAAM-Welfare Committee and the chair of the VZT-Population Management Working Group.		Katrin.Baumgartner@stadt.nuernberg.de

Role of Marine Mammal Parks in advancing research

Studies on marine mammals under human care and on wild populations are critical to understanding these animals' cognition, communication, physiology, and general biology. Both approaches provide valuable information about conservation challenges. This round table includes an introduction of different published studies on both managed and wild animals. The focus of it is to provide insights into experimental science used in managed and wild animals to show how the approaches can complement and inform each other, given the limitations of working with either group. For example, research on managed animals give a level of data independence and experimental control you cannot find in the wild; studies on wild populations, can give an opportunity to evaluate the effect of natural ecology on the biology and behavior of vulnerable groups. Semi-captive work can bridge some differences between the two kinds of study. We aim to bring together a discussion how to transform the vision and philosophy of marine parks that may help provide important information for scientific problems related to conservation, disease, pathology, and welfare; as well as to our basic understanding of animal biology, physiology, behavior, cognition and communication, and recommendations for the best approaches to solve specific conservation issues.

Moderator	Info	Photo	Contact
Andreas Fahlman	Dr. Andreas Fahlman is a comparative physiologist whose research projects revolve around the central question of how animals function in challenging environments. His research efforts have included laboratory and field studies in North and South America, Europe, and Africa, from Arctic to Antarctic regions. He is currently an independent researcher working for the Fundación Oceanografic in Valencia, Spain.		afahlman@whoi.edu
Peter Tyack	Professor of Marine Mammal Biology at the University of St Andrews. Proff. Tyack is a behavioral ecologist who studies acoustic communication and social behavior in marine mammals in the wild and in managed settings. Tyack has studied the role of vocal learning in individually distinctive contact calls in dolphins both in the wild and under managed care and in reproductive advertisement displays (songs) of wild baleen whales. Working with marine mammal bioacoustics at sea, it was always obvious to him that anthropogenic sound was ubiquitous. Tyack has served on the US Federal Advisory Committee on Acoustic Impacts on Marine Mammals, has advised international, EU and US government agencies on this topic, and has testified to the US Congress on the Marine Mammal Protection Act.		plt@st-andrews.ac.uk

Marine Mammal Nutrition: from the sea to the mouth

Description: As marine mammal caretakers, we need to provide the best food we can to the animals we have under our care. Adequate nutrition is one of the parameters of the animal well-being. The main objective of the nutrition is the health of the animals, to avoid nutritional diseases which can be frequent in zoo animals. Other important considerations when making diets, is the respect of a budget, and the respect of the oceans or long- term stock management. Taken these constraints all together, nutrition is one of the big challenges that our community will have to overcome within the next years. Sitting all around a table and sharing our experiences, our difficulties and our successes, is a first step towards this common goal.

Moderator	Info	Photo	Contact
Isabelle Brasseur	She is a Biologist and became a dolphin trainer in 1995 at Boudewijn Seapark. In 1999, she got hired at Marineland Antibes as a killer whale trainer, and in 2019, she became responsible for the Education, Research and Conservation department. She strives for raising visitors´ awareness about marine biodiversity, to valorize the daily work, the skills and the knowledge of professionals like you and me, to participate to scientific researches and to support conservation and in situ marine animals' populations thanks to our expertise and funds.		i.brasseur@marineland.fr
Theo Alario	Theo, Sales Manager Zoo & Aquarium - Seafoodia.		talario@seafoodia.com
Geraldine Lacave	Head of MMVS. She has 30 years of experience as a marine mammal veterinary consultant overseeing many aquaria and marine parks worldwide. She has devoted her carrier to the promotion of medical training and state of the art preventive health care programs, working very closely with trainers in the development of good husbandry procedures for the animals in human care. She has also a strong veterinary expertise in reproduction, ultrasound and pinnipeds anesthesia and gives regularly workshops on marine mammal subjects. Past President of EAAM, past Vice-President of IMATA and presented numerous papers over the years in scientific conferences.		Geraldine.lacave@icloud.com

Abstracts

Dolphin-WET (Welfare Evaluation Tool): a protocol for the evaluation of bottlenose dolphins (*Tursiops truncatus*) welfare

Baumgartner K.[1]*, Clegg I.[2], Garcia-Párraga D.[3], Delfour F.[4], Garcia Hartmann M.[5], Mercera B.[4], Monreal-Pawlowsky T.[6], Pilenga C.[7], Tallo-Parra O.[8], von Fersen L.[1], Vaicekauskaite R.[9], Manteca X.[8]

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[2] *Animal Welfare Expertise, Sydney, Australia.*
[3] *Fundación Oceanografic, Eduardo Primo Yufera 1B, 46013 Valencia, Spain*
[4] *Parc Asterix, BP8, 60128 Plailly, France.*
[5] *MarLab, Place du Chateau 7, Mougins, France.*
[6] *International Zoo Veterinary Group, Keighley, UK.*
[7] *Zoomarine Italia, Via Casablanca 61, Pomezia (Rome), Italy.*
[8] *Zoo Animal Welfare Education Centre, School of veterinary Science, UAB, Spain*
[9] *Klaipeda University, Lithuania.*

The need for research regarding the objective assessment and monitoring of animal welfare is a central issue within Zoos and Aquaria. In response to this demand the Welfare Committee of the European Association for Aquatic Mammals (EAAM) started the development of a protocol to scientifically evaluate the welfare of bottlenose dolphins. This protocol integrates multidimensional aspects of welfare and contains a hierarchical structure known from the Welfare Quality®, following: Overall welfare assessment > Principles > Criteria > Sub-criteria > Welfare indicators. The present protocol prioritizes animal-based indicators and does not allow compensations between Principles or between Criteria or Sub-Criteria from the same Principle. Where one section is missing or failing, the upper-related level cannot be considered optimal or fulfilled and will need to be addressed. Dolphin-WET is intended to assess the welfare status of the dolphins on a regular basis by combining animal and resource based validated welfare indicators. The aim of the protocol is to provide a tool for the evaluation, protection, and improvement of dolphins´ welfare under human care. It could be used as regular welfare monitoring tool, including identification of welfare concerns, and daily welfare management. The protocol is currently being developed and will be implemented in animals and resources of EAAM members.

Dolphin Welfare Assessment under Professional Care: 'Willingness to Participate', an Indicator Significantly Associated with Six Potential 'Alerting Factors'

Monreal-Pawlowsky T.[1]*, Vaicekauskaite R.[2*], Pilenga C.[3], Garcia-Parraga D.[4], Rödel H.G.[5], García Caro N.[6], Perlado Campos E.[7], Mercera B.[8], Delfour F.[5,8*]

[1] *International Zoo Veterinary Group, West Yorkshire BD21 4NQ, UK; T.monreal@izvg.co.uk*
[2] *Klaipeda University, H. Manto 84, 92294 Klaipeda, Lithuania; ruta@fox-zooconsulting.com*
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[6] *Barcelona Zoo, 08003 Barcelona, Spain; encmmarins@bsmsa.cat*
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[8] *Parc Asterix, 60128 Plailly, France; birgitta.mercera@parcasterix.com*

In dolphinaria, dolphins and their trainers build relationships and bonds due to the nature, closeness and repeatability of their interactions, hence training sessions are deemed appropriate to evaluate dolphin welfare. Qualitative Behavioural Assessments (QBAs) have been used to study human–animal relationships and are included in several animal welfare assessments. We introduce here the first QBA aiming to analyse dolphin–trainer interactions during training sessions in terms of dolphin welfare. Our results show that "Willingness to Participate" (WtP) was significantly associated to six other parameters: high-speed approach, high level of excitement, high number of positive responses to trainers' signals, rare refusal to perform certain behaviours, rare spontaneous departure behaviours and fast approach once the trainer entered into the pool. Therefore, we suggest using WtP and those "alerting factors" when assessing dolphin–trainer interactions under professional care. The evaluation should also consider the time of day, the dolphin's age, trainer experience level, the nature of the training sessions and to a lesser extent the sex of the dolphins, as contributing and modulating factors. These results demonstrate the pertinence and feasibility of this approach, the ease of use of this methodology by professionals in zoo/aquarium settings and the appropriateness of the obtained results within the holistic frame of animal welfare.

