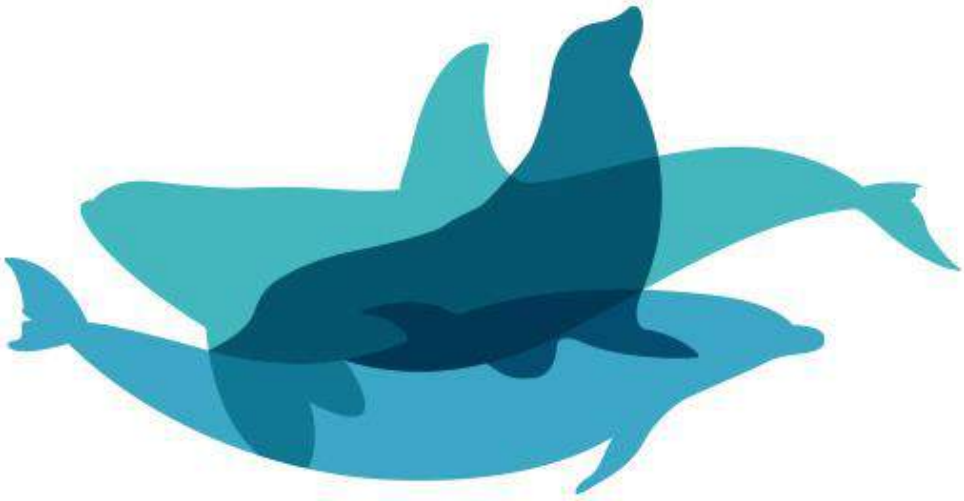

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Case series of fatal interspecific interaction between three cetacean species in Mediterranean waters.

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Aggressive interaction between different dolphin species is considered as part of the normal behaviour in mixed groups having been interpreted differently depending on authors. Additionally, fatal interactions have been commonly reported as a consequence of violent encounters between bottlenose dolphins (Tursiops truncatus) and harbour porpoises (Phocoena phocoena) in different populations but seldom or rarely reported with other larger cetacean species. We are presenting six cases of stripped dolphins (Stenella coeruleoalba) and one Risso´s dolphin (Grampus griseus) from the Valencia region (western Mediterranean basin) during the 2011-2015 period where the most probable cause of death established at post mortem examination was a traumatic interaction with bottlenose dolphins. Complete necropsy, histopathology, microbiology and molecular diagnosis reinforced presumptive diagnosis and ruled out evidence of other possible death causes including by-catch. All seven cases included rake marks, with inter-tooth spacing compatible with bottlenose dolphins, as well as severe internal damage such as skull and ribs fractures, brain haemorrhage, haemothorax or haemoabdomen. Microscopically main findings were severe tissue haemorrhages with related secondary changes. Main hypothesis for this presentation and potentially triggering local factors will be discussed during the communication. These data provide key information to better understanding of dolphin behaviour in the wild.

Health assessment project on free ranging dolphins in the Gulf of Mexico

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The project has been taking place in the Mexican southern portion of the Gulf of Mexico. This ecosystem is one of the largest natural wetland reserves in Mexico, and the population of bottlenose dolphins in this area is considered to be one of the largest and most studied concentrations of dolphins in the country. Since it is known that dolphins, among other charismatic marine mammals, are a sentinel species of the oceans and human health, the objective of this project is to perform a health assessment by collecting samples from them. Many harmful elements that may have possible impacts on the ecosystems will remain in their bodies permanently, so by understanding this and knowing their health status, we can indirectly assume the health status of the ecosystem. The knowledge acquired by working with dolphins maintained in human care allows veterinarians, researchers and specialists to develop proper techniques for their correct handling and sample collection. The development and adaptation of diagnostic techniques in marine mammal medicine is one of the greatest contributions to conservation; and the only way to develop such advances is by knowing and coexisting with individuals under human care.

Dynamite fishing and cetaceans in the Philippines

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Major effort has been invested into understanding the effects of anthropogenic sound on marine life. Small local and regional activities are rarely quantified or taken into account when investigating population and ecosystem stressors. Blast fishing is an illegal and unsustainable fishing method that is often reported in South East Asia and Africa. The effects on fish and reef building corals are well documented, yet there is limited information on the effects on other larger species and near shore predators. Several recent strandings in the Philippines have increasingly been linked to underwater explosions associated with blast fishing. The goal of this study was to measure the hearing of stranded dolphins including spinner (Stenella longirostris) rough toothed (Steno braviceps) and Indo-Pacific bottlenose dolphins (Tursiops aduncus) that were rehabilitated in Subic Bay at Ocean Adventure, in cooperation with the Philippine Marine Mammal Stranding Network and Wildlife in Need. Hearing measurements were conducted using non-invasive auditory brainstem responses (ABR) on a total of seven animals.All the results on stranded animals with vestibular clinical signs indicated elevated thresholds and various degrees of hearing loss. These results provide evidence of hearing loss associated with impulsive sound exposure due to blast fishing.

Dolphins in isolation: Irrawaddy Dolphins of Songkhla Lake

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The Irrawaddy Dolphin (Orcaella brevirostris) is globally at risk. Its occurrence is limited to certain areas of the Bay of Bengal and Southeast Asia. The freshwater populations of the species are threatened with extinction in the near future, and urgent conservation measures are required for long-term survival. There are few populations of Irrawaddy Dolphins in Thailand and the only freshwater population in this country is found in Songkhla Lake, which has three parts: Thale Noi (area=27 km2, depth=0.5 to 1.9 m); Upper and Middle Thale Sab, sometimes collectively referred to as Thale Luang (area=830 km2, depth=1 to 4 m); and Lower Thale Sab, or simply Thale Sab (area=185 km2, depth=1.4 to 2.0 m). Only the Upper Thale Luang is currently known to harbor Irrawaddy Dolphins. The other parts south of this are almost entirely covered with fishing gears which have become barriers for dolphin movement within and outside the lake. Fishing gears can harm dolphins directly (entanglement) and indirectly (competition for fish); but sedimentation and other environmental pollutants are highly probable threats as well.

Together with our colleagues from the Chulalongkorn University and the Department of Marine and Coastal Resources (Thailand), we conducted boat-based and aerial surveys of this population from 28 April to 02 May 2015. There was only a single pod of at least 20 individuals. Of these, images of the dorsal fins of 12 animals were taken for photo-identification. A cow-calf pair was also sighted, indicative of at least one breeding female.

Measures are currently in place to reduce, and ultimately stop, the use of fishing gear in Thale Luang. However, other environmental threats and the extremely low population may warrant more drastic measures including translocation. Determination of distinct population segments may help in long-term survival plans.

