EAAM

EUROPEAN ASSOCIATION FOR AQUATIC MAMMALS

MADRID 40TH SYMPOSIUM

9TH - 12 TH of MARCH 2012

FUNDACIÓN PARQUES REUNIDOS

ZOO AQUARIUM MADRID

Dear Colleagues and Friends,

In 2012 we probably celebrate the 40th anniversary of the European Association for Aquatic Mammals.

Our beloved Association was founded four decades ago with a very special aim: to congregate individuals interested in fields of knowledge related to all Aquatic Mammals species. As such, through the years the EAAM was able to combine the efforts, the expertise, the drive, the strength and the ethics of hundreds and hundreds of experts who dedicated countless hours to hundreds of scientific, educational, conservation, husbandry and ethics-related projects.

Alone or in groups, throughout months or even years, our membership was able to boost the technical knowledge in areas which, in 1972, would seem, to many of us, too hard or even impossible to achieve. However, through hard work, vision, patience and cooperation (most frequently, with the marine mammals themselves), our Association was able to open new technical and scientific horizons to researchers, educators, legislators, journalists and, of course, the public. These tremendous achievements should make us all, current and past members, extremely proud! We did, indeed, what our founding fathers initially envisioned.

In these 40 years our Association has grown, inspired by always renewed perspectives and through the development of many research activities both *ex situ* and *in situ*. The EAAM has humbly but also determinedly increased the awareness about the welfare of these creatures, which are so perfectly adapted to many different and challenging aquatic environments.

In the meantime, the EAAM always celebrated its goals and its achievements through an Annual Symposium. And this year would not be the exception. As such, and as President of the EAAM, it is my great honour to welcome you all to our 40th Annual Symposium, which is hosted, once again, by *Zoo Aquarium de Madrid*.

Thanks to the engagement of *Grupo Parques Reunidos*, this year's programme, which was organized through the dedication and hard work of several colleagues, will certainly allow us to, once again, join a very dedicated and clearly international group of professionals who enthusiastically share technical information, efforts and achievements. The Scientific Programme was organized by our President-Elect, Jesús Fernández Morán, in strong cooperation with the EAAM's Scientific Committee.

As we all know, and I cannot help but to reinforce, the development of a consistent programme requires an incredible amount of work by the Local Organizing Committee, the EAAM Board, and all the EAAM Committees. But it's certainly worth it, as it is dedicated to the sharing of knowledge which increase awareness and operational effectiveness in all aspects related to the complex biology of Aquatic Mammals and, more importantly, to all the potential applications of such knowledge to the Conservation of the different species in the wild: our ultimate and most noble goal!

With this in mind, allow me to recommend you to relax and fully enjoy our special Symposium, the amazing Madrid and the Friends who are joining us from so many countries around the world!

In the meantime, congratulations! You, as a member of EAAM, certainly deserve it! And may you, 40 years from now, continue to be as proud of the European Association for Aquatic Mammals as we all are today.

Sincerely,

Claudia Gili
President of EAAM

Dear Colleagues,

It is a great honor for us to welcome you to the 40th Symposium of the EAAM, which will take place in the Zoo Aquarium Madrid and the Parque de Atracciones de la Casa de Campo.

The first Zoo in Madrid opened in 1770 in the Retiro Park, making it the second oldest Zoo in Europe after the Zoo of Vienna. The current Zoo of Madrid which opened its doors in 1972 in the Casa de Campo has continued this long tradition of keeping and taking care of wild animals derived from its predecessor. Although not included in the initial plans, fifteen years later, a dolphinarium was built and later on a modern aquarium was added to the complex. Every year around a million people visit us, getting in contact with our fantastic nature and its species, out of which most are in danger of extinction. This allows us to share our conservation efforts with a broad audience. Moreover, we provide a special place, where adults and children can experience defining moments while getting close to nature.

Madrid Zoo is known to have one of the most diverse, endangered and impressive animal collections worldwide that ranges from the unique giant pandas, to others such as the great horned and white rhinos, koalas, dolphins, sea lions, seals, gorillas, orangutans, chimpanzees, Asian elephants, giraffes, tropical fish, birds and a large collection of reptiles. Our commitment with the conservation and research in this field, led us to also include highly endangered species in our Zoo such as the European mink, the Spanish Iberian eagle or the Malayan sun bears.

Our education department was inaugurated in 2002 and has ever since carried out a very important task for the nature conservation, protection and care of our ecosystem and the planet. The concept of education, while enjoying and visiting our animals and their facilities allows us to direct our message to more than 40.000 children per year. Another important department dedicated to the care of our valuable animals is the veterinary department that has the most modern diagnostic equipment and facilities, as well as well-trained veterinarians with cutting-edge knowledge at their disposal.

The Symposium's organizing committee will do their best to offer you a pleasant, comfortable, environmentally friendly meeting, and will be glad to assist you in anything you may need during your stay in Madrid.

We thank all EAAM members, zoo and aquarium professionals, as well as the students, for their attendance, and our sponsors for their generous contributions. We are convinced we will all benefit from the splendid programme presented by the EAAM Scientific Committee.

This is the second time Madrid organizes this symposium. Some of you might still remember the first one that took place in 1993. We wish this one is at least as well organized and that it will leave a good memory for all of us. We also hope that this symposium will produce important outcomes for the science and conservation of aquatic mammals.

Welcome to Madrid!

Jesús Fernández
Zoological Parks Division Director
Parques Reunidos

Ricardo Esteban
Director
Zoo Aquarium de Madrid

ENVIRONMENTAL POLICY

Madrid Zoo is aware of the environmental impact involved in organising symposiums. As a result, it has endeavoured to implement environmentally sustainable criteria and it has undertaken socially responsible actions:

Conference Registration on line (no paper)
Programme printed on recycled paper
Name tags printed on recycled card
Name tag ribbons made from recycled plastic
Recycled cardboard notebook
Non-woven multi-purpose bag, reusable and 100% recyclable material
Proceedings provided on USB pen drive. No paper
Use of durable dishes and glasses
All the selected hotels are within walking distance of each other.
We provided information about public transport system from airport.

ORGANIZATION

Organizing Committee
Jesús Fernández – President-Elect of EAAM
Ricardo Esteban – Director Zoo Aquarium de Madrid
Agustin López Goya – Zoo Aquarium de Madrid
Adeline Cassuhn – Parques Reunidos
Amparo Fernández – Zoo Aquarium de Madrid
Carlos de las Parras – Zoo Aquarium de Madrid
Eva Martínez – Zoo Aquarium de Madrid

Scientific Committee
Manuel G. Hartmann
Sabrina Brando
Stan Kuczaj

SPONSORS

The Organising Committee of the 409th Symposium of the EAAM would like to thank the following sponsors:

Scientific Programme

INVITED SPEAKER

NEW ADVANCES IN THE DIAGNOSIS OF VIRAL DISEASES IN DOLPHINS

Prof. JM. Sánchez-Vizcaíno
Visavet Center. Universidad Complutense. Madrid
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Virus infections are still not well studied in dolphins. Only a few DNA and RNA genome viruses have been reported until now. From these, Morbillivirus is the most known virus which affects wild and captive dolphins and has caused important losses among different populations of these animals during the last 25 years.

In the past, the diagnosis of most viral disease was mainly carried out by immunohistochemistry but the limitations, especially in sensitivity, of this method yielded to molecular diagnosis that has been proven to be more sensitive. The new early and rapid detection and characterization of specific nucleic acids have demonstrated invaluable for diagnostic purposes in herpesvirus, papillomavirus or poxvirus. Although there have been many advances in dolphins' virology diagnostics in the last years, relations of these viruses with pathologic states of dolphins have not been found in all cases. For this reason, viral metagenomics provide a powerful technology to investigate sick dolphins with unknown diseases. Using these methodologies, we'd gain a better understanding in the etiology and pathology of dolphin viral diseases.

Another important topic related to virus infection is the evaluation of the immune response, e.g., how infection can affect the cell mediated and humoral immune response in wild and captive dolphins. To achieve this, several techniques are already available to evaluate these effects that could give importance information of the real role of this type of infection.

Finally, the molecular epidemiology is another tool that improves our understanding of the pathogenesis of disease by identifying specific pathways, molecules and genes that influence the risk factor for developing the viral disease.

A complete review of these technologies and a summary of the main results obtained until now will be presented during this talk.

CURRENT STATUS OF APPLICATION OF ASSISTED REPRODUCTIVE TECHNOLOGY IN CETACEANS

TR Robeck, KJ Steinman, GA Montano and JK O'Brien
SeaWorld and Busch Gardens Reproductive Research Center, San Diego, CA

Research with ex-situ populations serves as a foundation of biological information necessary to improve and influence decisions concerning *in-situ* population management and policy and provides tools for active intervention when passive management strategies are unsuccessful. The development and application of assisted reproductive technology (ART), including artificial insemination (AI), gamete cryopreservation, sex-selection (using sperm sorting), IVM, IVF and embryo collection and transfer, has the potential to integrate small, genetically isolated populations of a species into a sustainable *ex-situ* population. Once developed in *ex-situ* populations, these technologies can be applied towards the rescue and/or genetic management of endangered *in-situ* populations. Among cetaceans, the development of ART began with semen collection and analysis research in the bottlenose dolphin during the 1970s. Since then, ARTs have progressed to become viable tools for population management in several cetaceans including the bottlenose dolphin, killer whale, Pacific white-sided dolphin and the beluga. The modification of extenders and the evolution of cryopreservation techniques from pellets and straws to directional freezing have lead to enhanced cryopreserved sperm quality. Methodologies for artificial insemination have also been developed and successfully applied in the aforementioned cetacean species. Together, these semen cryopreservation and AI methodologies allow for the use of ART in cetaceans globally. Currently, sex-sorted, frozen-thawed spermatozoa are routinely used for AI in bottlenose dolphins with 13 calves being produced globally to date. One of those calves was produced using sex-sorted spermatozoa derived from previously cryopreserved semen, which further increases the utility of this ART. Although successes with these techniques are significant to cooperative population management strategies, continued improvements and widespread application will be dependent on standardization of techniques between operators, systematic data collection and reporting. Future application of ART to endangered cetaceans will first require the formation of temporary or permanent *ex-situ* populations from at risk in-situ populations for research on their basic reproductive physiology.

COMMUNICATING ABOUT OUR ANIMALS

Jeff Jouett
Chief Executive Officer, Dolphin Quest / Quest Global Management

Effective communication is both an art and a science. When the subject matter is as sensitive and multi-layered as “animals” in general, or “marine mammals” in particular, effective communication becomes a fine art, and the science of it becomes more psychology than biology. How we express what we do and why we do it has a tremendous impact – positive or negative – on public understanding and approval, and, ultimately, on how successful we will be in reaching our animal care, education and conservation goals.

Whether we are talking to the media, to a government panel, to guests in our parks and aquariums, or to the person standing in front of us in the grocery store check-out queue, we have an opportunity to deliver key messages that will make a difference for our animals. Like a true performance art, good communication is a skill and a talent that can be developed and improved with training and practice.

