

# Report on the research activities with *Orcinus* *orca* in Loro Parque

**DATE:** MAY 23<sup>RD</sup> 2013

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# REPORT ON RESEARCH ACTIVITIES

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## 1 INTRODUCTION

The scientific research activities of Loro Parque are based on the Research and Conservation Program 2010-2015, which has been submitted to the Spanish authorities in compliance with the Spanish Zoo Law (31/2003) and the European Zoo Directive (1999/22/CE). This Program is the official document in which the fundamental criteria and guidelines for the research and conservation activities are established. These criteria are based on the recommendations of different zoological institutions related with Loro Parque (World Association of zoos and aquariums -WAZA-, European Association of Zoos and Aquariums -EAZA-, Iberian Association of Zoos and Aquariums -AIZA-, European Association of Aquatic Mammals -EAAM-), and several biodiversity conservation organizations related with Loro Parque Fundación (International Union for Nature Conservation -IUCN-, Survival Species Comission -SSC-, Conservation Breeding Specialist Group -CSBG-, etc.).

The main goal of this Program is to optimize the financial, material and human resources that Loro Parque dedicates to the research and conservation activities, either directly or through Loro Parque Fundación. As no zoological institution can run research and conservation projects for every single species in its zoological collection, the Research and Conservation Program of Loro Parque establishes the criteria to distribute the resources among the most appropriate projects and species, based on their biodiversity conservation potential and the suitability of the group of animals hosted at Loro Parque.

The selection of the scientific and conservation activities is based on an analysis of the potential value for research and conservation (either *in situ* or *ex situ*) of every single species in the zoological

collection. To determine this potential value several criteria will be used, such as: status in the IUCN Red List of Threatened Species, availability of breeding programs (ISB, EEP or ESB), level of knowledge about species biometrics, biology or physiology, number of individuals available in the collection, level of husbandry, suitability of the holding facilities, etc.

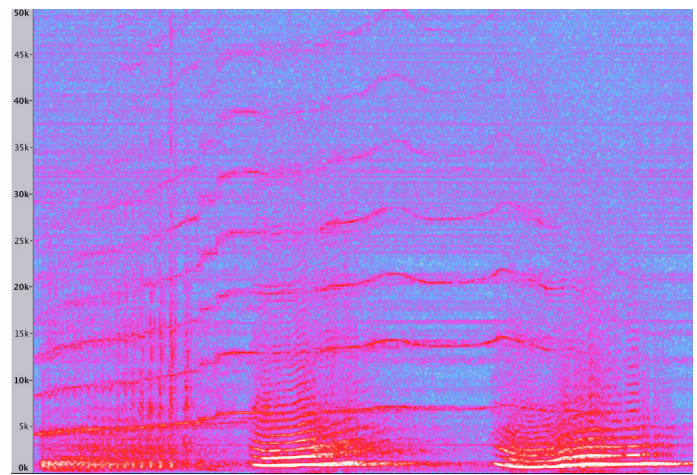
Based on the potential conservation and research analysis, a set of research proposals are submitted every year to the Board of Directors of the Loro Parque Fundación. All the proposals are evaluated in the Advisory Board Annual Meeting, using the most up-to-date scientific knowledge of the board of independent members. The Board of Directors approve the research and conservation proposal (*in situ* and *ex situ*), which are published in a final document.

In 2012 the LPF Advisory Board decided to fund 36 projects during 2013 with a total of 1.136.036 US\$, of them 30 were focused on parrots and terrestrial ecosystems, and 6 of them focused on cetaceans and marine ecosystems. Four out of the six marine projects are mainly focused on *Orcinus orca* research activities *ex situ*, with applications for *in situ* conservation of this and other cetacean species.