First evidence that environmental enrichment could help assessing dolphins' welfare

Vaicekauskaite R.[1]*, Livet J.[2], Monreal-Pawlowsky T.[3], Delfour F.[4,5]

[1] *Marine Research Institute, Klaipeda University, Lithuania, delfinruta@gmail.com*
[2] *Fox Consulting, Haguenau, France*
[3] *International Zoo Veterinary Group, Keighley, United Kingdom*
[4] *Laboratoire d'Ethologie Expérimentale et Comparée E.A. 4443, Université Paris 13, France*
[5] *Parc Asterix, Plailly, France*

Studies of animal welfare indicators have increased recently and environmental enrichment (EE) could be considered an important welfare parameter; although this remains to be scientifically proved.

Bottlenose dolphins under human care display behaviours and cognitive abilities that could be linked to different affective states and welfare status. We present the first evidence of EE as a possible tool to evaluate dolphins' behaviour and attitude changes. We also discuss interindividual associations in the general context of dolphins' daily management while assuring good welfare. We scored the dolphins' attitude using a straightforward method applicable by care staff.

Results highlighted the important role of interactions with familiar humans and showed attitude changes during educational presentations after EE sessions. Results showed a positive correlation between the use of EE and dolphin responses to asked behaviours; when dolphins paired during presentation were interacting together during the precedent enrichment session, these individuals were significantly more motivated and showed positive attitude.

We suggest considering the importance of evaluating not only the parameters of each item but also the many aspects encompassed in EE sessions. Animal attitude scoring could help enhancing EE strategies and might be a relevant criterion to include in animal welfare assessment under human care.

Do marine mammal trainers perceive a bond with the cetaceans they care for?

Brando S.[1], Welsh T.[2,3]*, Ward S.J.[3], Hosey G.[4]

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The possibility of a human-animal bond (HAB), which can be interpreted as a particularly good human-animal relationship that is reciprocal and promotes wellbeing in both parties, has largely been restricted to companion animals. Few studies have investigated the possibility of HABs occurring in zoo animals, with none examining the possibility of a HAB between a trainer and the marine mammals in their care. This study aimed to investigate if marine mammal trainers perceived themselves as having a bond with the cetaceans they cared for. A modified Lexington Attachment to Pets Scale (LAPS) was electronically distributed to holders of cetaceans worldwide. Responses were obtained from 131 trainers from 33 institutions across a range of countries. 90% of trainers reported a bond with an animal they worked with. Female trainers scored significantly higher than male trainers, suggesting gender is a factor in the strength of attachment. An exploratory factor analysis extracted three factors from the responses to the modified LAPS, which were "personal wellbeing", "people substituting" and "animal welfare". This demonstrates that marine mammal trainers gain a sense of positive wellbeing by interacting with individual animals that they care for, which can have positive implications for job satisfaction. For this perceived attachment to be considered a HAB, further research is required investigating dyadic relationships and their implications on both the trainer and the individual animal.

Visitor attachment to dolphins during an interaction programme: are there behavioural implications?

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Millions of people visit zoos and aquariums globally each year, with some participating in animal interactions that allow closer contact with individual animals. The learning experience in zoos has been well studied; demonstrating that forming emotional connections and memorable experiences with animals are effective learning tools for conservation education. Human-animal interactions (HAIs) during these programmes are generally positive experiences for the human participants, however are there implications for the individual animals involved? This research aimed to investigate visitor attachment to the Bottlenose dolphins *(Tursiops truncatus)* they interacted with, whilst assessing possible behavioural implications. Here, we surveyed 41 visitors to a Spanish dolphinarium who participated in an interaction, using a modified version of the Lexington Attachment to Pets Scale (LAPS) to assess the visitors' sense of attachment to the dolphin. Alongside this, 96 15-minute continuous focal sampling behaviour observations were carried out for three female dolphins aged 22 - >40, split into pre, during and post interaction. 80% of visitors reported forming a bond with the dolphin. An exploratory factor analysis extracted three factors from the survey; these were "relationships", "emotional attachment" and "non-attachment". A Friedmans Two-Way ANOVA produced significant results for some behaviour categories for each individual, including locomotory (D1: F_2=9.556, $p<0.01$), rest (D2: F_2 =14, $p<0.01$, D3: F_2=10.889, $p<0.01$) and individual play (D1: F_2=11.677, $p<0.01$ D2: F_2=6.353, $p<0.05$). However, pairwise comparison showed no differences pre-post interaction. Therefore, it can be implied that for these dolphins, participating in the HAI was neither enriching nor aversive for them. As visitors reported a sense of attachment post HAI, this could have applications in improving conservation education. This study has provided scope for research into methods facilities can use to utilise the emotional attachment developed to individual animals to facilitate learning about conservation issues.

D-Track – a method to quantify animal movements. What can it say about the welfare of dolphins?

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The D-Track is an open-source, semi-automatic tracking system able to quantify the 3D trajectories of dolphins, non-invasively, in the water. Using two video cameras above the pool, this software produces a three-dimensional reconstruction of the pool and tracks one animal at different depths, allowing the determination of spatial preferences, speeds and the identification of movement routines. The system was implemented separately with two dolphins during different periods of the day. The results showed that both animals spent around 85% of the time at the surface of the deep area of their pool (5- meters depth) and had stable average speed throughout 31 sessions, with slow speeds predominant (maximum 1.7 ms-1). Circular swimming was highly variable, with significant differences in the size and duration of the "circles". Having a baseline of the normal movements of captive dolphins facilitates the detection of bizarre behaviours that may emerge. Stereotyped movements and bizarre behaviours are one of the major welfare concerns of trainers and facilities holding captive animals, and such a tracking system may be very useful.

Why do they yawn? Potential functions of spontaneous yawning in *Otaria flavescens*

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Yawning is a multifunctional behaviour observed in most vertebrate species. Here, we tested for the first time some hypotheses regarding the potential functions of spontaneous yawning in a marine mammal species, the South American sea lion (*Otaria flavescens*). The performance of yawning was context-dependent, being higher during resting/sleeping contexts (*Drowsiness Hypothesis* supported). When assessing the distribution of this behaviour according to the sex of the subjects, we observed that it was equally distributed in both sexes, supporting the *Dimorphism Hypothesis* postulating that species with low levels of sexual dimorphism in canine size do not show sex differences in yawning distribution. The immediately increase of yawning and self-scratching after conflicts in both aggressors and victims demonstrates that both behaviours are reliable indicators of short-term anxiety in sea lions (*Social Distress Hypothesis* supported). Moreover, the lack of correlation between yawning and the subjects' dominance status shows that long-term anxiety was similarly experienced by subordinates and dominants. These last two findings can be explained by the social competition of the species involving individuals independently from their age, sex or ranking status leading to an equivalent behavioural distribution in response to short- and long-term anxiety. Our findings unveil that spontaneous yawning in sea lions share similar functions with other mammals, suggesting the plesiomorphic nature of this behaviour.

Optimization of a Platelet-Rich Plasma Centrifugation Isolation Protocol for South American sea lions (*Otaria byronia*).