Today we’ll talk about how we can be better communicators by being aware of the process and context, being mindful of our true audiences, identifying our key messages, saying what we want to say and then saying it again, seizing the emotional high ground, and giving a healthy nod to our ever-present non-verbal communication. We’ll talk about some things to look out for in media interviews, and how to take advantage of them. With just a little coaching, we can all do our best job of spreading the good word about all of the good things that we do.

PROGRAMME

SATURDAY, 10TH OF MARCH 2012

Opening of the Symposium by the host Grupo Parques Reunidos, ZooAquarium Madrid and the EAAM board

CHAIRMAN: **Dr. Jesús Fernández Morán**

Invited speaker:
New Advances in the diagnosis of viral diseases in dolphins.
Prof. J.M. Sánchez-Vizcaíno

Unusual striped dolphin mass mortality episode related to cetacean morbillivirus in the Spanish Mediterranean Sea.
Consuelo Rubio-Guerri*, Fernando Esperón, Mar Melero, Edwige Nina Bellière, Manuel Arbelo, Jose Luis Crespo, Eva Sierra, Daniel García, Jose Manuel Sánchez-Vizcaíno

Elevated presence and variability of Herpesvirus in cetaceans stranded during 2010 and 2011 in the Region of Valencia.
Mar Melero*, Consuelo Rubio-Guerri, Edwige Nina Bellière, Fernando Esperón, Jose Manuel Sánchez-Vizcaíno

The rehabilitation of a stranded killer whale.
C.E. van Elk

Suspected side effects of medication in bottlenose dolphins: a review.
Manuel García Hartmann

CHAIRWOMAN: **Dr. Arlete Sogorb**

Effect of Platelet Rich Plasma (PRP) treatment on wound healing in bottlenose dolphins (*Tursiops truncatus*) and loggerhead sea turtles *(Caretta caretta)*.
D. García-Párraga*, J.L. Crespo, T Alvaro, M. Valls

Establishing ophthalmic ultrasound in bottlenose dolphins (*Tursiops truncatus*).
K. Baumgartner*, I. Hoffmann, H. Will

Within-day and between-day variability of transthoracic echocardiography in the bottlenose dolphin (*Tursiops truncatus*): an anatomic M-mode study.
J. Lichtenberger*, M. Mellin, B. Mercera, F. Delfour, A.C. Hoffmann, G. Chaix, E. Trehiou-Sechi, C. Misbach, A. Petit, H.P. Lefebvre, R. Tissier, V. Chetboul

The bottlenose dolphin from Torvaianica (Italy, Rome): the account of a rescue.
G. Roncon*, V. Manfrini*, S. Mazzariol, F. Marcer, F. Scholl, C. Eleni, R. Meoli, C. Cocumelli, C. Di Francesco, G.Di Guardo

Presentation of the Mom project, a conservation project funded by the EAAM.
Mediterranean Monk Seal Conservation

CHAIRMAN: **Dr. Andrew Greenwood**

Medical management of an acute renal insufficiency in a 15 year old male *Arctocephalus pusillus pusillus* after a bilateral lensectomy.
Carla Flanagan*, Nuno Silva, Joana Silva, M. H Carmen Colitz, James Bailey, Antonieta Nunes, Miguel Silveira, Márcia Neto Lucie Palma

A few ideas about aquatic mammals' ophthalmology.
Huguet, E.*, C. Colitz, M. Pérez-Orrico, G. Lacave*, D. García

Ultrasound in pinnipeds – a review.
Geraldine Lacave

Identification of a C2 fracture and rachis deviation through CT scan in a California sea lion (*Zalophus californianus).*
Geraldine Lacave*, Laurent Marescaux, Jean-Luc Bourgain, Justine Deschamps, Corinne Godet, William Gournay, Aurelia Pouille, Virginie Roy

Treatment of traumatic lesions in stranded harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) under rehabilitation.
G.J.Sánchez Contreras*, A. Rubio García, N. Osinga, D. Morick

Field anaesthesia of leopard seals (*Hydrurga letonyx*) at Cape Shirreff, Western Antarctic peninsula
Nicola Pussini *, Michael E. Goebel, Ray Bucheit, Kevin Pietrzak, George Watters

SUNDAY, 11TH OF MARCH 2012

CHAIRMAN: **Dr. Daniel García Párraga**

Invited speaker:
Current Status of Application of Assisted Reproductive Technology in Cetaceans.
T.R. Robeck*, K.J. Steinman, G.A. Montano and J.K. O'Brien

Medical management of 4 bottlenose dolphins' calves from 0 to 12 months.
E. Guglielmi*, M.García Hartmann, M. Vella, B. Biancani

Handling of neonate dolphins – the past and the present.
Ulf Schönfeld*, Kerstin Jurczynski

Veterinary management of neonate dolphins at the Duisburg Zoo.
Kerstin Jurczynski*, Dimitri Widmer, Sandra Langer, Ulf Schönfeld

CHAIRWOMAN: **Dr. Kathleen Dudzinski**

Automatic localization by acoustic methods of "*Orcinus orca*" individuals at Loro Parque facilities.
Jose Carlos Sanluis, Jonas Phillip Luke, Fernando Rosa, Javier Almunia

Bridging: Beyond the Basics.
Thad Lacinak*, Angi Millwood*

Marine mammal feeding ecology and adaptations: feeding opportunities for marine mammals housed under human care.
Sabrina Brando

MONDAY, 12TH OF MARCH 2012

CHAIRWOMAN: **Birgitta Mercera**

Invited speaker:
Communicating About Our Animals.
Jeff Jouett

Personality assessment and social dynamics in two groups of bottlenose dolphins (*Tursiops truncatus*).
Sabina Birgersson*, Steven Birot de la Pommeraye, Fabienne Delfour, Birgitta Mercera

Comparing Object Play in Captive and Wild Dolphins.
W.E. Greene*, K. Melillo-Sweeting, K.M. Dudzinski

Development and reciprocity in pectoral fin contact between dolphins.
Kathleen M. Dudzinski

Beyond operant conditioning: Results of applying a cognitive-emotional training model to sea lions.
Carlos Alfonso López García, Carlos De las Parras Domingo, marine mammals staff

CHAIRMAN: **Kai Mattson**

Large repertoire of behavior training with harbor seals in a multiple species context.
Jérémy Ferrier*; Marjorie Flamey*; Jérémy Nemoz; Elodie Sene; Géraldine Lacave

Solving a life-threatening regurgitation problem in a California sea lion through training and satiation.
Pablo Joury*, Alexandre Le Blanc, Emilie Treviglio, Christilla Bouchet, Candice Jourdan, Claudia Mahtali, Alexis Maillot, Vanessa Alerte, Geraldine Lacave

Training of Geriatric Marine Mammals.
Márcia Neto*, Elsa Quintino, Lucie Palma, Hugo Camacho, Carla Flanagan, Luís Roque, Joana Silva, Miguel Silveira

Round tables (free participation, no registration necessary):

Dolphin neonatology.
Chair: Dr. Manuel García Hartmann

Training as a tool in the introduction of cetaceans into a social group
Chair: Lindsay Rubincam

Dolphin transport methodologies update.
Chair: Dr. Daniel García Parraga

ROUND TABLE DISCUSSIONS

Training as a tool in the introduction of dolphins into a social group: Lindsay Salerno

From a training and management point of view, different strategies are being used when introducing a new dolphin (bottlenose dolphin, killer whale, etc.) in a variety of situations. Such introductions can be related to the integration of new arrivals due to exchanges within breeding programs, re-introduction of an animal which has been separated from the group for health or other reasons, and integration of new calves to the group, after separation of the mother-and-calf dyad for birth and the first weeks of life of the calf.

The aim of this round table is to compare the different strategies used and discuss experiences with them.

Dolphin transport methodologies update: Daniel Garcia

The transport of dolphins for breeding purposes is becoming more and more frequent in recent years, and the aim of this round table is to make a review of transport methodologies and exchange experiences – about veterinary care and transport management, but also the difficulties encountered with obtaining the necessary permits within Europe.

Dolphin neonatology: Manuel Garcia Hartmann

[I plan to give a talk about the experiences in recent years with treating dolphin calves, and the positive outcome of all cases. In fact, this round table is an update of the Malta reproduction workshop.]

Dolphin neonate mortality has been long identified as a key factor to increase population size of captive dolphins in Europe. Recent changes in management, allowing for interaction with the calves and therefore making veterinary treatment possible have shown great success in several European facilities. The aim of this round table is to exchange experiences of cases, recent findings, as well as our increase in the understanding of the reasons for calf mortality.

Oral presentations

UNUSUAL STRIPED DOLPHIN MASS MORTALITY EPISODE RELATED TO CETACEAN MORBILLIVIRUS IN THE SPANISH MEDITERRANEAN SEA

Consuelo Rubio-Guerri* (1), Fernando Esperón (2), Mar Melero (1), Edwige Nina Bellière (2), Manuel Arbelo (3), Jose Luis Crespo (1), Eva Sierra (3), Daniel García (4), Jose Manuel Sánchez-Vizcaíno (1)

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(2) National Institute for Agricultural and Food Research and Technology, 28130 Madrid, Spain
(3) Unit of Histology and Veterinary Pathology, Institute for Animal Health, Veterinary School, University of Las Palmas de Gran Canaria, 35416 Canary Islands, Spain
(4) Veterinary Services, Oceanographic Aquarium of the Ciudad de las Artes y las Ciencias, 46023 Valencia, Spain

Between March and April 2011, 37 dolphins stranded along the Mediterranean coast of the region of Valencia, Spain: 26 striped dolphins (*Stenella coeruleoalba*), 3 bottlenose dolphins (*Tursiops truncatus*) and 8 undetermined. The annual mortality rate in this territory has been 28.4 dolphins per year over the last 20 years. Therefore, an unusual episode of mortality should be considered. Because of the poor conservation code, only thirteen animals could be necropsied, revealing a high frequency of pneumonia, proven on microscopic examination as bronchointerstitial and non-suppurative, with atelectasic lung areas and enlarged/congestive lymph nodes. Three animals presented non-suppurative encephalitis with astrocytes containing characteristic viral inclusion bodies in two. Of all individuals tested for cetacean morbillivirus (CeMV) using a novel RT-PCR technique based on amplification of a 192-bp conserved region of the gene for the viral fusion protein, seven proved positive. Viremia was suggested because in five of the seven positive animals, CeMV was detected in at least two tissues. Phylogenetic analysis based on partial sequencing of the phosphoprotein gene revealed a viral strain closely related to the dolphin morbillivirus (DMV) isolated in the mobillivirus epizootic of 2007. Therefore, given the nature of the lung and brain lesions and the detection of morbillivirus with high frequency, infection by DMV appears the cause for this new peak of strandings.

ELEVATED PRESENCE AND VARIABILITY OF HERPESVIRUS IN CETACEANS STRANDED DURING 2010 AND 2011 IN THE REGION OF VALENCIA.

Mar Melero* (1), Consuelo Rubio-Guerri (1), Edwige Nina Bellière (2), Fernando Esperón(2), Jose Manuel Sánchez-Vizcaíno (1).