The role of aquariums in conservation research, education and public outreach

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Trained animals under managed care are an important resource for public outreach, education, and conservation research. However, coordination between researchers, trainers, veterinarians, students and staff is crucial to create a successful program. At the Oceanográfic, Valencia, Spain, we have created a new Research Master Plan with 5 research programs (physiology and energetics, marine animal health, conservation research, animal welfare, and marine mammals and humans). Each program consists of several research lines, and animal training is vital for many of these lines. This new initiative includes an extensive investment in a new research department with 5 full-time researchers and 3 PhD students to coordinate the research effort. We have a new team of senior trainers to lead and develop our new philosophy of animal training and husbandry, a team of 5 full-time veterinarians to supervise animal welfare, and research trials. The Oceanográfic has a new ethics and welfare committee that approves research proposals, and a new focus on public education and conservation through research with a strong support on public media. This presentation will outline our strategy for this new vision of Oceanográfic as a leading research, conservation and education center in Europe, integrating the activity of the different aquarium sectors and especially highlighting the role of animal trainers.

Medical training in polar bears

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Introduction:

The medical training of polar bears at the Nuremberg Zoo was established a few years ago. Positive reinforcement was applied and the training sessions took place in the indoor enclosures.

Training steps:

Our aim was to improve the animal-trainer relation and the animal management. We wanted to be able to call the animals in and so the gating was one of the main goals. In a next step the handling of pregnant females was improved and this helped a lot during the management after birth. In the following years the medical training was enhanced and gives us now the possibility to: (1) weigh the animals, (2)have an excellent crate training and therefore a relaxed way to transport the animals,

(3) participate in scientific research (e.g. the hair cortisol study), (4) sample the animals in case of diseases and (5) have a relaxed access in case a veterinary visit becomes necessary.

Conclusion:

In our situation it proved to be important to have a good training location (with the necessary cage adjustments) and single responsible trainer for every new training exercise.

Swimming like a Dolphin – a bionic project

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Technical swimming fins for humans are in use for more than 100 years, but the efficiency always remained below 20% even with competition-grade-monofins.
On the other hand, Whales and Dolphins transform 90 – 95% of the applied force into thrust.

As an engineer's approach I had a close look at the involved mechanisms, resulting in a mermaid-style monofin which makes the kinematics and fluiddynamic "secrets" of a Dolphin's fin available for human swimmers and divers. The presentation also explains how conventional artificial fins work (or don't work) and why a Dolphin can swim with 80% less effort (even with an animal trainer hanging on its fins).

The whole bionic project is based on experimental engineering and research at dolphinariums. The result is proven by a video-documented swimming performance which water sports experts still would consider to be "impossible". The resulting product might also allow completely new possibilities in animal training and presentation.

Personality in Zoo-Housed Killer whales: a rating approach based on Five Factor Model

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The comparative study of animal personality has received a large interest in recent years. One of the goals is to detect whether nonhuman species have personality structures homologous to ours. So far, some studies have analyzed from different perspective personality in cetaceans (mainly in bottlenose dolphins), however none of them have analyzed the factorial structure of any species in this order. Our objective was to evaluate a sample of zoo-housed killer whales (n = 6) adapting one of the most widely used questionnaire in humans and animals: the Five Factor Model (FFM). Thirty-eight personality descriptive adjectives were rated by 21 keepers, researchers and complementary staff. Principal components analysis and Regularized Exploratory Factor Analysis revealed three statistically significant factors with acceptable standards of inter-rater reliability and validity, accounting for 54,17% of the variance. The first factor reflected a Conscientiousness factor with an Agreeableness component, the second one revealed an Extraversion factor, and the third one indicated a Neuroticism factor. The results were similar to those obtained for human and chimpanzees in previous studies, indicating that may possibly be explained as a result of convergent adaptive traits despite a deep evolutionary divergence, adaptation to physically dissimilar environments, and very different neuroanatomical organization.

Comparing day and night vocalizations in *Orcinus orca*

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Very little is known about the nocturnal vocal behavior of killer whales (Orcinus orca). Using data from a continuous recording hydrophone network we were able to investigate differences between day and night vocalizations of a group of captive specimens.

Our study compares the vocalizations of a group of four orcas over a period of 24 hours for three consecutive years (2009 – 2011). Vocalizations were recorded by the build in hydrophone network in the Orca Ocean facilities at Loro Parque (Tenerife, Spain). The calls were detected, clipped and stored in a database with an ad hoc detection software and later on classified according to the 2015 version of “Loro Parque Dialect”, which includes a total of 34 different call types in 11 classes.

More than 14.500 sound events could be classified for these three days, which were analyzed based on different criteria, including day times and allocation of the animals, while considering both abundance and distribution of the different call types.

This study is one of the first to focus on nocturnal differences in vocalization in Orcinus orca.

Through the eyes of the beholder - social representations of visitors and educators about dolphins

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It seems deeply rooted in our nature to identify and define behavioral patterns for all living animals. Mentioning words as ‘snake’ or 'spider' usually conjures negative emotions and stereotypes amongst many zoo visitors. On the contrary, words as 'dolphin' or 'seal' usually generate good emotions and mental images. Considering this, 150 young Zoomarine visitors and 150 international environmental educators were asked to participate in a free association recall task. Participants were asked to produce the first four words to come to mind that were related in any specified way to the presented cue word 'dolphin'. 229 different words were collected within the two groups, with under 30% word similarity between groups. As expected, children shared less than half of word heterogeneity and mostly positive emotional words. Educators, in contrast, showed a broader range of word heterogeneity and a dominance of classification and environment related words. Co-occurrence was also measured to identify prevalent word associations and saliences.

Results call for a philosophical discussion about the importance of word choice in conservation messaging. It also imposes a fundamental question: are educators, both operating within zoological settings and those not associated to the zoological community, unintentionally misleading visitors to possible learning obstacles?

A big challenge: training staff and animals for contemporary pregnancies of two primiparae females of bottlenose dolphin (*Tursiops truncatus)*

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Quina and Leah, two young females of bottlenose dolphin, remained pregnant in September 2011 at the same time; they had the same delivery estimated date. To manage one was already a challenge, manage both it looked like an odyssey, specially being the first time. Our biggest priority was to train all staff to manage two hypothetical simultaneous births, the possible scenarios, how to handle the calves and train our dolphins to the event. Thanks to several simulations, we reached a high level of preparation. After births, our simulations were essential to manage procedures in safety and without hesitation from staff.

Development, evaluation and results of a yearly enrichment plan in Bottlenose Dolphins (*Tursiops truncatus*)

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The development and implementation of an enrichment plan allows for better welfare in controlled environments, promoting natural behaviours and reducing behaviours associated to captivity.

An enrichment plan for Bottlenose Dolphins started in Mundomar in 2014, in a group of 4 males and 6 females, with ages varying between 1 and 36 years.

The enrichment devices were developed, constructed and evaluated. A total of 42 devices were newly made, not bought, and made available to the animals during the year. The final objective was to create a catalogue with all the available devices once evaluated, classified by types of environmental enrichment.

Evaluation of the set goals for each device was done through visual observations. Types of enrichment were differentiated using a colour code, as well as the responses or attitudes obtained from the animals. The final evaluation produced a list of recommendations per individual or for the entire group, based on the success of each individual enrichment device.

Enrichment is used currently as another reinforcement tool during animal training. An increase in the number of interactions with the enrichment items and in the expected responses of the animals has contributed to a better health status of the dolphins this past year.