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Accelerated wound healing in wild or captive sea lions is a key tool to help minimize infection and complications associated with open wounds. PRP is an autogenous source for Growth Factors, obtained by centrifuging whole blood. Currently, there are well defined PRP isolation protocols for humans and most companion animal species. However, there is no clear centrifugation protocol for PRP obtention in most marine-mammal species. This study aimed the comparison of platelet concentration in plasma centrifuged using centrifugation speeds ranging from 700 to 1500 rpm at centrifugation times ranging from 3 to 6 min. Blood was drawn from 5 adult sea lions, 6 ml of each, which were then divided into 1 ml sodium citrate tubes and centrifuged following the different centrifugation protocols. PRP was designated as the lower third fraction of the centrifuged plasma. Platelet counts were performed using flow cytometry and statistical analysis was carried out in order to stablish a well-defined protocol for efficient PRP isolation. PRP electron microscopy analysis was performed in order to evaluate a possible platelet degranulation during centrifugation. Absolute number of platelets in PRP significantly increased compared to whole blood and significant differences were found between the different centrifugation speeds and times.

An Investigation into the Use of Signature Whistles in the Local Subpopulation of Short Beaked Common Dolphins (*Delphinus delphis*) in the Eastern Aegean Sea, Greece

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Delphinid species live in fluid societies. First described in bottlenose dolphins (*Tursiops truncatus*), unique frequency modulated whistles (signature whistles, SW) have been putatively linked to the fluid delphinid social structure. Limited research exists on the presence of SWs in other delphinid species; hence the current study investigated the potential occurrence and purpose of SWs by common dolphins (*Delphinus delphis*). SWs were defined as three or more subsequent whistles of the same shape, duration and frequency range, produced with less than two seconds interval. Additionally, group size and social structure were looked at, when SWs were produced. A total of six SW types were categorized. There was no significant difference between the number of adults and juveniles found in the pods when SWs occurred. Significantly more calves were present when SWs were absent compared to when present. Furthermore, total group size was significantly larger when there was no production of SWs. Initial findings suggest SWs are mainly produced in smaller groups with no calves, yet, further sampling is required to explore this relationship. Nonetheless, it is a starting point on the function of SWs with evidence of interspecies, and potentially intraspecies, variability.

Trajectories of Vocal Repertoire Development in Beluga (*Delphinapterus leucas*) Calves: Insights from Studies a Decade Apart

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There is a shortage of literature regarding beluga vocal development as there has been only one published study to date. Here we offer the second longitudinal study on beluga vocal development, comparing the vocal progression of our calf, Kylu, to the previous research. We also report on changes in acoustic energy distribution and source levels of early calf sounds for the first time. From his day of birth, Kylu produced broadband pulse trains with upper frequency limits past our study's Nyquist cutoff (128 kHz); higher than what was reported by the previous study, limited by lower sampling rates. Pulse repetition rate, source level, and third quartile frequencies of Kylu's pulse trains increased significantly over his first month. First and third quartile, center, and peak frequencies, pulse repetition rate, and call duration increased significantly over his first year. Pulsed signals were his most common sound type during the first year, while mixed calls and tonal sounds were not regularly produced until later in the first year of life. Kylu developed a contact call most similar to his mother's, as found in the previous study. Through comparing beluga calves and employing new technology, we reveal parallels in development and provide new information.

Xenobalanus globicipitis as an indicator of cetacean hydrodynamics: spatial distribution and aggregation on the flukes of striped dolphins, *Stenella coeruleoalba*

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Xenobalanus globicipitis is an epibiont of cetaceans that typically attaches on the edge of fins. Recent evidence showed that its distribution on the tail flukes of striped dolphins, *Stenella coeruleoalba*, was non-random; most barnacles occurring on the dorsal side, and the central third, of the trailing edge of the flukes. These patterns were interpreted based on functional demands regarding barnacle structural integrity and/or filtering performance. The present study provides evidence that this seemingly preferential use of some fluke areas may actually be a consequence of dolphin hydrodynamics. We performed an analysis of the distribution and aggregation of *X. globicipitis* on the flukes of 55 Mediterranean striped dolphins, and interpreted the patterns obtained using published evidence on swimming dolphin models based on computational fluid dynamics. Nearest neighbor analysis strongly suggested that new barnacle recruits actively seek placements along the trailing edge beside already settled individuals, presumably to facilitate copulation. However, the gross distribution of *X. globicipitis* on the flukes was directly related with cetacean hydrodynamics, preferentially settling on areas of higher pressure and lower shear stress. Accordingly, these results suggest that *X. globicipitis* could be used as a natural tag to shed light on cetacean hydrodynamics.

Phagocytosis functional assays in the Sarasota Dolphin Health Assessment Project.

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In the context of oceans increasingly contaminated by humans, there is a growing interest in understanding the effects of these pollutants in marine mammals. With this objective, our group participated in the project Sarasota Dolphin Health Assessment, in Florida (USA), in order to detect differences in the immune status between dolphins under human care and wild dolphins, exposed to different contaminants present in the Bay.

The staff involved in this project throughout the year, identify the dolphins and monitor the group and once a year, veterinarians and researchers from around the world participate in the health assessment of the animals, capturing for a brief period of time the individuals of interest, performing veterinary tests and taking samples or measurements for different research projects. In our case, we obtained heparinized blood samples and we studied the phagocytic capacity of the animals, in the same way that we do with our control population of healthy dolphins inhabiting in the Oceanogràfic Aquarium of Valencia. After the sampling, the animals were released to the sea. After 10 days of work in the boat, 16 wild dolphins have been checked and their phagocytes functionality were assessed and compared with the animals under human care results.

Lessons learned from necropsies done on harbour porpoises (*Phocoena phocoena*), live stranded on the Dutch and adjacent coasts from 2003 to 2016

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Autopsies on stranded cetaceans are a valuable opportunity to assess health problems in cetacean species. We carried out 61 autopsies on live-stranded harbour porpoises, which died following admission to a rehabilitation centre (SOS Dolfijn) between 2003 and 2016. We were able to assign probable causes for stranding based on clinical and pathological criteria. The main causes of stranding were similar as observed by previous research: pneumonia and separation of mother dependent juveniles. We observed most animals suffered from lesions in multiple organs whereas multiple previous published surveys listed single causes of death. We found a much higher prevalence of aspergillosis than was observed previously. The implication of this finding is that the harbour porpoises we investigated suffered from an impaired immunocompetence.

The purpose of this survey was to make an overview of current health problems in harbour porpoises in the North Sea. Impairment of the immune resistance due to persistent organic pollutants is a potential and widely feared threat to the species. If we are to note changes in health problems that might be the result of impaired immunity then we must repeat regularly necropsy overviews and report in a standardized extensive manner so reports can be compared and analyzed fruitfully.

Statistically refusing the popular belief that orcas under human care live less than their wild counterpart

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Currently it is essential to have valid statistical information about cetacean survivorship under human care. Thus, we have compared the annual survival rates of wild and captive orcas using the method developed by Trent and Rongstad in 1974 and widely used later. Our data is based on information from the Center for Whale Research for wild orcas of the North Pacific (1998-2019), the Marine Mammal Inventory Report (1964-2011) and current news (2011-2019) about captive orcas. To be able to use this methodology, we had to use information of animals that have lived at least for 1 year and data from facilities that comply with the unit of 10.000 animal-day. 128 wild orcas, 147 captive orcas, 4 wild pods and 15 zoological parks were included. Results showed that there is not significant differences in the annual survival rates of wild and captive animals (F=1.16 p=0.2971), and comparing facilities from different continents, only Asian parks present annual survival rates smaller than wild pods (significantly difference of 0.036335 in the test of multiple comparisons of means). According to this, we can statistically refuse the popular belief that orcas hosted in European and American parks live less than their wild counterpart.