(1) VISAVET Center and Animal Health Department, Veterinary School, Complutense University of Madrid, 28040 Madrid, Spain, mar.melero@sanidadanimal.info
(2) National Institute for Agricultural and Food Research and Technology, 28130 Madrid, Spain

In order to investigate the presence and diversity of herpesvirus in four species of cetaceans: striped dolphin (*Stenella coeruleoalba*, Sc), bottlenose dolphin (*Tursiops truncatus*, Tt), common dolphin (*Delphinus delphis*, Dd), and fin whale (*Balaenoptera physalus*, Bp) stranded

during 2010 and 2011 in the Region of Valencia (Mediterranean Sea), a total of 301 tissues belonging to twenty-eight individuals were analyzed by nested consensus polymerase chain reaction (PCR) for herpesvirus (HV). Fifty-six samples from twenty-three animals (15/20 Sc, 6/6 Tt, 1/1 Dd and 1/1 Bp) were positive to HV, representing 82.14% .Sixteen of the HV positive animals presented more than one tissue positive. Phylogenetic analysis of the HV sequences showed sixteen different gamma and alphaherpesvirus in all of the animals except in one Tt in which the sequence found was repeated. This is the first worldwide description of Gammaherpesvirus systemic infection in a bottlenose dolphin (skin, brain and genital mucous) and the first identification of HV detected by PCR in fat, muscle, meninges, gingival tissue, pancreas, thymus, adrenal gland, urinary bladder and testicles in cetaceans. Furthermore, no HV had been published before in fin whale. In conclusion, this work suggests that HV are very frequent in cetaceans stranded in the Mediterranean Sea and that the HV sequences present are exceptionally diverse.

THE REHABILITATION OF A STRANDED KILLER WHALE

C.E. van Elk
Dolfinarium Harderwijk, Strandboulevard Oost 1, 3841 AB Harderwijk, The Netherlands,
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On the 23rd of June 2010, a juvenile killer whale stranded on the Dutch coast. The animal was severely undernourished and had dermatitis. Treatment and recovery were straightforward and greatly facilitated by her spontaneous and ravenous appetite. The animal adapted quickly to her new surroundings. Training and enrichment were deemed essential for her mental health and helped with ease of veterinary monitoring. The killer whale was highly interested in humans and in social interaction.
A panel of experts was assembled to evaluate the question whether she could be released. Tey decided unanimously that she could not be released. This decision led to a frantic media campaign and lobby efforts by persons that did not agree with this decision. They united themselves in two organizations that had links to international animal rights campaigners and a Dutch political party (Party for the Animals). The conflict was extremely appetizing for the media and huge coverage resulted in intense political involvement. The parliament even discussed the fate of this single animal and voted on the release of information by the Dolfinarium in Harderwijk. Two court cases where the CITES permit given by the Dutch authorities was disputed finally resulted in the verdict that the killer whale was authorized to be transported to Loro Parque in Tenerife.
The introduction of the killer whale was smooth and within a couple of weeks she had met with all group members. She again adapted quickly to her new surroundings. The present groups of animals and trainers were just as enthusiastic as she was.
This killer whale which was abandoned and given up by nature now has a bright future. Her charisma has caused an enormous amount of public attention. May it now inspire the public to protect and preserve the harsh wilderness she came from.

SUSPECTED SIDE EFFECTS OF MEDICATION IN BOTTLENOSE DOLPHINS: A REVIEW

Manuel García Hartmann
Marineland Antibes, Grupo Parques Reunidos, 306 Avenue Mozart, 06600 Antibes, France, vet@marineland.fr

Veterinary drugs are highly active substances which are used for their beneficial pharmacological effects, but they may have other, unwanted effects in a certain number of patients as well. Such unwanted effects, known as side effects, are very incompletely known in the bottlenose dolphins: on one hand, dolphin veterinary medicine is a relatively new field, dating back only about 50 years, in comparison to human medicine and veterinary medicine with centuries of history and experience. On the other hand, the number of dolphin patients is very small. While in human medicine, hundreds of thousands or more persons use certain medicaments per year, dolphin medicine will never reach such numbers –necessary for statistical analysis and confirmation of veracity of suspected side effects.
In this presentation, we present suspected cases of side effects of certain medicaments we used in bottlenose dolphins, like omeprazole, sucralfate, ceftiofur, doxycycline, Baytril S, paracetamol, voriconazole and others.
The declared aim of this presentation is to inform colleagues about these suspected side effects, while at the same time calling for caution to 'blame' medications for "unwanted effects" which are in reality unrelated to medication.

EFFECT OF PLATELET RICH PLASMA (PRP) TREATMENT ON WOUND HEALING IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) AND LOGGERHEAD SEA TURTLES *(CARETTA CARETTA)*.

D. García-Párraga*, J.L.Crespo, T. Alvaro, M. Valls
Oceanogràfic. Parques Reunidos Valencia S.A. Ciudad de las Artes y de las Ciencias. Junta de Murs i Valls sn. 46013. Valencia. SPAIN. dgarcia@oceanografic.org

In an aquatic environment, most common topical treatments are not very useful. Sometimes healing takes longer than expected and wounds can become infected, especially in cases of immune-suppressed individuals or poor water quality.
During the last years, several dolphin and sea turtle lesions were treated at the Oceanográfic of Valencia from different approaches in order to determine the potential use and effectiveness of different techniques. Low Level Laser Therapy and Platelet Rich Plasma were the most innovative ones. Different trials included treating recent non-infected uncomplicated injuries as well as infected torpid ones.
PRP was always produced from autologous blood collected on sodium citrate and applied in a single treatment. Plasma was directly injected into the injured tissue or the clot applied over the clean wound and covered with a water proof ointment. Evolution of treated wounds was evaluated during and after each therapeutic procedure.

Preliminary results in both species suggest that wounds treated with PRP have a tendency towards a better healing result in comparison to the standard topical treated and non-treated wounds, inducing a faster resolution and fewer complications.

ESTABLISHING OPHTHALMIC ULTRASOUND IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

K. Baumgartner* (1), I. Hoffmann (2), H. Will (1)
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Purpose: To establish ophthalmic ultrasound in bottlenose dolphins.
Methods: Using a new training approach and a 15 MHz probe we were able to perform ultrasound in our bottlenose dolphins.
Results: The animals tolerated the method well after a few training sessions. All contents of the eye globe could be visualized. Also, we were able to obtain values for anterior chamber depth (ACD), anterior-posterior lens diameter (LD) and bulbus length (BL) in all patients.
Conclusion: With special training ophthalmic ultrasound can be performed in bottlenose dolphins and may be a useful aid in the diagnosis of ocular disease in this species.

WITHIN-DAY AND BETWEEN-DAY VARIABILITY OF TRANSTHORACIC ECHOCARDIOGRAPHY IN THE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*): AN ANATOMIC M-MODE STUDY.

J. Lichtenberger* (1), M. Mellin (2), B. Mercera (2), F. Delfour (2), A.C. Hoffmann (1), G. Chaix (1), E. Trehiou-Sechi (1), C. Misbach (1), A. Petit (1), H.P. Lefebvre (3), R. Tissier (4), and V. Chetboul (1).
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The use of transthoracic echocardiography in dolphins has been limited so far owing to technical and anatomical specificities. Anatomic M-mode (AMM) is an echocardiographic technique capable of generating M-mode studies from two-dimensional (2D) cine loops

independently of the ultrasound beam orientation. The aim of the study was to determine the within-day and the between-day variability of AMM in bottlenose dolphins (BD, *Tursiops truncatus*).
our healthy BD trained to lie on left lateral recumbency (9-31 years; 180-250 kg) were included. A total of 96 echocardiographic examinations were performed by the same observer on 4 days with 4 dolphins examined 6 times/day. AMM measurements were performed from 2D left parasternal long-axis views showing the left ventricle (LV) ventrally and the aortic root dorsally. The within-day and between-day coefficients of variation (CV) were determined using a general linear model.Ten AMM variables (ventral and dorsal LV myocardial wall thicknesses, LV and aortic diameters at end-diastole and end-systole; mean aortic diameter and shortening fraction) were calculated. Most within- and between-day CV values (16/20) were <10%, the lowest being observed for the end-diastolic LV diameter (1.6%).Conclusion: AMM provides a non-invasive evaluation of left heart morphology and function in BD with appropriate repeatability and reproducibility.

THE BOTTLENOSE DOLPHIN FROM TORVAIANICA (ITALY, ROME): THE ACCOUNT OF A RESCUE

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On June 29, 2011 a live specimen of *Tursiops truncatus* (Montagu, 1821) stranded on the Roman coast of Torvaianica. Before stranding the dolphin was spotted and tracked along the 15 kilometres long stretch of coast between Ostia and Torvaianica. The case described was an

exceptional event on the Roman coast (it is the first time a live cetacean has been stranded there).
The specimen was a three meters long adult male which weighed 270 kg, did not have external injuries but tilted to the left side while swimming and appeared to have obvious difficulties of flotation in association with tremors and occasional convulsions. Despite the support provided, at 10:00 on June 30 the animal died.
The post-mortem investigation pointed out that the animal died for a widespread infection by *Staphylococcus aureus*, characterized by a purulent bronchopneumonia, widely distributed in the right lung, and a suppurative meningitis and chorioditis with a subsequent severe cerebral edema. A moderate, multifocal, non-purulent multifocal meningo-encephalitis was also detected. Bio-molecular and immunohistochemical (IHC) investigations identified a CeMV infection. The absence of any *Toxoplasma gondii* infection was also assessed by PCR and IHC. The morbilliviral infection likely predisposed the *S. aureus* septicemia, representing the principal cause of death.

MEDICAL MANAGEMENT OF AN ACUTE RENAL INSUFFICIENCY IN A 15 YEAR OLD MALE *ARCTOCEPHALUS PUSILLUS PUSILLUS* AFTER A BILATERAL LENSECTOMY

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A 15-year old, healthy, 149 kg, intact, male South African fur seal underwent elective bilateral lensectomy. Pre-surgical physical examination and blood work were normal.
The subject was immobilized with medetomidine (0.01 mg/kg), midazolam (0.2 mg/kg) and butorphanol (0.08 mg/kg) and maintained on isoflurane. Hyperkalemia developing late in the procedure was controlled with sodium bicarbonate and fluids. Pre-anesthetic medications were reversed in recovery. Perioperatively, carprofen, tramadol, enrofloxacin, cefovecin and cefazolin were administered.
Acute postoperative convalescence was uneventful with the subject eating 50% of normal base within 24 hours and accepting all medications orally. Anorexia developed 6 days postoperatively. Blood analysis revealed an intrinsic renal insult. Administration of carprofen was suspended immediately. Intensive fluid therapy (12 L 0.9 % NaCl daily) was maintained and renal parameters monitored for efficacy in order to adapt treatment daily. Urine production was confirmed and urinalysis performed by catheterization. Recovery was slow but consistent.
The subject's condition resolved within 7 days and is now clinically well. Looking at this case retrospectively pre-surgical urine analysis would have been ideal to rule out pre-existing undetected renal abnormalities. A postoperative blood sample might have allowed earlier detection and treatment of renal insufficiency and possible myopathic conditions.