Schedule of training sessions affects specific dolphin behaviours

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Animals kept under human care perform daily behavioural routines dictated to varying extents by human-controlled events or periods of time. However it has not been studied how schedules affect behaviour during their "free-time"; we chose to investigate this in bottlenose dolphins (*Tursiops truncatus*), selecting 29 individuals from four groups within three European facilities. Time budget analyses revealed that among the behaviours studied the dolphins spent the most time engaged in synchronous swimming, 35% of observation time on average, and within this spent most time (on average 22%) swimming at slow speeds and in close proximity to each other. Slow-close synchronous swimming peaked shortly after training sessions and was low shortly before the next session. Play behaviour was seen more frequently in juveniles although only when time was a considered factor, and anticipatory behaviour (6% of observation) was significantly higher shortly before sessions and lower afterwards, allowing us to propose a set of validated anticipatory behaviours for dolphins. We further conclude that the schedule of training sessions affected important social behaviours, slow-close synchronous swimming and age-dependent play in bottlenose dolphins, which has implications for managing the animals' environment in their free-time, designing congruous schedules, and could aid in measuring positive welfare.

Mental vs Medical:small facility,large impact, creative strategy for expanding and implementing husbandry practices with limited resources.

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Onmega Dolphin Park, located in Turkey, houses five bottlenose dolphins, displaying undesired conducts e.g refusal and avoidance towards previously trained husbandry behaviors. Since 2011, the trainer team persuit the same goal, to increase the welfare of the animals using positive conditioning to retrain the dolphins consistently.The challenge was the lack of effectiveness in husbandry practices, and a Preventative Medical Training Program (PMTP).We focused in developing trust among dolphins and with the trainers, introducing a socialization as well as increasing the positive association of the trainers, achieving a high comfort level while performing husbandry training. This allowed us to concentrate at create a PMTP using this methods (among others, e.g):

1) Extinguish aversion
2) Desensitization of a variety stimulation.
3) Avoid involuntary procedures.
4) Set the animal to succeed.

We experienced success observing animal´s willingness to cooperate increasing, extinguishing behavioral problems (avoidance, refusal, aversion), despite aspects such as age, conditioning level or individual character.

Through our PMTP we diminished stress levels allowing us to complete a variety of voluntary medical behaviors (samplings, ultrasounds, endoscopies, injections, etc…), increasing safety, while increasing welfare for the animals.

Environmental enrichment positively impacts Black Sea Bottlenose dolphins' behaviors

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Dolphinaria use different kinds of environmental enrichment, defined as any technique designed to improve animals' biological functioning and to encourage animals under human care to display their complete ethogram. However, few facilities follow a rigorous scientific program to evaluate and monitor the effectiveness of this practice. This study aims to assess how various objects introduced to dolphins in their free-time impact their behaviors while participating in educational programs.

The present study was conducted on 6 Black Sea bottlenose dolphins (*Tursiops truncatus ponticus*) in Attica Zoological Park (Greece). Twenty different objects, 4 types of ice - cubes with and without fish inside and gelatin were provided randomly to the animals. Using object - focal - sampling method, we collected behavioral data before educational presentations during forty 15 min sessions with various enrichments and forty 15 min sessions without enrichment. Then we compared dolphins' behaviors during the eighty public presentations (sessions with vs without enrichment). Preliminary results show that providing the dolphins an enriching environment in their free-time reduces aggressive behaviors between males, enhances social interactions and decrease stereotypical behaviors during the following educational program. In conclusion, we discuss ways to assess the effectiveness of environmental enrichments on dolphins under human care.

Five cases of Dirofilaria immitis natural infection in pinnipeds in a zoological context in Portugal

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Despite the importance of cardiopulmonary parasites in pinnipeds, few studies are currently available in literature. Therefore, a study was conducted in the pinniped group of "Zoomarine", an oceanographic park in Algarve, southern Portugal.
Blood samples were collected from 19 individuals from three species: 6 common seal (*Phoca vitulina*); 2 grey seal (*Halichoerus grypus*); 6 California sea lion (*Zalophus californianus)* and 6 from the subspecies, South African fur seal (*Arctocephalus pusillus pusillus*). Samples were tested using a Dirofilaria immitis commercial antigen test (WITNESS®Dirofilaria) and modified Knott's technique. Two samples from common seals tested positive in the antigen test and one sample from a South African fur seal showed microfilariae consistent with D. immitis.

Additionally, two necropsies of South African fur seals showed several nematodes in the right ventricle and pulmonary arteries, morphologically confirmed as D. immitis adult specimens, with concomitant histopathological lesions of heartworm infection.

To the authors knowledge, this represents the first world report of D. immitis infection in *A. p. pusillus* and the first report of this nematode in a pinniped group in Portugal.
Considering the worldwide emergence of dirofilariosis, these data will increase the awareness of veterinarian practitioners to adopt control measures against vectors and perform preventive therapy in pinnipeds kept in zoological contexts.

Follow up of a complicated case of fungal tracheobronchial mass in a bottlenose dolphin (*Tursiops truncatus*).

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A bottlenose dolphin was diagnosed of tracheobronchial mass that was removed with flexible bronchoscope and a cryosonde. A Zygomycete was confirmed as the causative agent. Posaconazole therapy (5 mg/kgPO-BID) was prescribed based on sensitivity testing also being the most effective reported treatment for Zygomycosis in dolphins.Post-surgical follow up consisted of serum levels of posaconazole, specific serum fungal tests, blowhole cytology, sputum cultures, CT-scans and bronchoscopies. After five months of treatment, posaconazole dose was reduced to 2 mg/kgPO-BID based on in vitro sensitivity and antifungal blood levels. Fungal growth restarted on culture one week later and posaconazole dose was increased again to previous regime, without therapeutic success. Bronchoscopy revealed new lesions that were also bronchoscopically removed with argon plasma coagulation. This time identification of Rhizopus microsporus showed higher sensitivity to amphotericin-B and terbinafine. Posaconazole was then discontinued and nebulized liposomal amphotericin-B (25mg/BID) was started showing evident improvement of lesions and stopping fungal growth on culture just after one week of treatment. Amphotericin was maintained for three months without any side effects leading to a full resolution. Since then close monitoring has been established revealing no signs of recidiva in the following three months at the moment of submitting this abstract.

Development of a fluorescent microbead-based immunoassay for anti-Erysipelothrix rhusiopathiae antibody detection in captive and free-ranging cetaceans

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Detection of anti-E. rhusiopathiae antibodies allows for the assessment of vaccine efficiency in captive cetaceans permitting also the detection of previous exposure in unvaccinated captive and free-ranging cetaceans. Fluorescent microbead-based immunoassay (FMIA) main advantage is the capacity to be multiplexed to allow the determination of up to 100 different analytes per sample, reducing cost, time and sample volume required. In the present study, FMIA for detection of anti-Erysipelothrix rhusiopathiae antibodies was adapted to cetaceans. The technique was validated and adjusted using serum samples from 10 vaccinated and 20 unvaccinated captive bottlenose dolphins (*Tursiops truncatus*). The FMIA was then used to analyze samples from 15 free-ranging dolphins stranded alive in the Valencian Mediterranean coast, Spain, between 2006 and 2014, which comprised 11 striped dolphins (*Stenella coeruleoalba*), 3 Risso's dolphins (*Grampus griseus*) and 1 bottlenose dolphin which died from E. rhusiopathiae septicemia, detecting antibodies in one of the Rissos's dolphin evaluated.
The determination of antibodies against E. rhusiopathiae in all vaccinated individuals and in none unvaccinated dolphin validated this novel technique. Furthermore, its diagnostic application in free-ranging cetaceans is particularly interesting for health assessment monitoring in wild populations.