Towards a new conservation strategy for the Franciscana Dolphin (Pontoporia blainvillei)

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The Franciscana Dolphin (*Pontoporia blainvillei*) is regarded as the most endangered small cetacean species in South America. Its distribution is confined to coastal waters of Argentina, Brazil and Uruguay. The main threat is bycatch in gillnet fisheries, taking 3-6^{+}%/year of some of the populations. In the last decades several In Situ Conservation strategies have been put into practice in order to improve the conservation status of the species. However, abundance estimation in some areas has shown that the population is still declining. Last November 45 biologists, veterinarians and population managers met in Argentina in order to evaluate the use of new conservation tools to preserve the Franciscana. During the talk some of these tools will be presented and discussed.

Non-invasive monitoring of the reproduction in Tursiops truncatus males: evaluation of testosterone levels and reproductive behavior in "males only" and in a mixed male-female group

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Marine mammals' conservation and concern for their welfare under human care are orientating researches towards cetaceans' reproductive physiology and ethology.

The aim of this study is to analyse the seasonal testosterone and progesterone fluctuations in Tursiops truncatus and to investigate a possible correlation between testosterone and the occurrence of aggressive interactions.

Sixteen bottlenose dolphins divided between a mixed group of male and female subjects, and a "males only" group, were sampled with non-invasive blow sampling between March and October. Hormone analyses were conducted with an enzyme immunoassay kit. In order to describe the incidence of aggressions, behavioural observations were organized between July and September. In parallel, tooth rake marks inflicted by conspecific were used to examine the relative rates of received aggression.

Although sample sizes were small, the results provide the expected hormonal trends for both testosterone and progesterone. Then, according to the observed patterns, the study supported but did not provide conclusive evidence for the absence of a correlation between testosterone and aggression, suggesting that testosterone is not directly connected to the aggressiveness in dolphins. Other factors, such as social and environmental aspects, could be responsible for the increase in agonistic interactions. Further studies are needed to confirm these hypotheses.

Collection, refrigeration, and subsequent evaluation of dolphin (*Tursiops truncatus*) spermatozoa

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Semen collection for reproductive studies in wild animals is a challenging procedure limited in most cases to capture/sedation and/or electroejaculation or postmortem gamete rescue. Trained animals under human care allow us to obtain essential information regarding their reproductive behavior in a holistic view.

Bottlenose dolphin sperm collection and further analysis at the Oceanografic is of relevance to evaluate the general health and reproductive status of the animals. Sperm samples are obtained periodically through the standard approach for semen collection in cetaceans, placing the animal in a dorsal recumbency and indicating the behavior with a hand signal. Manual stimulation is made to finalize the behavior, which occurs almost immediately after.

The ejaculates collected have been used for gamete cryopreservation and to develop a new effective method optimizing fresh semen refrigeration for further artificial insemination. The effect of temperature (5 ºC vs. 15 ºC), use of diluent and sperm concentration (20x106 vs. 100x106 spermatozoa/ml) on sperm quality and stability was evaluated during 7 days of storage. Dolphin semen can be refrigerated for a short to medium period of storage maintaining better functionality when stored in BTS commercial media at 100x106 spermatozoa/ml at 5ºC.

Preliminary validation of radiographic assessment of skeletal ossification in pectoral fins to estimate chronological age in a wild bottlenose dolphin (*Tursiops truncatus*) population.

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We recently published a new non-invasive technique to accurately determine the age of common bottlenose dolphins (*Tursiops truncatus*) via pectoral radiography. The predictive algorithms and curves were based on bone maturation scoring of 16 anatomical regions of the pectoral fin, from a database of 126 radiographs from 94 managed care dolphins of known chronological age and medical history. Precision oscillated within 3 months in animals < 5 years old, and within 5 years in animals > 30 years old. Age estimation in wildlife conservation is an important diagnostic tool in the interpretation of biological data, necropsy examination, environmental contaminant accumulation, reproductive status and population demographics, however it was unclear if the present study could be directly applicable in wild populations. For a preliminary validation, we obtained flipper radiographs of 6 wild individuals (3 males and 3 females) from Sarasota Bay, Florida. This population has been closely monitored by photo-identification over the last 50 years, enabling known age of most individuals. Wild individuals accurately fit the predictive curves/algorithms calculated based on managed counterparts, suggesting this new age determination technique could be easily validated in wild populations, minimizing invasiveness and increasing accuracy compared to previous techniques based on dental extraction.

The use of posaconazole delayed-release tablets and serum drug monitoring in the clinical approach of respiratory mucormycosis in a bottlenose dolphin (*Tursiops truncatus*) calf

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Posaconazole is an azole antifungal drug, available as an oral suspension and the more recent delayed-release tablets and intravenous formulation. Although there is a lack of pharmacokinetic studies conducted in cetaceans, posaconazole (5 mg/kg BID of the oral suspension) seems to be the drug of choice when dealing with mucormycotic fungal infections, given the overall good clinical results in the few successful cases described in the literature.

As we presented last year, a one-year-old bottlenose dolphin calf (*Tursiops truncatus*) was diagnosed with respiratory mucormycosis and posaconazole tablet treatment (10 mg/kg SID) was prescribed. There was a good overall response to treatment and antifungal therapy was discontinued. However, the infection relapsed and posaconazole tablets were reinitiated.

As part of a master's thesis, serum drug concentrations were retrospectively analysed in order to evaluate the possibility of subtherapeutic concentrations as the cause of relapse. As described in human studies and in contrast to the oral suspension, the delayed-release tablet formulation resulted in more stable and significantly higher concentrations (reaching levels of 11.63 mg/L). The promising results of the posaconazole tablets therapy and the lack of severe side effects may support the future use of this newer formulation in dolphins.

Long term ovarian ultrasonography study in killer whales

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Daily, alternated-day or bi-weekly ovarian ultrasounds (General Electric – Logig e BT9, BT12 & Logiq V2 with a 2,5-5MHz convexe probe) have been performed for at least 7 years in 3 female killer whales (*Orcinus orca*) kept at the Loro Parque facility in Tenerife. To render the study possible, 12 killer whale trainers were taught how to recognize killer whale's ovaries by ultrasound and record the data. Since 2012, about 4500 sessions with over 32.000 ovarian ultrasound videos have been obtained. The review of the data has permitted to identify antral follicles of much smaller sizes than previously described[1] and the confirmation of their presence in ovaries both with or without Regumate®. Once growing follicles were identified they were tracked daily throughout their entire cycle and up to 3 times per day allowing for a detailed visual follow-up. Corpus albicans' scars could consistently be seen long-term post ovulation, a structure not yet reported in marine mammals' ultrasounds. In this study, the following irregularities and/or pathologies have been identified post Regumate® removal: shorter cycles, longer cycles, delayed cycles, prolonged anoestrus (one animal), development of follicular cyst, retained corpus luteum and spontaneous galactorrhea. Future research will correlate endocrine data from daily urine samples taken during the time lapse of this study.