A FEW IDEAS ABOUT AQUATIC MAMMALS' OPHTHALMOLOGY

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How do I perform a proper ophthalmic exam in aquatic mammals? What instruments will we need, for instance? How to approach our animals? How to refer and report to a specialist?
The authors will explain the importance of trainers' work for early detection of eye problems, how to help the veterinarian for an eye exam, and why correct administration of treatments is so fundamental that it will make the difference between success or failure.
The authors will provide the basic steps to perform a basic ophthalmological exam by reviewing the basic anatomical details, focused on the principal diseases of the captive aquatic mammal eye. Prevention of predisposing factors will be discussed and first line treatments reviewed.
The purpose of the talk is to help veterinarians and trainers to improve their skills in clinical eye diseases in order to achieve a higher standard in animal welfare.

ULTRASOUND IN PINNIPEDS – A REVIEW

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Ultrasound has become a very useful tool for the medical control of marine mammals in the last years. It was mainly used for the follow-up of gestation – but has nowadays become a mean to control organs such as lungs, liver, kidneys or the reproductive tract, among others – or at least in dolphins. With the progress of medical training it is now also used in other marine mammal species, such as sea lions and seals. A couple of facilities have included it in the monthly physical check-ups of their pinnipeds and the purpose of this presentation is to show the different systems we are now able to see and some physiological differences or pathologies already identified.

IDENTIFICATION OF A C2 FRACTURE AND RACHIS DEVIATION THROUGH CT SCAN IN A CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS)*

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In November 2009, a bulging was identified on the left side of the neck in a 15y old male California sea lion by the training staff at Nausicaa. Though originally a reaction to a bite wound was considered, the animal seemed to show no discomfort and the swelling showed no reduction by the use of anti-inflammatory drug. Several differential diagnosis were then advanced, among others the development of a cold abscess, an enlarged lymph node (infectious, tuberculosis) or the presence of a tumoral mass. Blood results, x-Rays and ultrasound of the mass were unremarkable. Eventually it was decided to perform a CT scan and a biopsy at the same time, which would necessitate only one anesthesia. The procedure occurred at Oncovet, a small animal diagnostic imaging reference clinic. The good animal husbandry and training techniques used at the department helped tremendously to make the procedure a success. Contrast product was injected in the jugular on the R side through an IV catheter. Neck and thoracic scannings were performed in dorsal decubitus. No mass was visible in the cervical region. A deformation of the second cervical vertebra, with embedment in C1, due to an ancient fracture by compression, was identified, with development of a bony cal. This deformation provokes a lateral deviation of the rachis at the level of C1-C2 and the visible bulging. Consequent to this result, no biopsy was done. Recovery was uneventful and still shows no symptoms or reduction of the mass. Comparable fractures are described in dogs as a consequence to a heavy shock. The hypothesis is that the animal either felt hard on some rock or possibly had a heavy fight with some other animals in the group, being the lowest in the social ranking of this all castrated male colony.

TREATMENT OF TRAUMATIC LESIONS IN STRANDED HARBOUR SEALS (*PHOCA VITULINA*) AND GREY SEALS (*HALICHOERUS GRYPUS*) UNDER REHABILITATION.

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The Seal Rehabilitation and Research Centre (SRRC), Lenie 't Hart in Pieterburen, The Netherlands, has rehabilitated more than 500 harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) during 2011. The majority of seals stranded on the Dutch coast are orphaned pups or juvenile seals with a parasitic pneumonia. However, net entanglements and other traumatic lesions affecting the extremities of the seals are often seen in animals admitted to the SRRC for rehabilitation. Juvenile grey seals appear to be more susceptible to the interaction with nets from the fisheries and posterior complications (*P. vitulina* n=1, *H. grypus* n=7). Traumatic lesions affecting the extremities have been reported more often in very young harbour seals during 2011 (*P. vitulina* n=6, *H. grypus* n=1). Treating those injuries is challenging. Most cases are treated with wound care and conservative systemic antibiotic therapy but surgical procedures including amputations can be needed. Successful results have been achieved resulting in the releasable aptitude of the animals. This presentation will focus on two recent cases of flipper injuries in harbour seals, discussing the diagnosis, the medical and surgical treatment, rehabilitation course and their outcome.

FIELD ANESTHESIA OF LEOPARD SEALS (*HYDRURGA LETONYX*) AT CAPE SHIRREFF, WESTERN ANTARCTIC PENINSULA

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There are few studies on chemical immobilization of leopard seals in the field and it is frequently associated with high mortality rates (5-38%).
Here we present the preliminary results of an ongoing study on chemical immobilization of leopard seals, part of the U.S. Antarctic Marine Living Resources (AMLR) pinniped research program at Cape Shirreff (62°28'S, 60°46'W), Antarctic Peninsula. We chemically immobilized 11 adult female leopard seals (some animals twice), weighing from 380 to 501kg with a combination of butrophanol (50 mg/ml, Zoopharm, USA) at 0.1-0.2 mg/kg and midazolam (50 mg/ml, Zoopharm, USA) at 0.1-0.2 mg/kg. If necessary, additional 10 mg boluses of Midazolam were delivered to maintain the appropriate anesthetic plan. A mask was used to delivered oxygen at 3-5 l/min throughout the procedures.
During the anesthesia respiration rates (mean 7 b/pm), heart rates (mean77 b/pm), and blood pO2 saturation (mean 98%), were monitored and recorded. Reversal agents, naltrexone (Zoopharm, USA) at 0.1 mg/kg, and flumazenil (Sandoz, USA) at 0.005 mg/kg, were used to antagonize butorphanol and midazolam respectively.
No mortality events were recorded and no emergency procedures, as resuscitation, mechanical ventilation were needed. This report suggests a novel protocol for safe immobilization of leopard seals in the field.

MEDICAL MANAGEMENT OF FOUR BOTTLENOSE DOLPHIN CALVES FROM 0 TO 12 MONTHS

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Management and handling of bottlenose calves has much improved during the last years, allowing veterinarians to obtain a vast amount of information on clinical aspects of these animals. With the present study, we intend to share clinical information gained during physical examination of 4 male calves within the first year of life. All animals were examined for the first time between day 1 and day 4. Within the first year of life a total of 62 blood samples were collected from all animals. Body measurements were collected during the first capture and body length among subjects varied between 106.7 and 108 cm. Body weight was performed at first capture (from 13.7 to 16.85 Kg) and followed body weights were recorded with different frequency according to the animal. At the age of 7-8 months the animals' body weight ranged between 34.4 Kg and 75.5 Kg. During the first capture, all animals were treated with antibiotic as preventive treatment. Milk was collected from two mothers during the first

month of milking and analyzed for proximal composition and caloric value (172-240 Kcal/100gr).
The aim of this presentation is also to present some clinical cases we had to face with mothers and calves (aggressive attitude of mothers towards calves and a case of gastro-enteritis and meconium retention in one of the calves) during the first month of calves' life and describe problems most frequently faced when handling calves of few days.

HANDLING OF NEONATE DOLPHINS – THE PAST AND THE PRESENT

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Physical handling of neonate dolphins has been discussed controversially for many years. Experiencing neonate mortality, the frustration of not being able to counteract and the positive reports of other institutions being pro-active with newborn dolphins has led the keepers at Duisburg Zoo to change their way of working. This report will give an overview of the development of handling baby dolphins from a trainers' view. Different approaches have been developed over time. Historical ideologies were rejected and new ideas led to progressive paths and a better overview of the current situation. Achievements and problems of the past and the present will be outlined.

VETERINARY MANAGEMENT OF NEONATE DOLPHINS AT THE DUISBURG ZOO

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In late summer of 2011 Duisburg Zoo was facing the unique challenge and opportunity of managing the births of three bottlenose dolphins (*Tursiops truncatus*). Duisburg had to deal

with neonate mortality in the past, therefore a different approach in veterinary management and changes in husbandry were undertaken. Regular handling of neonates, application of a hand-held blood analyzer, glucometer, and additional nutritional support as well as prophylactic application of antibiotics in the first weeks of life were implemented into the veterinary schedule and led to survival and successful growth of all three dolphin calves. This presentation will provide an insight in the decision-making processes, the application of diagnostic methods and an overview of the „physiological" data collected during the first months of life.

AUTOMATIC LOCALIZATION BY ACOUSTIC METHODS OF "*ORCINUS ORCA*" INDIVIDUALS AT LORO PARQUE FACILITIES.

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Source localization, using more than one hydrophone, is a common measurement technique in the marine mammals conservation research, where the measurements are useful to provide information on location and behavior of these species. The orca facilities at Loro Parque include four third-generation orcas born in captivity, one born there and one more rescued. It can be difficult to distinguish between the calls of six animals if the location of the sound source is unknown.
To reduce the managing effort to identify the caller, we have developed a method to estimate the location of the sound source with more than one hydrophone, taking into account the geometry and obstacles that are present during the data acquisition. The equal time calculation, isochronous surfaces, lets us infer the source position and thereafter the caller. This is the first step to build an automatic method to classify the orca's calls. We present the geometrical arrangement of the pools and hydrophones, the method we used to calculate the isochronous surfaces, the obtained surfaces, and some considerations about the application of the method.

BRIDGING: BEYOND THE BASICS

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In animal training, a bridge is defined as a secondary or conditioned reinforcer that pinpoints the precise moment of a desired response. The skilled application of this tool is critical for clear communication with animals in training. Virtually every marine mammal trainer is familiar with the bridge and its use, however, there are several frequent mistakes unintentionally employed that result in behavioral break-down or reduced criteria in animal behaviors. These mistakes include: bridging with body language, taking an anthropomorphic approach to bridging, following a no-bridge event with another reinforcer and consistently bridging the same topography of a particular behavior, among other things. These errors impart inconsistent information to animals which results in confusion, frustration and decreased motivation. Appropriate use of this essential animal training tool requires greater attention to details and advanced trainer skill. The result of which is crisp resilient animal behavior.

MARINE MAMMAL FEEDING ECOLOGY AND ADAPTATIONS: FEEDING OPPORTUNITIES FOR MARINE MAMMALS HOUSED UNDER HUMAN CARE

Sabrina Brando
Animal Concepts

Marine mammals, from polar bears, manatees to sea otters, dolphins and sea lions, have different feeding ecologies and adaptations. The different species can occupy specific habitat and niches, and have species-specific adaptations to locating, tracking, processing and sometimes hiding captured prey. Whiskers, eyes, ears, paws and noses as well as echolocation are some of the sensory systems being used in feeding. Adapted dive physiology and breath holding facilitate the foraging and hunting underwater and many species have species-specific hunting strategies. Different tooth structures reflect the different prey species in toothed odontocetes, and the vibrissae of manatees, dugongs and walrus the adaptation to surface and or bottom feeding. Otters can use either paws and/or sensitive facial regions to obtain food. An estimated 95% of the food marine mammals receive comes from the trainer's hands, severely reducing the large behavioral and cognitive repertoire provided through natural foraging opportunities. Foraging is often cited to be one of the important activities for captive animals.
This talk will explore and illustrate the feeding ecology of many marine mammals housed and 1) provide examples to increase feeding opportunities and 2) discuss the balance and power of food used in training and enrichment.