Poxvirus detection in skin lesions in wild and captive cetaceans

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Many poxvirus infections have been described in marine mammals. In cetaceans these viruses produce ring or “tattoo-like” skin lesions (different shaped blemishes). Given the epidemiological and ecological interest of disease in these animals as markers of marine health, it is important to find out more about this group of viruses.73 skin lesions were sampled from 34 cetaceans found stranded on the Valencian Mediterranean coast, Spain, between the years 2010-2015 as well as 14 cetaceans belonging to different Spanish aquatic animal centers, all belonging to the species: *Tursiops truncatus* (Common bottlenose dolphin), *Stenella coeruleoalba* (Striped dolphin), *Delphinus delphis* (Short-beaked common dolphin) and *Delphinapterus leucas* (Beluga whale). They were analyzed using a conventional PCR designed by Bracht et. al (2006) targeting the gene of the polymerase protein of poxvirus. The results obtained included 4 positive samples along with 33 dubious samples that had nonspecific bands, some of which showed a faint band at the right height. Positive samples were sequenced and a phylogenetic analysis was performed resulting in cetaceanpoxvirus 1. One of the dubious samples interestingly turned out to be a different strain of poxvirus confirming the suspicion that more molecular information is needed for the detection of these infections.

Determination of the Main Reference Values in Ultrasound Examination of the Gastrointestinal Tract in Clinically Healthy Bottlenose Dolphins (*Tursiops truncatus*)

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This study aimed to establish: a) Reference Intervals (RI) for normal cytological findings in gastric samples, b) provide a detailed description of the ultrasonographic appearance of the normal gastrointestinal region, c) set up a consistent and standardized method to measure the entire wall thickness in forestomach, fundic stomach, pyloric stomach, and bowel in healthy bottlenose dolphins (*Tursiops truncatus*). This project was based, for the first time, on the “Guidelines for the Determination of RI in Veterinary Species. 31 bottlenose dolphins were examined and sampled by voluntary behaviour: they were considered healthy based on physical examination, laboratory evaluation, and histological assessment of the mucosa of the gastric chambers. Ultrasonography plays an important role in modern-day preventative medicine because it’s a non-invasive technique, it’s safe, and it can be performed routinely using medical behaviors. This study expands the present knowledge in bottlenose dolphins, providing additional relevant data and allowing the clinician the possibility to evaluate gastric health more extensively.

Case Report: Deep Pyoderma in a Harbour Seal (*Phoca vitulina*)

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Most skin infections in pinnipeds are considered secondary to systemic bacterial infections, traumatic processes or problems during the moult. Seals are prone to suffer dermatological diseases due to their terrestrial locomotion method.

In July of 2014 a nine-year-old harbour seal showed apathy, anorexia, unilateral corneal ulcer and skin lesions concurrent with the moult. This animal had a previous medical history of recurrent dermatological processes.

The nature of the lesions, skin cytology and blood analysis provided a diagnosis of superficial pyoderma. An initial treatment with oral enrofloxacin was prescribed. After ten days, the problem worsened to a deep pyoderma. A new combined topical and systemic treatment was then established, consisting of clorhexidine baths, a change in salinity and temperature of the medical pool, intravenous fluids and clindamicin and cefovecin parenteral administration. This animal was trained for medical behaviours that allowed applying this combined treatment without the need of physical restriction, maximizing its success.

Remission of the dermatological process occurred within 8 weeks of starting the treatment. Combined topical and systemic treatments in skin diseases are well described in other species. They accelerate healing, diminish the duration of antibiotic therapy and reduce swelling and pain, improving the overall status of the patient.

New cetacean Morbillivirus with Atlantic origin circulating in Mediterranean Striped Dolphin population

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Dolphin morbillivirus (DMV) causes epizootic outbreaks with associated mass mortalities of dolphins. In the Mediterranean Sea there have been 3 outbreaks (1990, 2007, 2011) of DMV with phylogenetically related viral sequences. We report two striped dolphins (*Stenella coeruleoalba*) stranded on the Spanish Mediterranean coast of Valencia, infected with a sequence of DMV novel to the Mediterranean sea. Animal 1 stranded in 2012 and showed evidence of systemic morbillivirus infection (MI), according to histopathology findings and DMV detection (immunohistochemistry, PCR) in several organs. Animal 2 stranded in 2014, and had chronic encephalitis and DMV antigen detection confined to the central nervous system. Characteristic DMV nucleotide and amino acid sequences of viral phosphoprotein (P), nucleoprotein and hemagglutinin fragments were identical in both animals. The P sequence was closer to that from the strain that caused the 1990 epizootic outbreak than to the sequences of the 2007 and 2011 viral strains. Additionally, this sequence was identical to a sequence found in a striped dolphin stranded in the Canary Islands (Northeast Atlantic) with systemic infection in 2011. Our results indicate coexistence of different pathogenic viral sequences among these populations, and suggest an Atlantic origin for this novel sequence in the Mediterranean Sea.

Transthoracic echocardiography as a non – invasive tool to investigate cardiac physiology, health and physical performance

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The aims of this study were to measure recovery of cardiac function following exercise and to assess differences in cardiac function between animals that perform at least 4 daily high intensity activities (high intensity group) with those that do not (low intensity group). Heart rate (HF) , stroke volume (SV) , and cardiac output (CO) were measured in 12 Atlantic bottlenose dolphins (Tursiops truncates, six male and six female) and one female Black sea Bottlenose dolphin (*T.T. ponticus*) at rest and 1,3 and 4 minutes after high intensity exercise. There were no significant differences in resting and post exercise cardiac variables between the dolphins in the high and low intensity exercise groups. Thus, 4 daily high intensity exercises do not result in increased physical performance or alter cardiovascular capacity. These results provide novel information on the cardiac physiology of cetaceans during rest and following exercise and provide baseline clinical information for diagnostic purposes. In future studies we aim to investigate the relationship between cardiac and respiratory function and how stress alters cardiac physiology. Estimating changes of pulmoarterial and systemic pressures by assessing medium pressures of mitral- and tricuspid regurgitation aswell as determining systemic blood flow before, during and after breath hold up to 5 minutes will give vital data to understand how marine mammals may respond to environmental change or exposure to man made sound.

Artificial milk supplementation to manatee calves (*Trichechus manatus manatus*, Linnaeus 1758)

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The authors will present 2 cases of successful artificial hand rearing of Antillean manatee calves (*Trichechus manatus manatus*, Linnaeus 1758).