## 2 FINISHED PROJECTS AND SCIENTIFIC ACTIVITIES

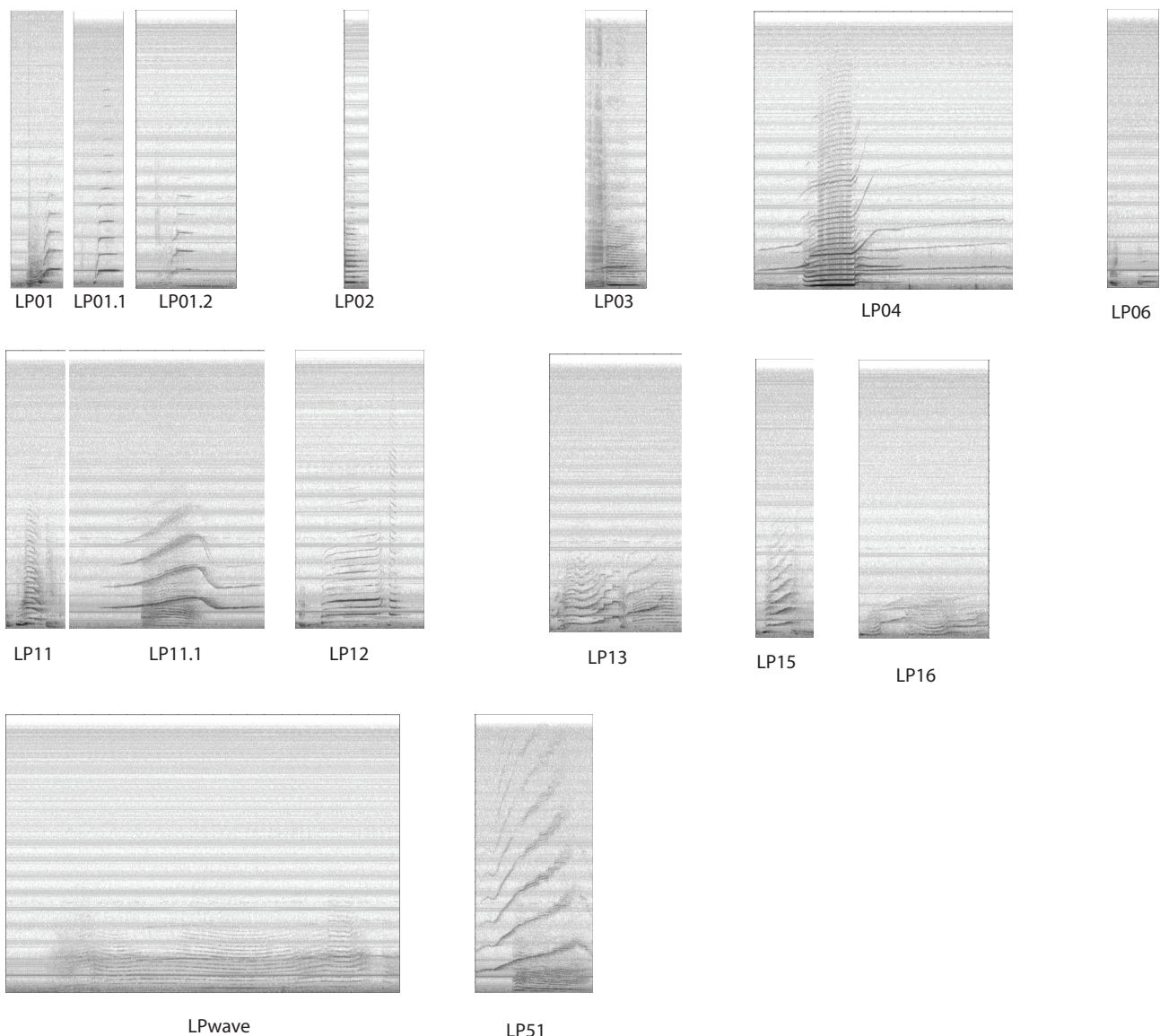
### 2.1 Research on bioacoustic tools

When Morgan arrived to Orca Ocean she was integrated into the two ongoing research projects, funded by Loro Parque Fundación, that implied *ex situ* research activities with *Orcinus orca*. One of them, aimed at the development of tools to improve bioacoustic research was finished in 2012. When Morgan arrived the project team was acquiring individually identified sounds in order to establish individual repertoires. As soon as Morgan could be held in the experimental pool (by then holding Pool 2), the setting-up of her individual repertoire was initiated. As a result, over 250 Morgan vocalizations were recorded and archived in 2012



**Figure 1:** Spectrogram of one of the sounds attributed to Morgan that were registered during the introduction phase.

for future analysis. These data were made available to the researchers of the University of St. Andrews studying the evolution of Morgan's dialect.