PERSONALITY ASSESSMENT AND SOCIAL DYNAMICS IN TWO GROUPS OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

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In recent years there has been an increased interest in measuring animal personality. It is argued that personality in animals is expressed through the behaviors they display. Here we present the results from two studies where personality has been investigated in two different groups of captive bottlenose dolphins (*Tursiops truncatus*). Data from focal samplings were analyzed by using behavioral codings and the Five-factor model consisting of Openness to Experience, Conscientiousness, Extraversion, Agreeableness and Neuroticism. The results revealed that the seventeen dolphins display both distinct personality differences as well as similarities in these factors. By calculating coefficients of association it was found that dolphins also prefer the company of certain individuals over others. Knowledge of individual personality differences and its implications can be helpful in aspects such as management and reintroduction programs, evolution and genetics, and in providing a complementary perspective to explain other behavioral and cognitive studies.

COMPARING OBJECT PLAY IN CAPTIVE AND WILD DOLPHINS

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The purpose of our study was to examine whether there is a difference in the frequency of object play exhibited by dolphins from two groups. Data were collected with underwater video, and event sampled for play involving objects. From 159 hr of data, roughly 102 min featured object play: 75 min from RIMS and 26 min near Bimini. A total of 304 bouts of object play were documented at RIMS, while 73 bouts were observed around Bimini. Juvenile dolphins engaged in solo and mutual play more than twice that of other ages from both study groups. Male dolphins at RIMS exhibited object play slightly more than females: at Bimini, male dolphins were not observed to play with objects during interactions with conspecifics (mutual) and engaged in object play about half as often as female spotted dolphins. Combining both groups, dolphins played with 23 different objects that were grouped into six categories: biological debris, human made objects, inanimate objects, other, people, and trash. RIMS dolphins played most with all objects except people while Bimini dolphins interacted with sand more than any other object. The role of play is considered important to development and maintenance of social relationships and to learning skills required for survival.

DEVELOPMENT AND RECIPROCITY IN PECTORAL FIN CONTACT BETWEEN DOLPHINS

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Pectoral fin contact among dolphins is a conserved behavior consistently documented between individuals. Social and hygienic functions have been identified with partner preference exhibited. Interactions of a group of captive bottlenose dolphins (*Tursiops truncatus*) were video-documented over a nine year period. Development of pectoral fin contact between individuals was examined among 33 dolphins, which were roughly divided into eight groups – one group of five unrelated males and seven matrilines with two to five offspring. Immediate reciprocity in pectoral fin contact was observed more among non-kin than between related dolphins, whereas overall reciprocity seemed not related to kinship. Pectoral fin contact between same-sex versus mixed-sex sibling pairs was not significantly different, although non-kin same-sex pairs exchanged significantly more pectoral fin contact than mix-gender pairs. Adult females were more likely to initiate pectoral fin contact with non-weaned calves than older offspring. These results, coupled with conclusions from a comparative study of the same behavior among three distinct populations, suggest that contact via the pectoral fin might be used to send different messages depending on context and, potentially, personality. It seems that young dolphins must learn proper social decorum for application of pectoral fin contact when socializing.

BEYOND OPERANT CONDITIONING: RESULTS OF APPLYING A COGNITIVE-EMOTIONAL TRAINING MODEL TO SEA LIONS.

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Learning and animal behavior are more complex process than previously thought. The potential affective relations, their emotions, cognitive abilities and the possibility that a learned behavior can be self-rewarding are elements not used in their training except occasionally and intuitively. In order to incorporate them systematically we propose a training model divided in stages and phases, with standardized protocols that can be reproduced and evaluated objectively. This model builds and evaluates the quality of the relationship and communication between animal and trainer, the teaching of behaviors, their understanding through the cognitive process of problem resolution, and, finally, that its execution activates internal reinforcement processes in the animal. The model has been applied to two California sea lions and three South American sea lions of Madrid ZooAquarium, showing a decrease in the 'effort' inherent to training, the use of primary reinforcements and in training time. Moreover, it has been achieved that training sessions and exhibitions turn out more rewarding for the animals, therefore we recommend incorporating cognitive-emotional processes to the training of these and other species.

LARGE REPERTOIRE OF BEHAVIOR TRAINING WITH HARBOR SEALS IN A MULTIPLE SPECIES CONTEXT.

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Seaquarium is an aquarium located in south of France. It host 5 harbor seals (*Phoca vitulina*), all born in the facility. They are age from 8 months to 3 years old. They all began their training program as soon as they were weaned. They live and are trained in a common pool with 3 Patagonian sea lion (*Otaria flavescens*). Day after day, they learn different kinds of behavior like medical, dynamic, cognitive exercises, which are used to take care of them or for pedagogic presentations. Harbor seals know approximately 30 behaviors on 50. We use operant conditioning to teach them all behaviors. They are used to work alone, in group and with the Patagonian sea lions. They work in different place of the facility and in several rooms, on land or in water. They are part of our different pedagogic presentations. Seaquarium's harbor seals are so motivated and dynamic, that trainers get lots of work for the future.

SOLVING A LIFE-THREATENING REGURGITATION PROBLEM IN A CALIFORNIA SEA LION THROUGH TRAINING AND SATIATION.

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In 2009 in Amneville Zoo, France, the 8-year old captive born castrated male California sea lion "Gipsy", started to play with fish and anticipate the end of the sessions. His diet and show participation was readjusted. However by August of that year, he was actually leaving all sessions to regurgitate and lost a lot of weight going from 130 Kg down to 86 Kg at his lowest point. He had a couple of fits and was even declared dead by cardiac arrest after one, but unexpectedly "resuscitated" when brought away.By then we knew we were facing a life-threatening regurgitation problem.
We implemented several protocols for, as main objectives, controlling his weight loss, maintaining his weight and diminishing his regurgitation. These consisted of: 1) keeping him without access to water, because we had identified that he regurgitated only when having access to water. However, because you can't keep an animal permanently on the dry we decided 2) starting a second protocol, based this time on training because the problem was much more probably related to boredom. We taught him a lot of new behaviors. However, even so, and although we had increased his weight and were maintaining it, we were still not successful in reducing the regurgitation enough. It had become extremely self-reinforcing and, as we realized, a permanent problem. So we decided to work on a 3rd protocol 3) based on satiation, to try to fix the problem. We kept on doing all those extra training sessions that we were doing before but very often we would offer him a huge container of fish, up to 20 Kg at once and we also developed many more secondary reinforcements or ways, for him, to have access to extra food through toys, games etc.
The satiation program worked very well with him. We also made some mistakes along the way and learned from it but eventually all our objectives were reached. Gipsy is alive and healthy. He is back with the social group and performing shows with them.
As a general conclusion, we can say that regurgitation is a complex behavior that may probably never be completely eliminated. The satiation protocol works very well. However it's a permanent treatment. It should never be stopped.

TRAINING OF GERIATRIC MARINE MAMMALS

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As part of the ageing process, physical capacities of marine mammals (e.g. eyesight, hearing) can become naturally altered over time. These changes should be taken into account in the training process. We believe that if the training process is not adapted to these alterations, one can expect degradation or even complete extinction of the previously trained behaviours.

This could mean that fundamental biological information would no longer be possible to obtain by voluntary behaviour.
With this in mind, Zoomarine-Portugal decided to implement a specially designed training program for geriatric marine mammals, specifically tailored to the physical alterations observed. This allowed us to maintain a number of important welfare behaviours (e.g. oral hydration, ultrasound examination, urine and blood sampling) in older pinnipeds and dolphins with strong visual, hearing and other physical limitations.
Since many institutions within our community have geriatric marine mammals under their care, we propose to share the main aspects of this training programme.

Posters

RELATIVE QUANTITY JUDGMENTS IN THE SOUTH AMERICAN SEA LION (*OTARIA FLAVESCENS*), THE BELUGA WHALE (*DELPHINAPTERUS LEUCAS*) AND THE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS)*

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Objectives: We investigate quantitative skills and their mechanisms in three species of marine mammals, the South American sea lion, the beluga whale and the bottlenose dolphin. We test if, in absence of training, they are able to assess and select the larger of two sets of quantities and explore the nature of the mechanism underlying this capacity.
Mehods: Two different experimental tasks were used. In experiment 1, the two sets of quantities were presented simultaneously as whole sets. In experiment 2, the two sets of quantities were presented item-by-item and the totality of items was never visually available at the time of choice. The Beluga was also tested with an echoic condition; a visually opaque but echoically transparent box for echolocation. For each type of presentation, we analysed the effect of the ratio between quantities, the difference between quantities, and the total number of items presented.
Results: We provide empirical evidence (1) that the three species can make relative quantity judgments successfully and (2) that sea lions and belugas can also make these judgments mentally.
Conclusions: These findings suggest that the capacity to make relative quantities judgments may be rather widespread and that it may be supported by similar cognitive mechanisms.
POSTER PRESENTATION

EXPERIMENTAL EVIDENCE OF ACTION IMITATION IN KILLER WHALES (*ORCINUS ORCA*)

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Objectives: Comparative experimental studies of the imitative skills of nonhuman animals have focused mainly on the great apes. However, cetaceans are promising candidates to display imitative learning as they have evolved in socioecological settings that have selected for large brains, complex sociality, and coordinated hunting tactics. Here we tested the imitation capabilities of killer whales, *Orcinus orca*. Methods: We used a "do-as-other-does" paradigm in which 3 subjects witnessed a conspecific demonstrator's performance that included 15 familiar and 4 novel behaviours. Results: The three subjects (i) learned the copy command signal "Do that" very quickly, i.e., 20 trials on average; (ii) copied 100% of the demonstrator's familiar and novel actions; (iii) achieved full matches in the first attempt for 8-13 (out of 15) familiar and for 2 (out of 4) novel behaviours; and (iv) accurately copied the remaining behaviours within trials 8 and 15, respectively.
Conclusions: This study provides experimental evidence of body imitation, including production imitation, in killer whales that is comparable to that observed in dolphins tested under similar conditions. These findings suggest that some of the group-specific behavioural signatures reported in field studies of killer whales may be supported by imitative learning, which may drive the transmission of non-genetic, potentially fitness-enhancing group-specific traditions.

DEVELOPMENT OF A COMMON REPERTOIRE IN A NEW SOCIAL GROUP OF ORCAS (*ORCINUS ORCA*)

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Orcas (*Orcinus orca*) are animals with a hierarchical and complex social system, and with an extraordinarily rich vocal behaviour. Their vocal behaviour is characterized by vocal repertoires (dialects) that are exclusive of one particular pod or family group, and can differ among geographically close pods. These dialects are transferred through the matrilineal social structures. So, some pods can share particular calls depending on their matrilineal genealogical proximity. Recent researches seem to suggest that, unlike bird dialects, orca individual repertoires can be modified after acquisition. This peculiarity allows the group repertoires to evolve within short periods of time following a complex mechanism as yet not described.
The establishment of a new group of orcas in Loro Parque with four individuals, coming from two different groups, was an opportunity to study the development of a common repertoire. Analysis of the group dialect evolution can shed light on the vocal communication of the species, the plasticity of its vocal culture and the evolution of the dialects in time.