Both calves were males, born in two different zoological settings, from young and inexperienced females who failed initial nursing. They were maintained together with their mothers, in a social context, and manually fed with artificial milk formulation.

All individuals showed dyad bonding and natural nursing approximation behavior from the first week, but natural suckling from their mothers started only after approximately 6 weeks from birth.

All the details on the husbandry (handling of the calves and mothers) and energetics (strategy of artificial milk preparation and scheduled administration) have been analyzed and will be presented. Information about the logistics of routine data collection, staff management and impact of such operation within the organization, will also be provided.

The outcome of both cases showed that human intervention has proven to be mandatory for their survival.

All relevant information and practical experiences are, therefore, shared with the audience, in order to further expand the current knowledge available on the captive management of such a challenging endangered aquatic mammal species.

Intravaginal artificial insemination in Bottlenose Dolphins (*Tursiops truncatus*) using refrigerated semen and medical training

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Advances in artificial reproductive techniques facilitate the development of genetically controlled reproduction programs in captive populations.

Three bottlenose dolphin females were artificially inseminated in Mundomar in the summer of 2015. The animals were between 20 and 27 years old. The semen used was fresh and refrigerated for less than three days post collection. All three females were trained three months prior to the actual procedure and allowed vaginal catheterization, replicating artificial insemination techniques.

The oestrus cycles were synchronised using oral altrenogest at 0.044 mg/kg. Appropriate timing of insemination was determined by ultrasound studies of the ovaries and hormone levels in blood and urine.

The time of ovulation was determined by follicle size and LH surge in urine, trying to ensure intravaginal presence of viable sperm from 12 hours before ovulation until the end of the ovulatory window. The frequency of insemination was every 12 to 24 hours, based on sperm quality and availability. Semen dosages varied in quality and quantity, as semen donor was not consistent with the collection behaviour.

In total, 35 artificial inseminations, 482 ultrasounds, 55 blood and 295 urine analysis were conducted. All these procedures were achieved using medical behaviours.

Lactation stage and milk composition in two bottlenose dolphin (*Tursiops truncatus*) females

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This study aims to determine the changes in milk composition during the lactation in two females (Q+L) of bottlenose dolphin (Tursiops truncatus) kept under human care. Samples were collected in different stage of lactation, early lactation (Q:4 months post-partum) and late lactation (L:27 months post-partum). Milk samples were collected by voluntary behaviour twice a day, once a week. 34 individual milk samples were collected during the whole study period, and analyzed for principal chemical and physical parameters. The results showed the following values for fat: 13.61±0.76%, protein: 10.41±0.15% and lactose: 2.58±0.10%. Gross energy in both dolphins was positively correlated with period of lactation ($P<0.05$). Gross energy mean was 195.20±6.76 Kcal/100g with significance difference ($P=0.004$) between the two stage of lactation (Q:180.48 vs L:218.97 Kcal/100g).

Among the various parameters analyzed, fat ($P=0.005$) and lactose ($P=0.015$) showed significant differences between the two periods. The collected data can be useful to the clinician to assess the quality of the milk suckled by the calf during the various stages of lactation. This study wants to be a first step on determination of composition changes in dolphins milk during lactation, however, more data are necessary to establish reference values.

Raising dolphins the Delphi-style – successful raising of *Tursiops truncatus* with a laissez-faire attitude

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Zoo Duisburg is keeping bottlenose-dolphins (*Tursiops truncatus*) for more than 50 years. In 1978 the first birth has occurred. Since then regular dolphin births have been observed and several calves were raised successfully. DELPHI herself was born in Duisburg in 1992 and has given birth to 5 calves. She additionally aborted her first calf. The last three calves are still alive (DONNA, born 2007; DOERTE, born 2011 and NAMELESS, born in december 2015). DELPHI takes care of her babies in a very minimalistic way. She nurses them and pays attention but at the same time the offspring has to swim on its own from the very first day on several occasions.

This very unusual way of rearing made a general observation and judgement of normal/abnormal behavior to determine the well-being of the calf very difficult. This presentation will include video material to show how DELPHI raises her calves in her own special way.

Development of an online survey for the review of Otariids reproduction control in captivity

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Facing a major reproductive success in human care, it seems increasingly necessary to better understand the physiology of reproduction of Otariids and to develop reliable techniques of birth control. Physical separation, permanent castration, reversible and immunological contraception are all means of control used by zoos and marine facilities to master the reproduction of their animals. The ultimate goal is to ensure optimal genetic diversity, reduce overcrowding and the risk of intraspecific conflicts that may arise. Currently, scientific information on the various methods and consequences of contraceptive methods used among sea lions are very limited in the literature.

This has lead to the development of a thorough on-line questionnaire, aimed at veterinarians and institutions holding these species, in order to review the different techniques used nowadays and their relative success and/or failures.

During this presentation, current knowledge on contraceptive methods in pinnipeds will be overviewed and the questionnaire will be explained. The information collected herewith and their analysis will permit, in the near future, the realization of a synthetic work on the control of reproduction knowledge in sea lions, but this can only happen if the information is made available.

Multi facility study to determine a salivary cortisol baseline in *Tursiops truncatus*

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The stress response is a complex behavioral and physiological mechanism developed by animals to cope with changes in their environment. The production of glucocorticoid hormones (GCC) has been described as one of the physiological reactions related with the stress response and, consequently, cortisol (the mayor GCC in cetaceans) has been widely used as a stress indicator for dolphins. Measuring the concentration of cortisol in saliva is common in humans and other animals. Recently it has been shown to be reliable in dolphins as well, and furthermore is a non-invasive, feasible technique.

Despite its potential use in many dolphin facilities, there is no reference baseline for normal levels of salivary cortisol, taking into account the daily fluctuations of the hormone or the precision of the technique.

The present study was aimed to establish a salivary cortisol baseline and its specific variability by means of a multi facility (11) simultaneous experiment with a large number of dolphins (85). The results obtained will allow facilities to use more consistent sampling methods and make more precise interpretations of the salivary cortisol concentrations.

Lung function in the harbor seal (*Phoca vitulina*): a non-invasive method for assessing respiratory physiology and health for animals in human care.

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Our current knowledge about marine mammal eco-physiology is limited. However, an increased understanding of physiology is required to assess how anthropogenic environmental impact may affect species. These air-breathing vertebrates perform extended breath-holds to obtain food underwater. Therefore, environmental or physiological alterations may disturb foraging efficiency and, consequently, their survival. Lung function studies on wild animals require restraint, and the subsequent stress limits data. Otherwise, work on trained animals enable us to study physiology under voluntary control, which minimizes stress. At the Oceanogràfic, València, we are working with trained animals to evaluate lung function in the harbor seal (*Phoca vitulina*) using a non-invasive ultrasonic flow-meter developed for humans (NDD Inc). The main objective of the present work is to obtain baseline data on respiratory flow for healthy individuals of different ages and in different positions (2 on land and 3 in water). These results will allow us to compare the pulmonary physiology with other diving mammals, identify changes with age, and diagnose respiratory health. Thus, our research is a project related to conservation aspects and veterinary diagnostic capabilities for both wildlife and animals under human care.