Lymphoblastic leukemia in a South American sea lion (*Otaria flavescens*)

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A 4 years-old captive-born, South American sea lion (*Otaria flavescens*) was presented on October 2019 with acute leucocytosis and a total lymphocyte count of 40.000 cells/ul on a routine blood analysis. Intermittent loose stools over the last months was the only clinical sign observed. Oral antibiotics were started while receiving results from viral PCR and serology, which were negative. White blood cell count raised to 199.000 cells/ul in 10 days. Based on blood count and blood smears revealing a large lymphoid cell population, an acute lymphoid neoplasia (leukaemia) was suspected and treatment was initiated with prednisone (2 mg/kg PO SID) and cyclophosphamide (500 mg PO once a week). Initial response to therapy was rewarding with a decrease in white blood cell count to 9.600 cells/ul after a week of treatment but as leucocytosis reoccurred a week later, L-asparaginase (20000 IU SQ) was added. Leucocytosis resolved with this treatment, but hepatic enzymes continued increasing. Clinical condition of the animal severely worsened after a month of treatment and euthanasia was performed. On necropsy, hepato-splenomegaly and generalized lymph node enlargement were observed. Histology showed large numbers of immature and atypical lymphocytes expanding the hepatic and splenic sinusoids, confirming the diagnosis of lymphoblastic leukaemia.

Successful treatment through a temporary nictitating membrane flap in Patagonian sea lion (*Otaria Flavescens*) with a bullous keratopathy.

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A 9-yr-old male Patagonian sea lion with previous history of gastroenteric signs presented sudden ocular affection including corneal opacity, blepharospasm and a severe bullous keratopathy. Preliminary ophthalmic examination suggested a descemetocele of potential traumatic origin.

Nictitating membrane flap was decided as temporary treatment to protect the affected eye. Water access restriction, systemic and topical therapy were also established to prevent bacterial ocular complications and reduce pain.

Nine days later, the other eye rapidly developed similar bullous corneal changes. This observation ruled out traumatic etiology in favor of other potential factors as causative of these severe bilateral corneal lesions including infectious, inflammatory, immunosuppressive or environmental agents. Treatment was readjusted based on potential herpesvirus involvement, and a second flap was performed. Despite some initial management issues, training strategies were adapted temporarily to deal with a blind animal.

Palpebral flaps were maintained for 2 and 4 months before being totally removed. However, several anesthesias were scheduled during this period to evaluate corneal evolution and to apply antivirals and ocular specific treatments through iontophoresis. During the following nine months, the animal progressively recovered full corneal transparency in both eyes, with just very mild central opacities and remodeled corneal surfaces but completely recuperating bilateral vision.

Contributions of osteopathy to veterinary medicine through a case of paralysis in a California sea lion (*Zalophus californianus*).

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When one of Nausicaa's 23-year old California Sea Lions presented symptoms suggestive of lower limb paralysis, various tests (blood analysis, x-ray, ultrasound) were performed. These examinations were unremarkable and no reason for this sudden paralysis could be identified. The animal eating well and showing no signs of pain, no treatment was installed. It was decided to attempt helping him through the use of osteopathy. In the last 15 months, the sea lion has recovered some of its hind flippers' mobility capabilities. This presentation describes the joint work of the various veterinary approaches and the results obtained.

The Sealion Rescue Project

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With a sealions' natural capabilities, such as being able to reach depths of up to 180 meters in a matter of minutes, having low light vision and not being affected by the bends are all key factors why the Oceanografic and its Pinniped team considered the possibilities of training their sealions to collaborate as a possible support system in rescue missions. Contacting the Ministry of Defense, the Oceanographic is now working to provide a demonstration that their sealions could play a fundamental role for the emergency services.

The Civil guard, Emergencies Military Unit along with other emergency control boards such as Maritimes are called out on search and rescue assignments throughout the year. Their searches can cover kilometers of open ocean surface and ocean bottom areas.

We selected out of 15 sealions 3 potential candidates that we thought had the right credentials to participate in the training program.

While assuring not to effect training/enrichment program or the daily needs of the Parks public presentations, we needed to create a separate program for the "Demo rescue sealions".

The training included: Transport desensitization, object discrimination and desensitization/acclamation to new facilities with exposure to variable animal species, and training outside of the sealions "normality" exposing them to new enriching depths/water visibility/habitats and out of the ordinary training schedules.

The beauty of communication between training and research: a chance for understanding energy expenditure while diving in the pacific walrus.

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Sea ice provides a critical platform for walruses to access benthic foraging habitat. Declines in summer sea-ice extent have resulted in walruses increasingly hauling out on land instead of remaining on ice over their primary foraging grounds. As a result, they travel greater distances to key foraging habitat, increasing their potential energetic costs. In this study, trainers and researchers worked together to investigate the diving energetic cost in 3 adult female pacific walruses (*Odobenus rosmarus divergens*) using flow-through respirometry. Once the animals were desensitized to the equipment and trained to breath inside the respirometer, we measured resting metabolic rate. We also measured diving metabolic rate during static (no swimming) and active (swimming) dives. The static dives were approximately 180 s in duration while the active dives were approximately 90 s. Our preliminary data showed no additional energetic cost for the static dives, while the metabolic cost increased between 1.7-3.3 times during active dives. These data provide a better understanding of walrus energetic costs, which has important conservation value. Additionally, the project highlights the value of intensifying the connection between training and science communities for investigating animals under human care that provide vital information to help animals in the wild.

Need a needle?

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The Selwo Marina team faced numerous problems with the contraceptive injection in a male South American sea lion. Unsuccessful sessions, refusals and avoidance were recurring due to a previous history of the blow pipe, and the individual´s low tolerance threshold when dealing with new stimuli.

In addition, the veterinary staff announced that it was necessary to vaccinate for *Erysipelas* the entire group of bottlenose dolphins and it was decided that a voluntary injection must be trained. This would avoid invasive capture in the medical pool and achieving this objective would be a first in the history of the park.

The team wishes to share their experience of training this behavior with you, using different techniques in challenging circumstances, and implementing it unexpectedly and successfully in a sick animal.

This is to show that through training it is possible to achieve goals that promote animal welfare.

To go or not to go: Training cognitive tasks across different modalities in bottlenose dolphins (*Tursiops truncatus*)

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Dolphinaria provide a unique opportunity to study cetaceans, and other marine mammals. Especially research on dolphin cognition and perception is hard to study with wild animals as controlled studies with trained animals under human care are necessary to be able properly assess the cognitive and sensory capabilities of dolphins. Such psychophysical experiments provide vital insight into how dolphins perceive their environment. The go/no-go paradigm is an often-used and straightforward method that has been used in various stimulus detection tasks. At Nuremberg Zoo, this paradigm was trained with four bottlenose dolphins (Tursiops truncatus) using different acoustic stimuli. In a second step it was then tested if the animals would also transfer the response behavior to novel stimuli within and across other modalities. The dolphins demonstrated generalization and transfer performances as they immediately responded correctly to the novel stimuli. It shows, bottlenose dolphins can learn an abstract concept of an initially trained rule. While various studies already demonstrated concept formation in dolphins, this multi-modal application of the go/no-go paradigm after it was learned only acoustically expands our knowledge on the cognitive abilities of the bottlenose dolphin.

The challenging training process for assessing the energetic cost of whistling in Bottlenose dolphin (*Tursiops truncatus*)

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Positive reinforcement is a method used in operant conditioning that uses reinforcing stimuli to enhance the chance that a behavior will occur again. This method is commonly used in managed care animals to allow voluntary participation in veterinary procedures or research studies. In the present project, the dolphin care specialists at the Oceanogràfic used positive reinforcement in a study aimed at investigating the metabolic cost of whistling in the bottlenose dolphin (*Tursiops truncatus*). Three dolphins voluntarily participated in 60 sampling sessions over a 6-month period. The animals were initially desensitized for the experimental environment, including staying stationary with the trainer while breathing into a flow-meter, with a PVC structure for the hydrophones array that measured the whistles. A training plan was created for the experimental procedure, which included an experimental procedure following an overnight fast and without reinforcement during the procedure. The experimental procedure consisted of a 5-minute period where the dolphin remained still while breathing into the flow-meter, followed by a 2 minute breath-hold where the dolphin was either whistling or being quiet, and a final 5 minute recovery period where the dolphin again was breathing into the flow-meter. The results highlight the value of experiments on managed care animals and the importance of research training.