**Figure 2:** Acoustic dialect of the orcas in Loro Parque before the introduction of Morgan



## 2.2 Genetic comparison between species of Marine Mammals

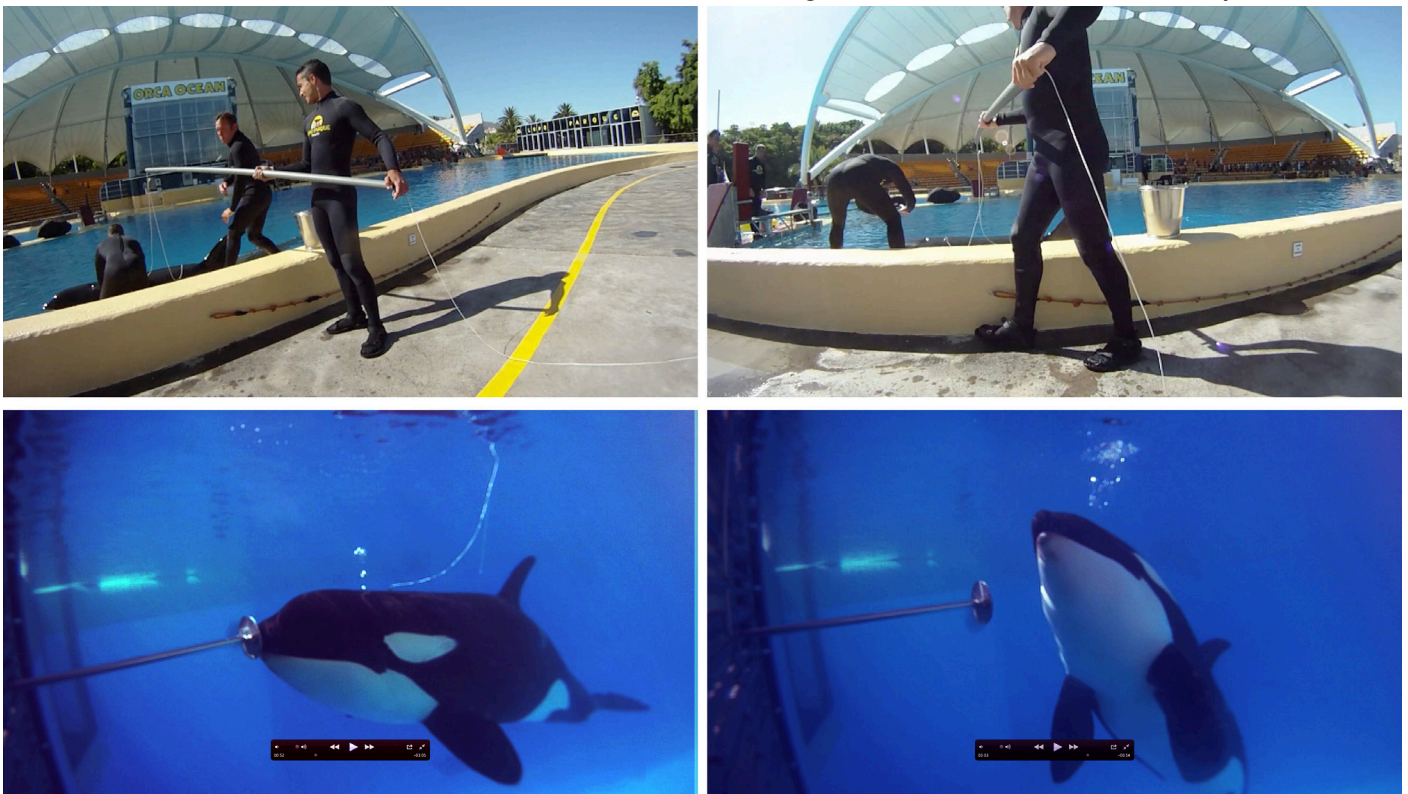
Short after the arrival of Morgan to Loro Parque, the Museum of Natural History of Copenhagen requested good quality genetic material to perform a comparison between different species of marine mammals. As they already used Morgan's genetic samples in a previous stage of the project (when they obtained the samples to determine Morgan's original pod), they wanted to use genetic material from the same individual to continue the project. Loro Parque requested the necessary CITES permits to export the blood samples, and sent them to the research team, which received and processed them accordingly. Morgan's genetic material was sequenced and provided a very high quality genome. Despite that the researchers are still working on the publication of the comparative genetic adaptations in different marine mammal species, the genome sequence obtained from Morgan has been published as reference for *Orcinus orca* as public domain: <http://www.ncbi.nlm.nih.gov/nuccore/ANOL000000000.2>