Metabolic rate and respiratory physiology in the walrus (*Odobenus rosmarus*)

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We measured breath-by-breath respiratory flow-rates, breath duration, airway pressure (Pair), esophageal pressures (Peso), and expired gas composition in three adult female walruses on land (body mass range: 640-841 kg) using a custom made pneumotachometer and fast response O2 and CO2 analyzers. A total of 194 spontaneous and 33 forced breaths (FB, trained forceful exhalations through the nose) were collected in Feb-Apr 2015. The average (± SD) expiratory duration was significantly longer (spontaneous: 1.96 ± 0.56 sec; FB: 0.69 ± 0.33 sec) and the expiratory flow-rates lower (spontaneous: 8.4 ± 3.3 sec; FB: 30.0 ± 11.1 L sec-1, paired t-test, P <0.05) during spontaneous breaths than during forced breaths. Maximum expiratory and inspiratory flow-rates exceeded 45 L sec-1, and 11 L sec-1, respectively. Flow-volume curves following FB indicated no volume dependence of maximal expiratory flow, and suggest that expiratory flows were effort dependent over most of the vital capacity. Lung compliance from one female (640kg) was 2.2 L cm H2O. Average end-expiratory O2 and CO2 during spontaneous breaths were 12.4 ± 1.4 %, and 7.5 ± 1.4 %, respectively. The average estimated metabolic rate during spontaneous breaths was 4.9 ± 1.0 L O2 min-1, which is approximately twice Kleiber's basal metabolic rate, and approximately 1/3 the field metabolic rate measured in wild males weighing approximately 1300 kg. Our results provide novel physiological data from walruses that may be useful for bioenergetics modeling and to understand physiological constraints during diving.

Presenting an Original 360° HD Audio-Video Device to be used in an Etho-Acoustical Analysis on Bottlenose Dolphins (*Tursiops truncatus*)

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Visual and acoustic observations linking sound emissions to individuals are necessary to better understand the bottlenose dolphins' social life. Although this task is challenging for human observers because human hearing is not adapted to locate underwater sound sources. To prevail over this difficulty, we designed and built an original underwater 360° HD audio-video device (i.e. BaBeL, Bio-acoustique, Bien-être & Langage) consisting of a five-hydrophone array attached to two spherical cameras to visually and acoustically cover 360°. The system was firstly tested on synthetic acoustic signals in a swimming pool to assess the level of performance, by providing errors on virtual locations. Tests showed that the direction of the acoustic source position may be provided with an error inferior to ½ dolphin body size. We conducted tests on bottlenose dolphins' sounds collected from moving individuals off Reunion Island (France) and Parc Astérix (France). We discuss in what cases it was possible to precisely locate the emitting dolphins and to describe their body postures and we present three etho-acoustic analyses of selected video sequences to demonstrate the scope of our methodology. Benefits of using BaBeL for underwater observations of cetaceans in clear waters are reviewed.

Bottlenose dolphin (*Tursiops trucatus*) sperm motion evaluation after 5 days of refrigeration at 5ºC and 15ºC degrees, a preliminary study

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Refrigerated semen is commonly used in different mammalian species keeping good fertility capacity for being used in artificial insemination; so this method of conservation could be extrapolated to dolphin species. The aim was to evaluate dolphin sperm motility kept at 5ºC and 15ºC during several consecutive days of refrigeration. Ejaculates (n=3) were collected voluntarily and immediately diluted (1:1) in Zoosperm-ND4 porcine extender (Import-Vet S.A.). Motion parameters were evaluated by CASA system at days 0, 1, 3 and 5 of refrigeration in both experimental groups (5ºC vs. 15ºC). A sample of 4 µl was placed on pre-warmed (38ºC) SpermTrack camera (Proiser®) for evaluation. The initial sperm motility (%) and progressive motility (%) was 89±4.04% and 47.33±5.78%, respectively. After 5 days of refrigeration these parameters were 54.33 and 28.33% for 5ºC group, and 77.33 and 33.66% for 15ºC. Regarding other motility parameters, sperm velocity (VCL, µm/s) was 139.33±2.40 µm/s at day 0, and 88.33 and 94.33 µm/s for 5ºC and 15ºC respectively for day 5. No statistic differences were found among groups in any parameter studied. Overall, dolphin bottlenose sperm samples keep a good motility at least after 5 days of refrigeration at 5ºC or 15ºC under the previously mentioned extender.

The effect of Inmuno-2865® (Natramune-2865®) and Shana vet® in Poxvirus lesions in Grey Seal (*Halichoerus grypus*): preliminary results.

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The family Poxviridae represents a large group of complex, highly epitheliotropic DNA viruses causing cutaneous and systemic diseases in different animal species and humans. In pinnipeds, Poxvirus injuries are especially common as a complication in the treatment of stranded animals in specialized marine mammal rehabilitation centers. The distribution of cutaneous lesions in pinnipeds includes the head, neck, and flippers. In harbour seals (Phoca vitulina) and grey seals (Halichoerus grypus), initial lesions begin as small, raised nodules, 0.5 to 1 cm in diameter which, over a period of approximately 1 week, may increase to 1.5 to 3 cm in diameter. During the second week, these lesions ulcerate and may suppurate, also rapidly spreading satellite lesions can develop around the initial nodules. After the fourth week, lesions begin to regress in both species of seal. Areas of alopecia and scar tissue may remain following resolution. This pilot study, conducted with grey seals in rehabilitation at the SRRC, aimed at evaluating whether dietary supplementation with Imuno-2865 (Natramune-2865®) and Shana Vet® besides Shana Vet® ointment local aplication, will improve and accelerate the resolution of Poxvirus lesions. Preliminary results are promising, showing a faster regression of the lesions.

Melatonin concentrations in captive bottlenose dolphins (*Tursiops truncatus)*

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Melatonin is one of the hormonal outputs of the biological clock and plays a role in the modulation of sleep-wake patterns. In humans and most other animals, melatonin is produced in the pineal gland and secreted in darkness. However, in dolphins, previous studies have shown that there are low blood levels of melatonin present without a night time increase, daily rhythmicity or seasonal variety, although the necessary night samples for exclusion were not always taken. The pineal gland is not or rarely ever detected in bottlenose dolphin brains.

In our study we have used both blowhole chuff and saliva measurements to collect samples for melatonin analysis. Samples were taken at 3-hour intervals during a 24-hour period in four dolphins.

Melatonin expression was found in low concentrations without the presence of a clear circadian rhythm. The absence of a functional pineal gland might account for this. Our methods have shown that it is possible to detect melatonin using saliva and/or blowhole chuff sampling instead of the more invasive blood sampling. Questions remain; such as where the melatonin present in our samples is formed if not in the pineal gland; and what are the explanations for, and implications of, a missing melatonin rhythm?

Evolution of the population of Orcas under human care from 1960s to the 2000s

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The population of orcas (*Orcinus orca*) under human care is in constant evolution, being conditioned by its specific environment, which has remarkably improved since the first individuals were captured. Changes in population dynamics are the reflection of that evolution.