New diagnostic approaches to detect cetacean Poxvirus: Non-invasive skin sampling procedure and molecular detection of Poxvirus in bottlenose dolphins (*Tursiops truncatus*) under human care.

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Tattoo skin disease (TSD) and "Tattoo like lesions" have been described in both under human care and free-ranging cetaceans. It is characterized by a very distinctive irregular, slightly in relief, hyperpigmented pinhole and stippled skin pattern that may occur more often on the dorsal body, dorsal fin, flukes and pectoral flippers. It has been reported that TSD in cetaceans is caused by poxvirus, classified within the *Chordopoxvirinae* subfamily and, more specifically, within the *Orthopoxvirus* genera.

In this study, the presence of poxvirus infection was examined in the skin of 18 bottlenose dolphins (*Tursiops truncatus*) from Loro Parque and Rancho Texas Lanzarote Park in Tenerife and Lanzarote islands respectively. Long term photographical surveys were handled in order to analyze the evolution of the lesions over time as well as the general appearance of the skin of the animals. Furthermore, with the objective to molecularly detect poxvirus infection in the skin of these dolphins whether macroscopically presenting tattoo like lesions or not, a non-invasive methodological sampling was settled. Cytological brushes were used to gently scrape the skin of the dolphins. Skin samples were correctly identified and stored with a tissue storage reagent for a later DNA extraction and PCR procedure. Results of this work will be presented and discussed.

Is gammaherpesvirus a new virus to study in the central nervous system of dolphins?

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A mass stranding of 14 striped dolphins (*Stenella coeruleoalba*) happened in Cantabria, Spain.

Post-mortem examination, both gross and histological, did not reveal the cause of the mass stranding.

Different tissue samples (cerebrum, cerebellum, spinal cord, lung, liver, spleen, kidney, skin, pharyngeal tonsils, lymph nodes and penis) were analysed for the presence of herpesvirus using a previously described nested polymerase chain reaction (PCR). A prevalence of 78.57% (11/14) was determined. Three males were coinfected with alpha and gammaherpesviruses. Four alphaherpesvirus amplicons and 30 gammaherpesvirus amplicons were detected. Alphaherpesviruses were detected in Central Nervous System and lymph nodes, while gammaherpesviruses were detected in Central Nervous System, lung, skin, penis, pharyngeal tonsils and lymph nodes.

To our knowledge, this is the first case of gammaherpesvirus detection in the central nervous system of dolphins, raising new questions for future studies.

Using spirometry to study respiratory physiology and detect lung disease in the bottlenose dolphin (*Tursiops truncatus*)

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As breath-hold divers, marine mammals rely on adequate gas exchange to maximize diving and foraging efficiency. However, global anthropogenic activity is resulting in an increased ecological change that could compromise the feeding behaviour of these animals. Studying respiratory physiology will allow us a better understanding about the diving limitations of these endangered species. Spirometry is used in human medicine to assess lung health through measurement of flow and volume during forced breaths. We are currently using this methodology in bottlenose dolphins (*Tursiops truncatus*) to study their respiratory physiology, create a baseline from healthy animals, and develop a diagnostic tool to detect lung disease. This project is performed under the voluntary participation of animals held under human care. The animals are desensitized and trained for the experimental procedures using operant conditioning and developing positive experiences together with trainers and researchers. Trained dolphins are providing valuable information that could be used to assess the health status of wild populations, and to better understand the physiological response to the environmental change. Dolphins are thought to be sentinel species that inform us of environmental health, therefore animals participating in these studies are acting as ambassadors of their own species and nature.

Lungworm communities in cetaceans from the Mediterranean Sea

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We investigated the diversity and host-specificity of lungworms in five cetacean species that stranded on the Mediterranean coast of Spain from 1987 to 2019: the short-beaked common dolphin (Dd), *Delphinus delphis*, the striped dolphin (Sc), *Stenella coeruleoalba*, the bottlenose dolphin (Tt), *Tursiops truncatus,* Risso's dolphin (Gg), *Grampus griseus*, and the long-finned pilot whale (Gm), *Globicephalae melas*. Three species were found: *Stenurus globicephalae*, a species associated with the Globicephalinae, was found in Gm and Gg; *Stenurus ovatus*, a species typically associated with the Delphininae, was found in Tt and Sc; and *Skrjabinalius guevarai*, another species typically associated with the Delphininae, was found in Tt, Sc and Gg, the latter of which is a new host record. The presence of *S. guevarai* in Gg raises an interesting question about transmission routes and potential intermediate hosts. This is further highlighted when taking into consideration the fact that the parasite diversity in our study differs from that of others. In Sc from the United Kingdom, Costa Rica and Australia, similar surveys only identified *Halocercus lagenorhynchi*; in Tt from Australia, only *Stenurus ovatus* and *Pharurus alatus*. Future research should be dedicated to identifying intermediate hosts and other factors that could regulate parasite diversity.

Epibiotic macrofauna of Antarctic minke whale, Balaenoptera bonaerensis Burmeister, 1867, in the Southern Ocean

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Antarctic minke whales, *Balaenoptera bonaerensis*, typically inhabit waters of the Southern Hemisphere and migrate alone or in small groups to higher latitudes during the austral summer, where they feed on Antarctic krill. We examined the epibiotic macrofauna of 333 Antarctic minkes captured in the Indo-Pacific region of the Antarctic by a Japanese scientific expedition between 2018 and 2019, and explored its potential use as biological tags of their hosts. Four of the taxa found were obligate epibionts of cetaceans, i.e. the amphipod *Balaenocyamus balaenopterae* (prevalence: 21.3%), the copepod *Pennella balaenoptera* (0.6%), and the cirripeds *Xenobalanus globicipitis* (11.1%) and *Coronula* spp. (9.5%); and two were generalist barnacles, i.e. *Conchoderma auritum* (9.0%) and *C. virgatum* (0.3%). Only *B. balaenopterae* and *P. balaenoptera* had previously been reported on Antarctic minkes. Interestingly, the epibiotic fauna was nearly identical to that found on a similar survey of common minke whales, *Balaenoptera acutorostrata*, from Icelandic waters, thus providing an excellent opportunity to perform future phylogeographic analyses. Most individuals of *X. globicipitis* were found dead and degraded. Since this is a typical temperate species, it is likely that barnacles died soon after whales entered Antarctic waters and, therefore, they could be used to trace whales' migration movements.

First steps towards building a regional stranding response network in Cantabria, Spain

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Cantabria is a province of the North of Spain. It has 210 km of coastline belonging to the Cantabrian sea and to the Southern area of the Bay of Biscay. There are historical records of the marine mammal standings along this shore. These standings, however, were not consistently assisted nor were they registered in a standardized way. Moreover, the carcasses of the dead animals were not usually used for further investigations, thus losing very valuable information.

In the year 2018, the Government of Cantabria took the first steps to develop a regional stranding response network, improving the assistance provided to stranded marine mammals. The network also established a thorough register and a standardized investigation of the causes of death of the stranded animals.

Here we present the structure of the Cantabrian stranding response network as well as the preliminary results of the investigations taken place during the first full year of work. A complete post-mortem examination followed by histopathology, microbiology and virology was performed to 22 animals, 14 of them belonging to a mass stranding.

Hopefully this network will continue to improve assistance to stranded animals and the ongoing research for years to come will contribute to the knowledge of the biology and mortality of the marine mammal casualties along the Bay of Biscay.

Characterization of Microbial Composition of Different Body Sites of Two Groups of Bottlenose Dolphins (*Tursiops truncatus*) and their controlled environment.