CITROBACTER FREUNDII SEPTICEMIA IN A NEWBORN STRANDED CUVIER´S BEAKED WHALE (*ZIPHIUS CAVIROSTRIS*)

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Citrobacter freundii has infrequently been identified in cetaceans, and although not reported as a cause of death, its likely importance in relation to dolphin health has been hypothesized. Here we report a case of a stranded neonatal beaked whale showing a bacteremia–septicemia caused by a systemic infection of *Citrobacter freundii.* Subcutaneous hemorrhage of the musculature of the dorsal area was observed without corresponding external skin marks. Lungs and pulmonary lymph nodes were edematous. The stomach was empty and meconium-like material was still present in the colorectal lumen. Microscopically, lungs showed diffuse marked congestion and alveolar proteinaceous edema. Diffuse subepithelial edema in the bronchial mucosa, epithelial cell debris, alveolar macrophages and squamous epithelial cells within the bronchial and bronchioalveolar spaces with numerous bacteria. Basophilic microorganisms with hematoxilin and eosin technique showed immunoreaction by using a serum anti-*C. freundii*. Microbiologic identification was done by morphologic and biochemical characterization of brain, lung, liver and kidney isolates. The isolates were identified definitively by molecular methods. The almost complete 16S rRNA gene of each isolate was sequenced. The 16S rRNA gene analyses revealed that the isolates were genotypically identical, and were 99.9% similar to the type strain of *Citrobacter freundii DSM 30039T (NR_028894).*

MOLECULAR DIAGNOSIS OF LOBOMYCOSIS-LIKE DISEASE IN A BOTTLENOSE DOLPHIN IN CAPTIVITY

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This study reports the diagnosis and molecular characterization of lobomycosis-like lesions in a captive bottlenose dolphin. The animal was born in the wild and brought from Cuba to a Spanish aquarium in 2002, arriving with two localized, slightly prominent whitish dermal lesions. During the winter of 2009, these lesions became active and ulcerated, taking on a

nodular and hyperplastic appearance. Cytology and histopathology analyses of two biopsies of the skin lesions showed the presence of yeast-like structures joined in chains, resembling those described for lobomycosis (*Lacazia loboi*). Results of sequencing the ribosomal DNA region and further phylogenetic analyses revealed that the fungus in the present case was more related to *Paracoccidioides brasiliensis* than to *L. loboi* observed in human patients. Moreover, the morphology of the yeast cells differed from those *L. loboi*. A successful treatment protocol based on topic and systemic terbinafine was administered.
Results suggest that the dolphin lobomycosis-like lesions might have been caused by a different fungus clustered inside the order *Onygenales*. Although dolphin lobomycosis-like lesion may be caused by a different yeast-like fungus than that previously described for human lobomycosis, the zoonotic potential of this yeast should be considered, especially related to captive animals.

CETACEANS IN GRAND COMORE: FIRST EVALUATION

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From the declaration of the Sanctuary of Cetaceans of the Indian Ocean, few information concerning cetaceans present in the Comoros Archipelago are available. Preliminary assessment on marine mammal populations showed the presence of 14 species of cetaceans (Kizka et al. 2006). The aim of the present research is to describe the distribution of cetaceans in the Grand Comore Sanctuary waters. We carried out surveys for a period of four months covering a surface of over 65 nautical miles square. Transects were done on a boat starting from the villages situated on the coast up to 2.6 miles offshore using photo-ID techniques and sound emission recording. We did 51 surveys for a total of 116 hours, 73 sightings and 721 miles. The more frequently observed species were *Tursiops sp*. (N=13) and *Stenella longirostris* (N=31). We analyzed vocalizations emitted by both species in order to compare the bio-acoustic structure of whistles with recordings done in the Atlantic Ocean. A deeper knowledge of distribution and behavior of cetaceans will allow to support the future development of educational and eco-touristic activities in the Marine Protected Area.

STUDY ON CETACEAN STRANDING, REHABILITATION, AND PATHOLOGY ALONG COASTS OF TAIWAN BETWEEN 2007 AND 2010

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The objective of this study was to describe cetacean strandings, conservation activities of volunteers, and pathology in south-western Taiwan. A total of 88 stranding events and 282 individuals (1990-2010) have been documented in this study. Sixty-six percent of these individuals stranded alive (including 31% of these released back to the ocean) and 34% were dead on the spot. The stranding events occurred at six coastal regions of Taiwan (Taichung, Chiayi, Tainan, Kaohsiung city, Kaohsiung country and Taitung). The bottlenose dolphin (*Tursiops truncatus*) was the most frequently stranding species (20 events, 21 individuals) in southwest of Taiwan. However, pygmy killer whale (*Feresa attenuata*) was the species with the largest number of stranded individuals (18 events, 126 individuals) because of several mass strandings. Detailed pathological examinations were carried out on 10 rehabilitated individuals between 2007 and 2010. These were four pygmy killer whales (*Feresa attenuata*), two Risso's dolphins (*Grampus griseus*), one rough-toothed dolphin (*Steno bredanensis*), one short-finned pilot whale (*Globicephala macrorhynchus*), one dwarf sperm whale (*Kogia simus*) and one melon-headed whale (*Peponocephala electra*. Apart from parasitic disease (70%), the most frequently detected lesions were of the pulmonary system (70%), the hepatic system (70%), the cardiovascular system (90%) and the GI system (90%). Two animals had histopathologic evidence of significant renal pathology, one had chronic pancreatitis (fluke infection), goiter, and focal fibrosis of the adrenal glands. From these cases of the rescued stranded cetacean, we found that cardiovascular diseases, pneumonia, and bacterial septicemia are the main factors resulting in the death of cetaceans. Based on these findings, it may be concluded that: key to the successful maintenance of these species include (1) Prophylactic anthelminthic and antibiotic therapy immediately postcapture; (2) Maintenance in microbiologically hygienic water quality at all times; (3) A proactive program of preventive medicine during the immediate postcapture and maintenance period of captivity.

IMPROVEMENT IN SAMPLING AND ANALYTICAL TECHNIQUES FOR ENDOCRINOLOGICAL AND TOXICOLOGICAL RESEARCHES IN MARINE MAMMALS

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During the last twenty years, the analysis techniques of contaminants and hormones in marine mammals improved significantly. In early years, the amount of samples necessary for the different analyses was very high and consequently the best way to obtain them was sampling during necropsies.

In recent years, new analytical techniques made it possible to dramatically reduce the sample amount required, so that now a biopsy of less than one gram of tissue can be enough for the majority of the researches. Also many non-invasive samples such us hair, urines and feces can give information about the endocrinological and toxicological state of animals, reducing at the same time the stress of constraint in live animals and the need of invasive sampling. Among the most exciting improvements is the possibility of sampling and analyzing urine from ice and

sand, obtaining the same valuable endocrinological result as from direct urine collection. In toxicology, new extraction techniques allow to treat with microwaves and solvent a small amount of sample, in a short time, for the analysis of heavy metals or organic compounds. Improvements in sampling and analytical techniques allow a better, easier and more continuous monitoring of animals' welfare and physiology. In this poster, we present the techniques currently used by our research group and provide information about sample size and storage.

USE OF RADIOGRAPHIC PARAMETERS FOR AGE ESTIMATION IN STRANDED CETACEANS.

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We used radiographic parameters to estimate the age of the wild cetaceans that strand along the Mediterranean Sea in the Region of Valencia (Spain). There are some descriptions of age estimation by bone maturation in bottlenose dolphins (*Tursiops truncatus*) until now. The use of radiographic parameters is a noninvasive technique which accurately assesses the age range of different species of cetaceans. In this study, pectoral flippers have been evaluated from five cetacean species which stranded along Spanish Mediterranean Sea (Region of Valencia): striped dolphin (*Stenella coeruleoalba)*, bottlenose dolphin *(Tursiops truncatus)* , common dolphin (*Delphinus delphis*), Risso´s dolphin (*Grampus griseus*) and fin whale (*Balaenoptera physalus*). The radiographic bone findings with anatomical findings found during the necropsy of these animals allowed us to establish the range of age. In conclusion, these preliminary results give us valuable information as a new tool for the future.

PULMONARY ANGIOMATOSIS AND HEMANGIOMA IN COMMON DOLPHINS (*DELPHINUS DELPHIS*) STRANDED IN CANARY ISLANDS

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Vascular tumors and disorders, like angiomatosis, are rarely described in cetacean species. The first report in the scientific literature of pulmonary hemangioma and angiomatosis in dolphins was described by Turnbull and Cowan (1999); however, the authors had firstly recognized angiomatosis in 1992, as an important factor of morbidity in common bottlenose dolphins

(*Tursiops truncatus).* This disease was characterized by proliferation of small, thick-walled blood vessels diffusely throughout the lungs, without inflammation, exudation, or alveolar haemorrhage and by proliferative hypervascularity in the visceral pleura as well as in lung-associated lymph nodes. Kuwamura et al., (2007) reported a chronic bronchopneumonia due to lungworm infestation in a common bottlenose dolphin associated with pulmonary vascular proliferation consistent with "pulmonary angiomatosis." They argued that parasites could play an important role on angiogenesis and/or acquired vascular anomalies. A retrospective histological study was carried out on lung samples from 35 common dolphins stranded in the Canary Islands coasts looking for morphological vascular changes and probable related causes. Twenty five out of 35 (71%) common dolphins showed focal or multifocal angiomatosis-like lesions. A high association between this type of vascular proliferation and parasitic infestation was observed. In addition, a single pulmonary cavernous hemangioma not previously reported in common dolphins is presented.

USE OF ENRICHMENT OBJECTS BY DOLPHINS IN CAPTIVITY.

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We presented 9 dolphins at Parc Asterix Delphinarium with 21 different objects in order to evaluate their effectiveness as enrichment objects. We classified the objects as simple or complex, and as floating or sinking, and we observed the behaviour of the dolphins in relation to the objects. During 17 hours of observations we observed 84.5 minutes of interaction between the dolphins and the objects and we noted that there was a large amount of individual variation in the use of the objects by the dolphins. The dolphins spent significantly more time manipulating the objects classified as simple and floating, by all the measures we used. There were significantly more manipulations using the head than with the fins, flukes or the body. Where we were able to observe which side the dolphins used to manipulate the object there was no significant handedness displayed either in relation to the body part used for manipulation, the object or the dolphin. These results indicate that dolphins show individual differences in the use to which they put objects presented as enrichment, there is no strong evidence of handedness and the best objects, in terms of the time spent manipulating them, are simple floating objects.