## 2.3 Audiogram of *Orcinus orca*

During 2012 Loro Parque requested a group of international experts to evaluate the hearing capacity of Morgan, as the trainers inferred that she was inconsistent in responding to the sound bridge (whistle). The research team suggested the use of Auditory Evoked Potential (AEP) as it allows a quick assessment of hearing capabilities, and has been extensively used with dolphins and other small cetaceans. In order to get the most scientific information possible from this hearing evaluation not only Morgan, but also the other four orcas, were trained to perform the auditory evoked potential measurements underwater, and the other two were made available to be measured out of the water.

As a result, the basic audiometric functions of six killer whales were assessed, becoming the largest test using Auditory Evoked Potential in this species ever. This methodology was used only once before in 1999 with two killer whales to describe the audiogram of the species. Before this research was performed, all the scientific information on the hearing capacity of the species was based just on three individuals.

The results of the test revealed a lack of click-evoked response in Morgan, which confirmed that this animal suffers from a hearing deficit. The magnitude and frequency range over which the hearing deficit occurs couldn't be specified with



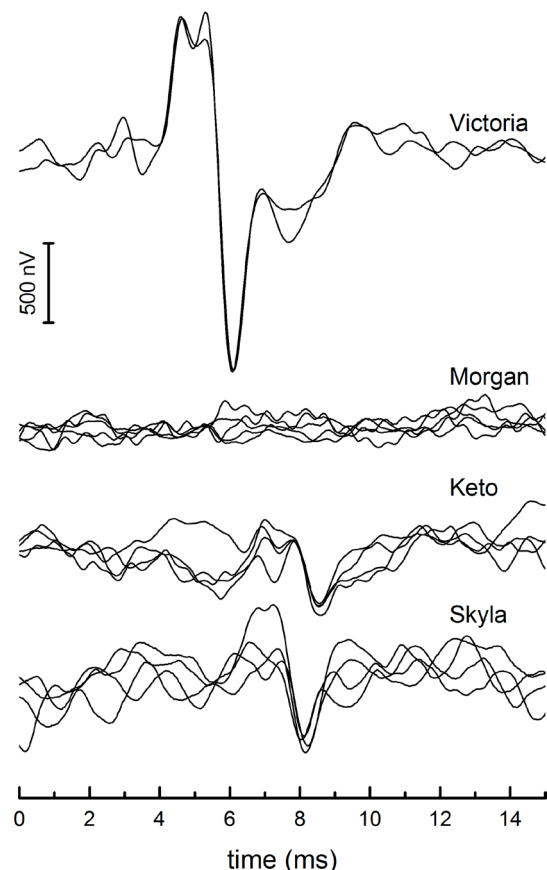
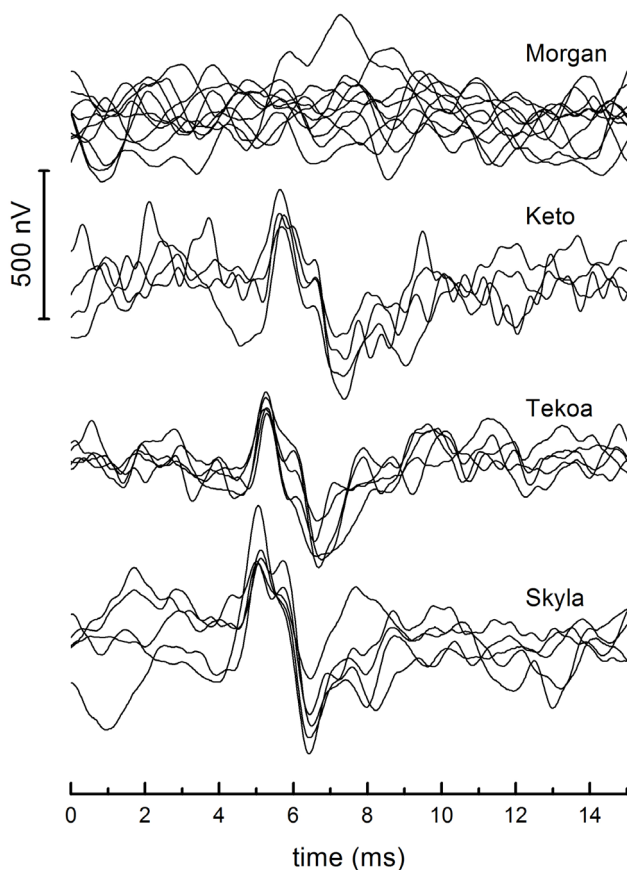
**Figure 3:** Morgan desensitization training session. From left to right and top to down: 1 attaching dummy suction cups, 2 giving signal to put nose on target, 3 correct position and 4 incorrect position.