In order to look for those changes, we have built a data base using the Marine Mammals Inventory Report, which contains information about the most of the worldwide population from early 1960s to 2010. The aspects we analysed were: how the origin of the animals and the global composition of the population have changed along the time; the proportion of death during the first year after birth or capture; and the general pattern of deceases.

One of the most remarkable facts is that the proportion of deaths during the first year drops from 70% in the 1960s to 11,63% in the 2000s. Besides, the pattern of deaths changes: in the 1960s the most of deceased animals (70%) lived less than a year under human care while in the 200s they lived more than 20 years (32,56%).

Our results show that the population has tended to be more stable along the time, descending the number of premature deceases and increasing the longevity.

Pharmacokinetic behaviour of cefovecin after subcutaneous administration in small clawed otters (*Aonyx cinerea*)

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Cefovecin is a third-generation parenteral cephalosporin used to treat most aerobic and anaerobic bacterial infections in pets. Its dosage, administration route and period of drug activity make it an alternative antibiotic of choice to reduce stress and handling in wild and zoo animals.
The objective of this study is to evaluate the kinetic behaviour of cefovecin in small clawed otters after a single subcutaneous administration.

Two females and one male were injected with subcutaneous cefovecin at 4 mg/kg body weight (BW). Blood samples were collected by jugular puncture prior to injection (t=0) and then periodically for 70 days, aproximately. All procedures were performed under anaesthesia, with Isofluorane, during the period between January and August of 2014 in Mundomar, Alicante. Plasma drug concentrations were determined by HPLC methods with UV detection, and pharmacokinetic parameters were calculated by a non-compartmental analysis.

The drug concentrations lasted over 1µg/ml (MIC90 calculated for habitual microorganisms from small domestic carnivores) for at least 60 days after drug administration. The mean half-life estimated for the drug was 18.45 d and the mean MRT, 22,95 d.

Our results show that a prolonged kinetic behaviour of cefovecin in small clawed otters allows for the maintenance of therapeutic levels during 60 days after a single subcutaneous injection of 4 mg/kg BW. Nevertheless, additional experiments with other dosages should be considered to establish a rational therapeutic regime in this species.

Case report: clinical approach to a severe traumatic palpebral laceration in a harbour seal (*phoca vitulina*)

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Extensive injuries in eyelids or conjunctiva accompanied by loss of tissue are considered ophthalmological emergencies as they could compromise eye viability in a short term.

In January of 2013 a seven-year-old harbour seal of Mundomar, Alicante, presented a bite wound in the left lower eyelid, with inflammation, bleeding, affection of the third eyelid and complete tear of the eyelid margin.

The extension of the lesion and surrounding affected tissues required surgical eyelid reconstruction. Two interventions were required because of wound dehiscence of first reconstructing suture.

The anaesthetic protocol in both surgeries was: midazolam (0.06 mg/kg IM) + butorphanol (0.2 mg/kg IM) as premedication, propofol (5 mg/kg IV) for induction and isoflurane (0.5-1.5%) for maintenance.

Enrofloxacin, doxiciclin and meloxicam was prescribed after the first surgery and cefovecin, clindamicin and meloxicam after the second. Both treatments included local cleanings and eye drop application.

Part of the postoperative period required keeping the animal out of the water until the healing of the edges of the suture allowed for the reintroduction into the aquatic environment.

The use of an interrupted suture pattern in the second surgery was possibly a determining factor for avoiding dehiscence.

Delaying surgery in these kinds of lesions may have undesirable scarring and a significant negative impact with the functionality of the eyelid.

Blood sampling in a wary California Sea Lion (*Zalophus Californianus*)

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Pinnipeds hosted at Zoomarine Italia are trained to participate in welfare behaviours voluntarily to avoid or minimize stressful circumstances. For training we use operant conditioning technique based on positive reinforcement and a very close relationship between trainers and animals.

Tao is a young male of California Sea Lion. He is very smart, but he is also wary and sometimes unpredictable. During the training process, each time we introduce something of new (e.g. object, toy, trainer) he uses to be scared and go away.

Sometimes he is aggressive and for this reason we interact with him in a very careful way. So, Tao is trained for blood sampling voluntarily through the gate of the enclosure, in safety for veterinarians and trainers.

In the first training steps, Tao is stationary very close to the gate in lateral position with the hind flippers under the gate. In this way, the trainers desensitize the animal touching the body and the flippers.

In the next steps, the trainers touch the hind flippers before with different materials (e.g. warm gloves and dry/wet gauze) then with nail and needle. After these steps, the vet is able to take a blood sample.

How to maintain our high standard of animal training during period of construction in keeping animals in their environment?

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Seaquarium host 2 different species of marine mammals in the same pool: 4 Patagonian Sea Lions and 4 Harbor Seals.

Seaquarium decide to expand the actual pool to give more of space to animals. The team decide to keep animals in the main enclosure during bulding work. We use the differents place of our facility and the various techniques of operant conditioning to keep the level of training of our animals

Whalewatching in Russia: Fact or Fiction

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In 2014 the requirement was identified and the social aspect - the level of Russian people being informed about scientific-expeditionary tourism (especially whalewatching tours) existence - was studied. Also marketing research of potential tourists was done (who, what, why). Methods: survey, expert assessment, data statistic analysis. In the research 178 participants were offered a questionnaire (20 questions). According to the research results 78.09% participants have heard about special tours and wildlife observing. 36.52% participants have recognised the term "whalewatching". 11.24% of participants have never watched sea animals. 46.63% have seen dolphins in the sea occasionally on vacation. 35.39% of participants go to the dolphinariums on purpose; and about 20% have visited a dolphinarium in their own city once or more. 8.99% of participants have taken part in the sea excursions or whalewatching expeditions. 85.96% of participants expressed their positive attitude to whalewhatching tours considering them as a good alternative way to captivity. 14% of participants wouldn't mind visiting dolphinariums and admiring the animals there. The participants' answer to the question "Would you like to watch whales in the pool in your city" lets us notice the high moral aesthetic value of the environmental expeditions. 93% of participants are against keeping the animals in captivity. Participation in different environmental tours can reduce the number of people with positive attitude to animal captivity. Such tours can increase the educational level and humanity of society, and also help towards contribution of important information through expedition members.

Dolphins: Training artificial insemination in small population

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We have a population consisting of five bottlenose dolphins, three males and two females. Until today, our breeding program based on natural conception was unsuccessful.
The main goal of the team is to conduct medical training with our population, so we planned an artificial insemination training program, challenged with the fact that of our five dolphins only two are good candidates for this project, and resolving any complications and difficulties encountered during the process.Our goal is to show that for small dolphinariums, it is possible to condition these processes, with whatever animal we have in our collection as long as they are sexually mature.

- Ultrasonographic diagnosis and monitoring.
- Flip and Frosia socialization.
- Flip, penis presentation and semen collection training.
- Frosia, genital desensitization for insemination.
- Trained assisted insemination in small population results.

This procedure promotes better reproduction programs of species under human care. All medical behaviors are trained to have the veterinarian job less hectic as well as significantly reducing the stress factor for the animals during medical procedures, hence creating a very good relationship between veterinarians and trainers where the welfare of the animals are concerned.