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The microbiota of marine mammals is used as a proxy for their environmental health and as a marker to measure changes on the ecosystem within the One Health approach. Thus, we collected samples of blowhole exhale (N=17), gastric juice (N=16), skin (N=17) and oral swabs (N=17), and fecal samples (N=16) from 17 bottlenose dolphins hosted in two European facilities, as well as water samples from their hosting tanks (N=4). With the intent of characterizing the microbial composition associated with the different body sites, sufficient microbial DNA was extracted from 35 samples. DNA was analyzed by sequencing the V3-V4 hypervariable region of the gene encoding for the 16S ribosomal RNA. Samples of gastric juice, oral and skin swabs did not provide sufficient material to proceed with analysis. Results indicated the general composition, in terms of bacterial phyla and families, of the characteristic microbiota associated to the different dolphin tissues and the water they live in. All the metrics used showed the greatest microbial diversity in the blow samples. Bacterial biodiversity of water samples was richer than in the fecal samples. These results provide useful data to correlate the health of the animals and their environment also from a clinical perspective.

Updates on the case of a Hepatocellular carcinoma in a South American sea lion (*Otaria flavescens*)

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During the EAAM Annual meeting in Portugal in 2019, we presented a case report on a South American sea lion (*Otaria flavescens*) that suffered from a Hepatocellular carcinoma with metastases in the lungs. Further research on the case has demonstrated that metastases were present also in the spleen and lymph nodes. Besides, the histopathological examination of the parathyroid revealed the presence of proliferative neoplastic phenomena that could represent the primitive site of the development of the adenocarcinoma of which metastases were observed in the liver and lymph nodes. The Immunohystochemestry (IHC) analysis confirmed that the tumor was of primary hepatic origin, metastasized in various organs, including the parathyroid which does not represent a usual site of metastasis.

First report of striped dolphin *(Stenella Coeruleoalba)* strandings due to dolphin morbillivirus In Greece

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In the past 30 years cetacean morbilliviruses (CeMV) infections have occurred in many marine mammal populations around the world, including major episodes of mortalities in striped dolphins (*Stenella coeruleoalba)* in the western Mediterranean Sea. Here, we present the resurgence of *morbillivirus* in the Greek coasts in the year 2018. Seventy 70 striped dolphins were found in an unusual stranding event between April and October 2018 in the Eastern Mediterranean, both in the Aegean and Ionian Sea. Fourteen stranded alive; they were calves or juveniles (0,80 - 1,60m TBL). All had similar clinical signs. Despite supportive first aid treatment, within a few hours all animals showed severe neurological signs, i.e., convulsions, seizures, leading to death. Full necropsy and tissue sampling were feasible in 12 animals. Gross pathological lesions were mainly found in the CNS, respiratory, lymphoid and digestive systems. Histopathology consistently revealed lesions characteristic of morbillivirus-associated broncho-interstitial pneumonia and non-suppurative meningoencephalitis. Samples from nine dolphins were examined using a real-time RT-PCR targeting the P/V/C gene of morbillivirus. The assay was strongly positive in brain and lung of all (9/9). These findings support the diagnosis of a fatal acute and subacute DMV infection in the striped dolphin populations of the Eastern Mediterranean Sea for the first time. Further research is needed to fully characterize the virus strain and elucidate its epidemiology.

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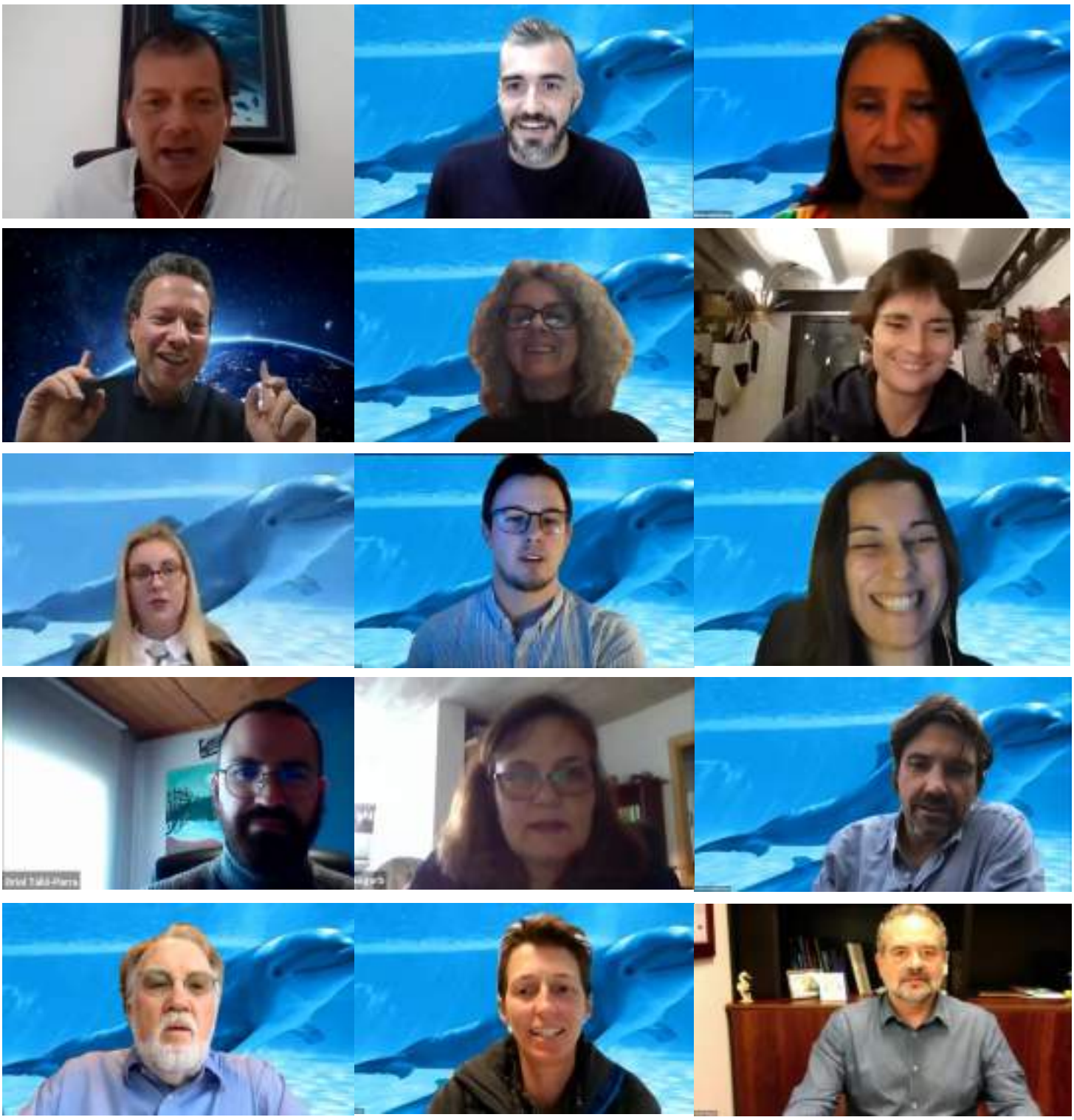