DECOMPRESSION LIKE SICKNESS IN RISSO´S DOLPHINS

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Although marine mammals appear to have developed adaptations to avoid most mechanical and physiological effects related to this type of diving disease (Kooyman, 1989), gas bubble-like lesions have been found in mass strandings of beaked whales (*family Ziphiidae*) associated in time and space with naval exercises (Fernandez et al., 2005). Several hypotheses have been proposed as a cause–effect relationship between MFA sonar use and these stranding events (Cox et al., 2006). One of them is the alteration of beaked whales' diving behaviour in response to MFA sonar exposure in such a manner that behavioural or physiological mechanisms employed for protecting against the formation of nitrogen gas (N2) bubbles are overridden (Fernández et al., 2005; Cox et al., 2006). According to this proposal, bubble evolution occurs as a result of severe alterations in dive behaviour (e.g. extremely rapid surfacing or remaining at the surface and possibly vigorously swimming).
Risso's dolphins inhabit deep oceanic and continental slope waters, generally 400-1,000 m deep, mostly occurring seaward of the continental slope. Risso´s dolphins have not been reported in atypical mass strandings linked to naval exercises, but "gas bubble" lesions have been found in single stranded Risso´s dolphins in UK and now in the Canary Islands. A description of these cases with the corresponding pathological findings will be presented in this communication as well as most likely linked cause/s.

PRELIMINARY RESULTS OF METAL CONTAMINATION IN CAPTIVE KILLER WHALES (*ORCINUS ORCA*)

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Most studies conducted on toxicology of killer whales (*Orcinus orca*) focus on the accumulation of organochlorine compounds. Few heavy metals studies have been published on stranded individuals. The aim of this work is to monitor the accumulation of inorganic compounds in live captive orcas, using non-invasive methods and the periodical veterinary blood sampling in animals kept in a controlled environment. Metal and metalloid contamination has been evaluated in four captive killer whales: one adult and one sub-adult male, and two adult females. Low levels of contaminants were found in all of the animals, with the exception of lead, showing higher levels than observed in wild bottlenose dolphins. One female gave birth during the period of study and showed a decrease in Pb and Fe concentrations and an increase in Zn levels after parturition (0.7 µg/ml vs 0.4 µg/ml; 303.6 µg/ml vs 273.90 µg/ml; 2.92 µg/ml vs 3.121 µg/ml respectively) These changes can be partially ascribed to lactation, as already found in humans and domestic animals.
This study is the first step for the definition of reference baseline values for hematic metals in killer whales, to be applied in the future in the study of wild animals.

SUCCESSES AND FAILURES OF A SYMBOLIC COMMUNICATION SYSTEM BETWEEN MAN AND DOLPHIN

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Symbolic communication offers the opportunity to develop an interspecific communication system. Such symbols can be visual, and we elected to assess dolphins' ability to learn visual symbols in the air and in the water. At Parc Asterix (France), two bottlenose dolphins (*Tursiops truncatus*) were tested in order to evaluate their capacity to associate three 2D-geometrical symbols with three arbitrary chosen objects and their ability to visually discriminate the symbols. Symbols were initially shown in the air, and subsequently in the water. The trainers' methods slightly differed; however they both used two symbols and carried out eight sessions. We calculated the rates of success for each situation (air *versus* underwater presentation). Both dolphins correctly associated each symbol with the corresponding object, either in the air or in the water. There was no significant difference in the dolphins' scores between these two situations. However, the animals performed better in visual discrimination when the symbols were presented underwater *versus* in the air: they found it difficult to discriminate the symbols in the air. Dolphins demonstrated an ability to understand and assimilate this symbolic system, although they appeared to be more efficient when 2D symbols are presented underwater.

TRAINING OF A NEONATE BOTTLENOSE DOLPHIN IN THE FIRST YEAR OF LIFE

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Training of marine mammals is widely described, but very few publications exist about the training progress of neonate bottlenose dolphins (*Tursiops truncatus*) before they reach the age of one year. During this time, dolphin calves are still fully dependant on their mother for milk and protection. In some cases, the mothers are so protective that they do not allow contact to the calves by human handlers, in other situations the calves start eating at an early age and may benefit from the entertainment of play interactions with the trainers, which in a playful way can be used to train the calf, either by capturing behaviours or traditional training. The present poster shows the trained behaviours in the first months of life, in a rather speedy progress of a dolphin calf which was extremely motivated to interact with trainers. Within less than 9 months, the calf was successfully trained for a series of medical behaviours (fecal sample, blow sample, full body examination), is in the process of being trained for voluntary blood take (remaining up to 4 minutes in stable position), and has accomplished a series of other play behaviours which imitate the adults.

REPORT OF CATARACT SURGERY IN HARBOUR SEALS

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Based on observing the extraction of cataracts in three harbor seals, this presentation will describe clinical and anatomical differences between these and other animals. It is important to teach the trainers to observe the animals and detect early signs of vision loss. Training is very important for examinations, photography of the eyes, and treatment. Dr Colitz has performed over 150 cataract removals in pinnipeds and each surgery can have its complications. Overall, success relies on executing the surgery to the last detail, training of the animals, and overall preparation of all involved. Anesthesia is the most important detail aside from the surgery and experienced veterinarians or anesthesiologist must be involved. The anesthesia in these animals differs vastly from terrestrial mammals and lack of this knowledge may result in loss of life.

OLFACTORY DISCRIMINATION ABILITY OF SOUTH AFRICAN FUR SEALS (*ARCTOCEPHALUS PUSILLUS*) FOR ENANTIOMERS

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Marine mammals are traditionally considered to have a poorly developed sense of smell. However, increasing evidence suggests that fur seals may use their sense of smell in a variety of behavioral contexts including social communication, foraging, food selection, and reproduction. Using a food-rewarded two-choice instrumental conditioning paradigm we assessed the ability of South African fur seals to discriminate between the odors of 12 pairs of enantiomers, that is, of odorants that are identical in structure except for chirality.
We found that the fur seals were able to discriminate between the majority of these perceptually similar odor pairs and thus were at least as proficient as human subjects in detecting subtle differences in odor quality. Further across-species comparisons of the fur seals' performance with that of other species tested in earlier studies on the same enantiomeric odor pairs support the notion that the relative size of olfactory brain structures is a poor predictor of olfactory discrimination capabilities. These results suggest that the sense of smell may play an important and hitherto underestimated role in regulating the behavior of fur seals.

STUDY REGARDING THE DETERMINATION OF BIOMETRIC CORRELATIONS IN BOTTLENOSE DOLPHIN *TURSIOPS TRUNCATUS* (MONTAGU, 1821)

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This research is carried on through the collection of 27 standard body measurements from wild animals (stranded specimens) and from animals hosted in controlled environment. The collected data include the following additional information: sex; date of birth (where available); age (if known); origin (if wild, the geographical area of origin must be known); injury (e.g. tip of rostrum or tip of pectoral/dorsal fin missing) and weight.
In order to have a statistically significant sample, measurements from specimens hosted in over 20 Italian and international facilities have been requested. The data is being studied using methods of descriptive statistics to highlight possible trend lines and subsequently using finer statistical methods to point out possible correlations between the measured parameters.
The study is aimed at developing a reliable age determination method for wild specimens; at designing and adjusting adequate prosthesis for injured specimens; at realizing anatomic stretchers and at constructing a dolphin model based on animation technology both for marine research and as a new original device for environmental education projects (Manfrini, V., Bellotti, D., 2009).

ORGANOLEPTIC EVALUATION OF THE DECAY OF VITAMIN TABLETS IN FISH OVER TIME

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Maintaining of cetaceans in captivity implies a change in their nutrition, using frozen fish. The freezing process leads to an elimination of parasites which otherwise would be transmitted to the mammal, but also to the loss of part of the fishes' nutritive components. Thus, a supplementation of certain compounds, especially vitamins, is necessary and current standard. However, some of these supplemented compounds, especially the hydrophilic ones, are easily washed out and degraded once inserted into food, i.e. when in contact with liquid and tissue. Research into the decay of the supplementation products and knowing how long they remain stable seemed important to us for good food management: The aim of the presented research was to find out the timing between for vitamin supplement insertion into the fish, and its decay, to ensure supplementation without loss of quality. Reference food for the trial was adult herring. Vitamins tablets were inserted into each individual fish through the gill openings,

one for each fish. At fixed times (from 5 minutes to 2 hours) an organoleptic control of oxidation level and dissolution of each tablet was performed, through a lateral opening. Differences were observed in oxidation status between male and female fish, so measurement of tissue pH was performed, to verify if this parameter could have influenced the stability of the tablets. Differences in pH value were found, with females presenting a lower pH. Inserting the tablet into gelatin provided a more stable environment for the tablets, at pH 5.5.
Given our preliminary results, it seems recommendable to integrate the vitamin supplements into food fish just prior, and no more than 20 minutes before its feeding.

INTEGRATION OF A BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) MOTHER AND CALF DYAD TO A SOCIAL GROUP OF CETACEANS

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The aims of this research were to study the integration of a mother and her six month old calf to the community of cetaceans hosted at Oltremare Park. The present social group consist a juvenile female Risso's dolphin and 6 bottlenose dolphins (one adult male, 4 adult females and the male calf). Observations were performed between 17th November 2010 and 27th February 2011 for a total amount of 70 hours. Data were sampled utilizing Focal Animal Sampling and recorded using the Continuous Recording. The integration process consisted of four different phases to minimize potential aggression toward the calf. Many social interactions were observed with a diversity of social displays. Social interactions were studied by quantitative analysis of the affiliative, agonistic and sexual interactions between the dyad and the rest of the group. Affiliative interactions within the dyad remained higher for all the period, but were also noticed between calf and another adult female. Agonistic interactions within the dyad and toward another adult female occurred to establish control of the calf. Sexual interactions were registered since the second to fourth phase between the calf and his mother, but also between the calf and another adult female. The integration between the dyad and the dominant male occurred without agonistic display between the animals.

EPILEPSY IN A GREY SEAL

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In May 2011 a six year old female grey seal (*Halichoerus grypus*) started displaying intermittent generalized seizures at Muenster Zoo. The animal continued with these attacks roughly seven days apart with similar patterns. Seizures of about three minutes long included turning around its own axis, twitching of the head and cramping. The animal was not accessible or responsive during the whole time.

The installation of video cameras after the first seizure allowed monitoring the animal full-time. Daily medical training permitted general examination, faecal analysis, x-ray and ultrasound. Blood sampling was also performed without anaesthesia. Further diagnostics such as magnetic resonance imaging, computer tomography and cerebrospinal puncture required general anaesthesia at the Veterinary University of Hanover.
Diagnostics did not reveal any pathological findings. The working diagnosis *idiopathic epilepsy* has been assessed and treatment started immediately. The anticonvulsant phenobarbital was given twice daily (1 mg/kg BW) as well as supplementary 2 g sodium chloride per kg fish. No more seizures have been observed or recorded. For monitoring the phenobarbital level, blood was taken on a regular basis.
Many disorders can cause signs commonly confused with epilepsy such as cardiovascular or respiratory disorders, narcolepsy, anaemia, organic disease (e.g. hepatic/renal dysfunction), hyperthyroidism, hypoglycaemia (e.g. insulinoma), electrolyte imbalances, neuromuscular disease (e.g. myasthenia gravis), intoxication and even abnormal behaviour. In this case most of these causes were ruled out.