Bottlenose dolphins (*Tursiops truncatus*) semen banking project in France.

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As part of a veterinary thesis, the interests and feasibility of a bottlenose dolphin semen bank in France were investigated. We will discuss factors that speak in favor of the implementation of this project in dolphinariums (recent progress in the field, genetic diversity and practical issues). Moreover this project will be discussed in the international context of cryopreserved semen exchanges. The aim of the project is to establish in France an efficient method to cryopreserve bottlenose dolphin semen by collecting at least ten ejaculates in French institutions swilling to cooperate. Two freezing media, used in canine semen cryoconservation, will be used and their efficiency will be compared. The first freezing medium « Liposome 6% » is based on egg-yolk and is quite similar to the one used by Robeck et al. (2004). The second freezing medium « LDL 6% » includes low density lipoproteins which are the cryoprotective component of egg-yolk and showed better results than egg-yolk in canine semen cryopreservation in terms of spermatozoa motility, viability and integrity. Finally, we will raise awareness in French dolphinariums about challenges related to artificial insemination development to create in the long-term an artificial insemination center for bottlenose dolphins in France.

Variation of the sound emission rates in a captive group of false killer whales (*Pseudorca crassidens*) during feedings: possible food anticipatory activity?.

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This study examines whether a group of captive false killer whales (*Pseudorca crassidens*) showed variations in the vocal rate around feeding times. The high level of motivation to express appetitive behaviors in captive animals may lead them to respond with changes of the behavioral activities during the time prior to food deliveries which are referred to as food anticipatory activity. False killer whales at Qingdao Polar Ocean World (Qingdao, China) showed significant variations of the rates of both the total sounds and sound classes (whistles, clicks, and burst pulses) around feedings. Precisely, from the Transition interval that recorded the lowest vocalization rate (3.40 s/m/d), the whales increased their acoustic emissions upon trainers' arrival (13.08 s/m/d). The high rate was maintained or intensified throughout the food delivery (25.12 s/m/d), and then reduced immediately after the animals were fed (9.91 s/m/d). These changes in the false killer whales sound production rates around feeding times supports the hypothesis of the presence of a food anticipatory vocal activity. Although sound rates may not give detailed information regarding referential aspects of the animal communication it might still shed light about the arousal levels of the individuals during different social or environmental conditions.

Voluntary urine collection training in bottlenose dolphin (*Tursiops truncatus*)

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Animal handling and obtaining samples is facilitates by medical training.

The aim of the training was to monitor conjugated estrogens and LH levels in the urine for an artificial insemination program.

Urine analysis is a test often used in medicine to aid in the diagnosis of certain diseases or to monitor the substances secreted by the kidneys.

Three female bottlenose dolphins were trained to voluntarily and regularly give samples of urine by spontaneous urination in Mundomar, Alicante during 2015.

The training combined classical and operant conditioning. Positive reinforcement was used, first continuous and afterwards selective. Primary and secondary reinforcers were used, and also LRS (Least Reinforcing Stimulus) to reduce any possible frustrations in the animals and increase the training success.

A total of 295 urine samples were collected in six estrous cycles.

The sampling success increases with a bladder of minimum 4 cm in diameter measured by ultrasound, with previous food intake, handling of the animal with one single trainer and when the procedure was performed in the reproduction pool, much preferred by the animals.

Quantitative analysis of the normal gastrointestinal microbiota in captive bottlenose dolphins (*Tursiops truncatus*)

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Aims: evaluate normal culturable dolphin gastrointestinal microbiota discerning some possible implications on diagnostic and treatment.

Methods: decimal dilutions of gastric and faecal samples from ten clinically healthy bottlenose dolphins at the Oceanogràfic were plated using Digralsky spreaders. VRBG, VRBL, MacConkey, Enterococcosel, and Blood agars were incubated aerobically; mCCDA and Preston agars microaerobically and TSC and MRS agars anaerobically. Gram stain, catalase, oxidase, and API kits were used for identification. Colonies were counted and results expressed as mean log10 cfu/ml of sample.

Results: A total of 65 bacterial strains were isolated. The bacteria isolated from gastric samples were Clostridium perfringens (30% of prevalence) with an average concentration of 3.5, Plesiomonas shigelloides (20%) at 4.0, Staphylococcus aureus (20%) at 3.1, Escherichia coli (10%) at 3.4, and Edwarsiella tarda (10%) at 3.25. In faecal samples, P. shigelloides (100%) at 9.8, C. perfringens (90%) at 9.2, E. coli (50%) at 9.0, E. tarda (40%) at 9.5, and S. aureus (10%) at 8.7 were isolated.

Conclusions: quantification through direct culture could be an easy way to evaluate dolphin gastrointestinal microbiota, record specific individual patterns that can lead to predict illness before clinical signs appear, and elucidate normal saprophytic bacteria that could serve as a probiotic in order to minimize the use of antibiotics.

Increasing number of the encounters of short-beaked common dolphins (*Delphinus delphis ponticus*) in the coastal northwestern Black Sea waters in summer: a new trend in distribution?

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In the Black Sea, short-beaked common dolphin (*Delphinus delphis ponticus*) usually avoids coastal, shallow and brackish waters. Coastal waters of the northwestern Black Sea are known for their shallow depths and intensive human activities. However, visual observations, photo-identification and questionnaire survey of local residents about cetacean encounters during last decades off the Ukrainian coast in June-September 2015, showed that the common dolphin inhabits coastal waters of the region during summer period. Moreover, common dolphins were not only encountered in more pristine waters such as adjacent to the Danube Delta or Dzharylgach Bay, but also more areas of high human activity such as the Port of Odessa in the Gulf of Odessa and the Hryhorivsky (Maliy Adzhalik) Bay with its deep water Yuzhny Port. Group sizes of common dolphins varied from 2 up to several dozens with 95% of groups consisted of primarily females with calves and juveniles. Preliminary results of the coast-based photo-identification study at Hryhorivsky Bay showed up to 6 re-sightings of the same individuals during the summer period. A possible reason of increase in encounters of common dolphins in coastal northwestern Black Sea waters in last decades is the shift in feeding conditions.

Assessment of environmental enrichment on two Harbor seals (*Phoca vitulina*) under human care

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Traditionally, environmental enrichment is an approach designed to encourage animals under human care to display their complete behavioral repertoire and it is used to reinforce the appearance of desirable behaviors, decrease the frequency of undesirable behaviors and bad feeding habits, and reduce excessive self-directed behaviors. Numerous examples can be found in varying species. However, very little information concerns pinnipeds. We present here a study conducted on environmental enrichment on two Harbor seals (*Phoca vitulina*) in Attica Zoological Park (Greece). We introduced 10 floating objects and 7 sinking objects. Using an appropriate behavioral repertoire, we noted animals' levels (from 1: poor to 5: high) of interest and interactions during sixty- eight 20min sessions.

Preliminary results showed that one seal preferred floating objects and the other one sinking objects introduced in their environment. We discuss the importance of assessing environmental enrichment for animals under human care and the necessity to individually consider the impacts of those programs on groups of animals.