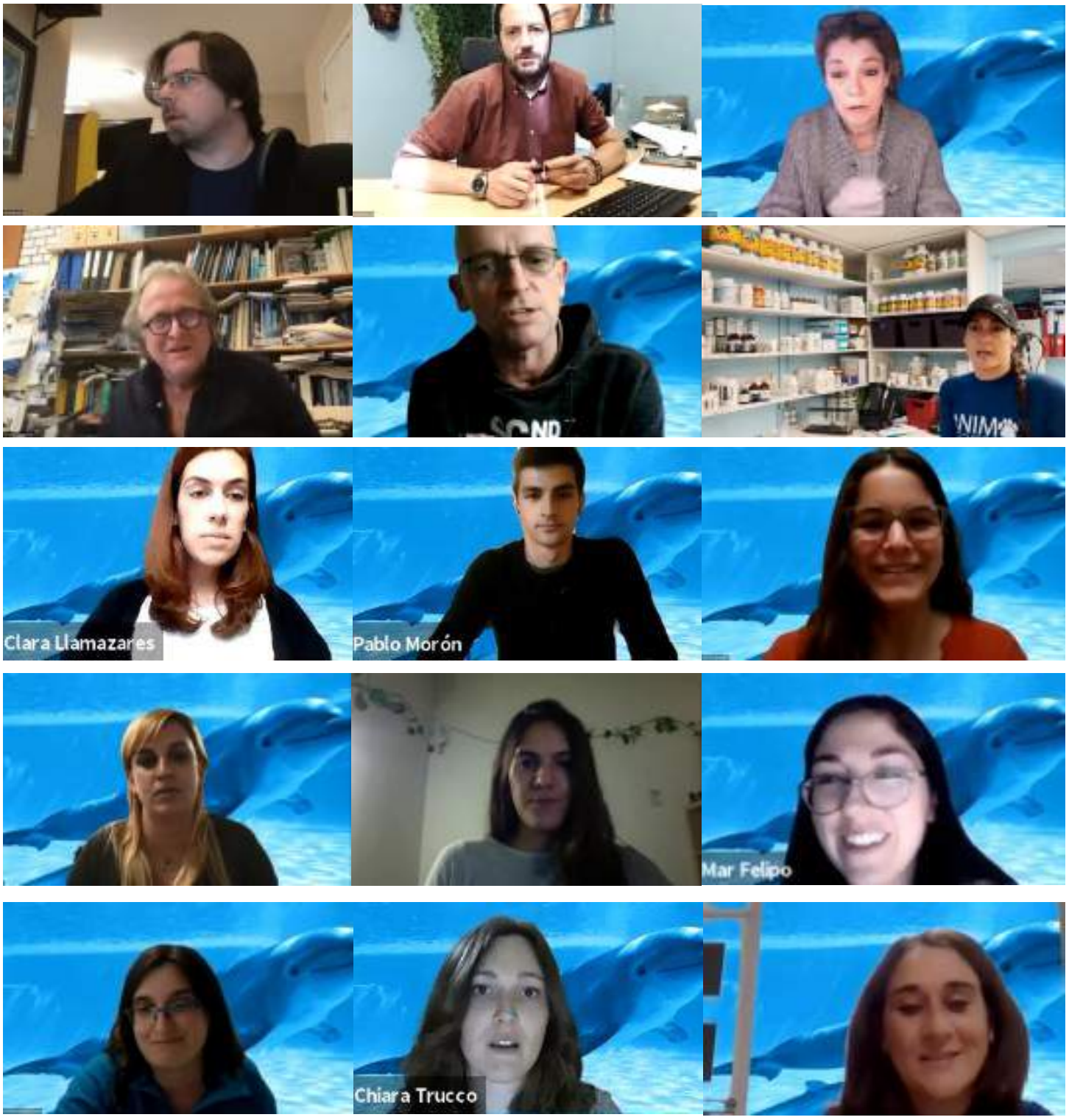
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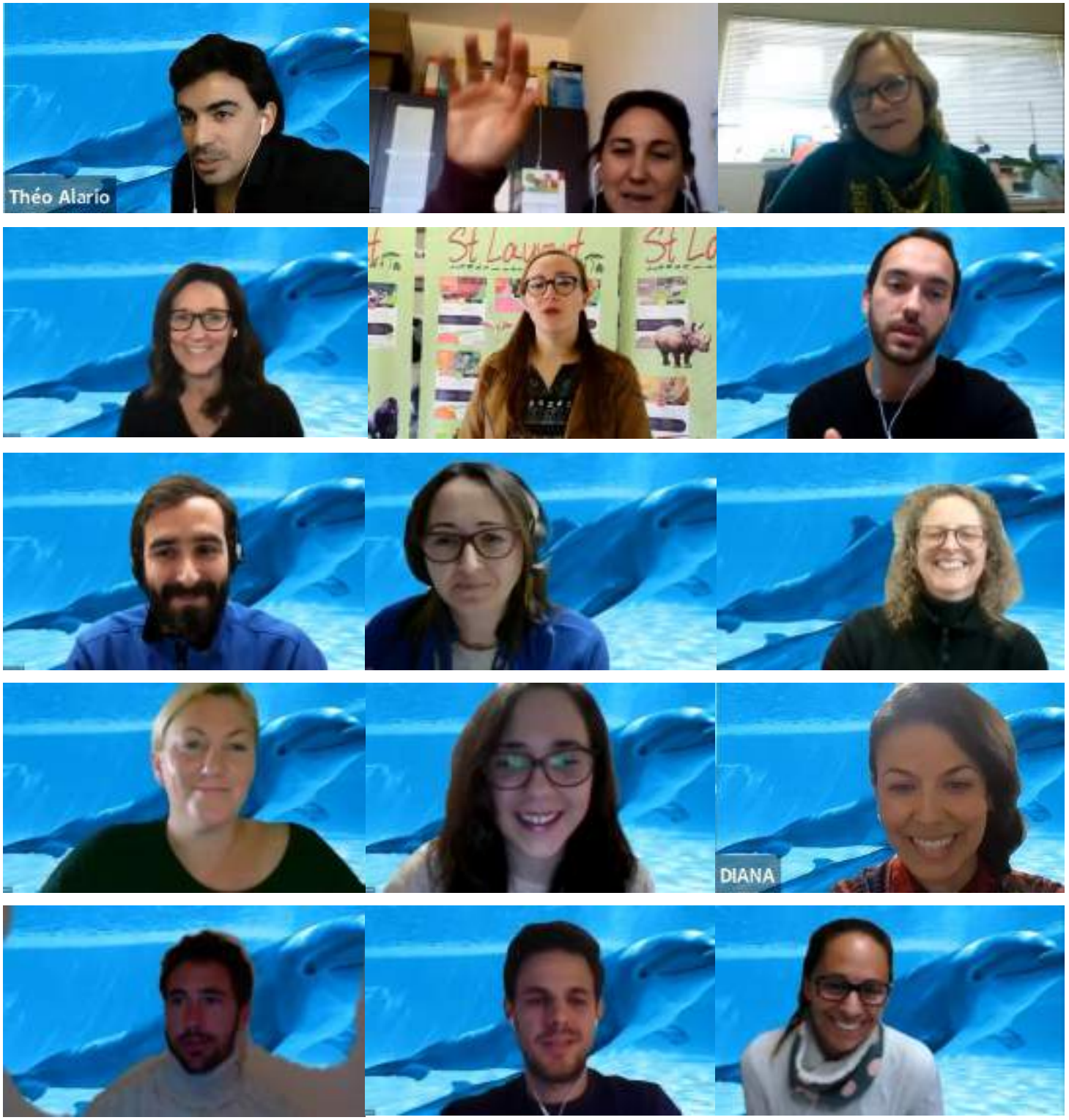
Chiara Trucco

“A Conference For The Future”



"A Conference For The Future"

**Images are sorted in order of appearance during the event.*

THANK YOU ALL FOR YOUR PARTICIPATION!

See you in Valencia in 2022!

SPECIAL THANKS TO: AVELINO MARTÍNEZ, EDUARDO GONZÁLEZ Y BEA PÉREZ

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's 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.