EDUCATION THROUGH CONNECTION: ZOOLOGICAL TOURS AND EDUCATIONAL DEMONSTRATIONS AT ZOOMARINE ITALY AS A TOOL TO GET PEOPLE INVOLVED IN NATURE CONSERVATION

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During the last few years zoos and parks are focusing their attention to engaging the visitors on important topics such as conservation, biodiversity and related issues. Modern Zoos and Marine Parks are good examples of how non-conventional teaching methods can help to capture the interest of the public to topics of global importance.
Increasing importance is given to the bond between education and the world of animals everybody can see in a themed animal park. The Department of Education and Science of Zoomarine Italy proposes a set of special programs and activities designed for and aimed at all ages public: the Zoological Tours and the Educational Demonstration.
During a Zoological Tour (Dolphins, Parrots or Pinnipeds), the visitor is escorted by a biologist directly inside the zoological area where he can meet the animals very closely and discover everything about them and about their husbandry.

During the Educational Demonstration, trainers and biologist work together to show to a big auditorium (3,000 pax) the specimen, the husbandry, the training, the all-important medical behaviors and particularly the differences among the species and the problems they face living in the wild.
More than 4,000 persons participated to a Zoological Tour and more than 18,000 attended to an Educational Demonstration during 2011.

CENTRAL NUCLEUS OF AMYGDALE, LOCUS COERULEUS AND PARAVENTRICULAR NUCLEUS OF DOLPHIN´S BRAIN: WHERE AND WHY?

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The brain is still nowadays a great mystery for researchers. There is a great lack of information concerning neuroanatomy of cetaceans. Based on our previous results (Arbelo, 2007; Herraéz et al., 2007), we have intended to investigate those nuclei related with acute catecholaminergic stress-response. Massive catecholamine's liberation from the adrenal glands has been reported in alive stranded cetaceans, as a capture response (alarm reaction) (Cowan and Curry, 2008). But what could trigger such a dramatic reaction? We focused on three nuclei that are known to be involved with the alarm reaction: central nucleus of the amygdaloid complex (NCA), locus coeruleus (LC) and paraventricular nucleus (PVN).
Serial 50 µm sections of the amygdaloid complex, hypothalamus and brainstem (at the level of the inferior colliculus) were made using a sliding-microtome with a cooling unit. Sections were later stained for Nissl substance with thionin and immunocytochemically stained for Calbindin (NCA), Tyrosine Hydroxylase (LC) and Vasopressin (PVN) using the free-floating technique. This is a preliminary study; the final goal of our research is to obtain a microscopical description of these nuclei, in order to later detect the expression of some acute stress biomarkers like c-fos and HSP70 (Heat Shock Protein 70).

THE RESIDENT BOTTLENOSE DOLPHIN POPULATION IN NORTH WESTERN ADRIATIC: STUDY BY A SMALL TRANSECT SURVEY

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The north western coast of Adriatic sea is characterized by high level of urbanization, an intensive touristic season, overfishing and high ship traffic. Such a strong fishing effort is severely impacting ecosystems and it is the major direct (bycatch) or indirect (food competition) threat for cetaceans. In the area the most common marine mammal is the bottlenose dolphin *Tursiops truncatus*. A series of random trips with small boats were carried out in April and May 2010 in the area between the river Po delta and river Reno estuary, for 268 hours and 1079 nautical miles of transects. Eleven percent of observation time was spent in areas between 1 to 5 miles from the shore, 38% from 5,1 to 10, 6% from 10,1 to 15, 29% from 15,1 to 25 and 16% from 25,1 to 35 miles of distance. Sightings were 2 in April, one with 1 adult specimen and one with 3 adults, and 4 in May, one with 3 bottlenose dolphins and three encounters with groups of 10, 15 and 35 specimens respectively. The distance from the coast was equally distributed between the distance ranges 1-5 miles, 10-20 miles and 20-30 miles. No correlation with meteorological and sea condition were found. This first results confirm the presence of a viable population of bottlenose dolphins in the area. Further observations will be dedicated to understand if there is one large group or independent sub-units along the whole 150 km coastal area and to start a photo identification activity that can help understanding movement, social structure and conservation necessities.

MUSCULAR ATROPHIES IN STRANDED CETACEANS IN CANARY ISLANDS BETWEEN 1996-2008

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Gross and histopathologic evaluation of skeletal muscle was performed in 148 stranded cetaceans of 21 different species, undergoing post-mortem examination in Canary Islands. Animals were evaluated for grossly evident muscle lesions, and muscle samples were fixed in formalin, processed routinely, and stained with haematoxylin and eosin, *Phosphotungstic Acid Haematoxylin,* periodic acid-Schiff for glucogen, *and Von Kossa* for calcium. In addition, immunohistochemical examination was performed using primary monoclonal (fast and anti-slow myosin heavy chain) and polyclonal antibodies (myoglobin and fibrinogen).

Generalized or focal/multifocal muscular atrophy was the second most common muscular finding and it was detected in 42 animals (28,4%). Morphological diagnosis of muscular atrophy was made based on: decrease myofiber diameter, increased fiber size variation, morphological myofiber alterations, endomisial fibrosis, fiber type involved and alterations in fiber type distribution.

The combination of histopathological findings and overall data concerning the cause of death was useful in order to establish an aetiological diagnosis of muscular atrophy in these animals. Cachexia/malnutrition and senility were the first common cause of muscular atrophy in stranded cetaceans (40,5%, respectively), followed by denervation atrophy (16,7%) and disuse atrophy (2,4%). Morphological and lesional pattern of each atrophy group will be discussed.

THE ROLE OF ECHOLOCATION IN THE DEVELOPMENT OF OBJECT PERMANENCE IN BOTTLENOSE DOLPHINS

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Object permanence is the ability to understand that an object exists even after it has disappeared from view. Piaget proposed that development of object permanence takes place in six substages during the sensorimotor stage of development in human children with complete development occurring between the ages of 18 and 24 months. Few animal species have been successful at higher level object permanence. Although capable of second order mental representations in other tasks, the one study of object permanence in dolphins failed to demonstrate stage 6 capability. The current experiment investigated the developmental track of object permanence in bottlenose dolphins (*Tursiops truncatus*) and California sea lions (*Zalophus californianus)*. Second, this experiment tested both species to see if differences in object permanence development were the result of the dolphins' reliance on echolocation abilities. Echolocation may preclude the development of stage 6 object permanence in dolphins.

DEVELOPMENT OF DIAGNOSTIC PROTOCOL TO HIGHLIGHT SUB-CLINICAL UROLITHIASIS IN BOTTLENOSE DOLPHIN *TURSIOPS TRUNCATUS* (MONTAGU, 1821) HOSTED IN CONTROLLED ENVIRONMENT

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Urate uroliths are frequently found in bottlenose dolphins maintained in controlled environment. The aim of this study was to apply a diagnostic protocol for the control of urolithiasis in bottlenose dolphins without overt symptoms and to describe the therapeutic protocol employed in a subject with nephrolithiasis.
The study was conducted on five bottlenose dolphins hosted at the Zoomarine Italy. All dolphins underwent voluntary urine sampling every fifteen days, voluntary blood sampling and ultrasonographic examination of the urinary tract every two weeks. The planned clinical and diagnostic investigations were well tolerated in all subjects. Crystalluria caused by urate crystals associated with an isolated renal lesion likely due to uroliths was found in a female 11-year-old dolphin. In this subject, the use of oral rehydration therapy was associated with progressive improvement of crystalluria that was no more appreciable at successive follow-ups.

Use of serial urine and blood testing in addition to ultrasound evaluation of the urinary tract was useful for identification of sub-clinical urolithiasis in bottlenose dolphins. The early detection of abnormalities related to urolithiasis, such as crystalluria, prompted adoption of prophylactic and therapeutic actions aimed at avoiding more serious complications such as hydronephrosis and/or renal failure.

SOCIOGRAM FOCUSED ON BOTTLENOSE DOLPHIN INFANTS ON THE BASIS OF AGONISTIC TACTILE INTERACTIONS

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Analogously to affiliative contacts, even touches exchanged in agonistic contexts represent ordinary behaviours of bottlenose dolphins (*Tursiops truncatus*) ethogram. Aggressive displays gradually start to appear and differentiate during the first year of infants' life. This work aims to outline sociodiagrams that structure calves/group interactions in such a sensitive period. Both active and passive patterns of following behaviours: "push", "body slam", "bite", "rostrum hit" and "tail hit" were collected in relation to four calves born in different periods (1995, 1997, 2003 and 2007) at the Rimini Delfinario (Italy). A total of 1440 sessions lasting 30 minutes each (total 720 hours) were carried out and analyzed by the software Observer 5.0 (Noldus). The results revealed how physical aggressions, registered in a maximum of 3-4 events per hour per calf, were extremely rarer than affiliative contacts, expressed often over 30 times in the same interval. Indeed, even with frequencies growing up with age, conflicts involving calves happened only marginally. The infants were given forceful contacts mainly by the mother (over 50% of received acts). However, they did not systematically reciprocate the attacks (less than 30% of active contacts) but elected as preferred targets the second youngest member of the group (over 40% of active touches). While in the first case, the punishing/educative role of the mother was clear, the interactions with juveniles were fuzzy mixed in an often hardly distinguishable play/aggressive context. As a matter of hypothesis, as well as affiliative contacts, agonistic interactions are extremely complex dynamics. Hence, aggressive touches, normally interpreted as defence mechanisms or threat feedbacks, could be also perceived as constructive elements useful for group social organization or skills' acquisition for adult life.

NON-INVASIVE MONITORING OF HORMONAL PROFILE IN TWO PUBERTAL CAPTIVE POLAR BEARS (*URSUS MARITIMUS*): THYROID HORMONES

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Little research have been published concerning endocrine profiles in bears, the existing literature mainly focusing on sun (*Ursus malayanus*) and Formosan bear (*Ursus thibetanus formosanus*). Almost no information is available on the endocrine profile of polar bears (*Ursus maritimus*). Our present research aims at evaluating thyroid hormones profile in captive pubertal polar bears through the non-invasive measurement of thyroid hormones in feces. Samples were collected from two juvenile polar bear, one male and one female, and stored frozen until analysis. Hormone extraction was performed on 0.3 g homogenized feces with a double ethanol extraction and analyzed with commercial ELISA kits for thyroxine (T4) and triiodothyronine (T3). In all samples T4 presented higher concentrations than T3. The female bear showed higher level of fecal T3 than the male (40.67 ± 55.49 ng/g vs 25.87 ± 4.78 ng/g respectively). Similarly, a higher variation in T3 and T4 excretion was observed in the female bear with respect to male. Finally, a decrease in T4 excretion was observed in both bears in summer months.

Our preliminary results strongly suggest that feces can be used as tool for monitoring thyroid function in bears. This is also the first study presenting data concerning thyroid hormones from non-invasive, fecal samples in bears.

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