**Figure 4:** Left: The research team measuring the AEP in water, this experimental arrangement is more complex and difficult to train, but gives better results as the sound reaches the animal in a more natural way. Right: Measuring AEP in a beached individual using a jawphone.

the methodology used in the study. Unfortunately, although AEP measurements allow a quick assessment of hearing capabilities, due to the size-dependent loss of the electrophysiological signal, this methodology was revealed to be inappropriate to detect the lower hearing threshold of *Orcinus orca*. As a consequence, the research team submitted a

new proposal to perform a behavioural audiogram of the same animals during 2013, the proposal was approved by the Advisory Board Meeting and partial funding granted by Loro Parque Fundación.



**Figure 5:** Results of AEP measurements in water (left) and beached (right), show a total absence of brain response to the sound stimulus in Morgan.



## 3 ACTIVE PROJECTS AND SCIENTIFIC ACTIVITIES

Currently, there are five active projects and one proposal, all of them focused on ex situ research activities with the group of *Orcinus orca* hosted at Loro Parque, thus all of them include Morgan.

### 3.1 Pollution markers in European bottlenose dolphins

This project is mainly focused on the effects of pollution in the pathology of the wild dolphin populations in Europe. The research team collected biopsies of wild and stranded dolphins around the Canary Islands simultaneously with other researchers in the Mediterranean and North Atlantic. The group of dolphins hosted at Loro Parque were used as a reference group, to measure the biodynamics and bio-accumulation of pollutants in a group of dolphins with a controlled diet.

When the research team started the sampling at Loro Parque, the opportunity to obtain blood samples from other marine mammals with the same diet came up. As a consequence, the researchers initiated a parallel data collection to study a poorly understood issue: the relationship between concentrations of Persistent Organic Pollutants (POPs) in blubber and those in blood of marine mammals, as a better indicator of organ exposure to pollutants.

Morgan's blood samples were obtained regularly (as for the rest the group of orcas, dolphins and sea lions) and shipped to the research team in order to be analysed and to track any possible variation of the pollution markers. During 2013 the research team is obtaining biopsy samples to evaluate the toxicological parameters in the blubber of the three species and compare them with the plasmatic concentrations. The measurement of the toxic concentrations in plasma, blubber and food should shed light on the bioaccumulation mechanisms in the three species.

### 3.2 Evolution of Morgan's Dialect

When Morgan arrived to Loro Parque a proposal to evaluate the changes on her dialect was received from the University of St. Andrews (Scotland). This was the same research team that made the initial comparison of Morgan's dialect

with the records of pod dialects from the three major orca populations in the North Atlantic.

The proposal tested the acoustic evolution of Morgan's dialect, as there is a hypothesis about the evolution of the orcas' dialect during their entire lifetime. If this hypothesis proved to be correct, Morgan's dialect should be modified with the incorporation of calls specific to the Orca Ocean group. Similarly, the rest of the animals in the group would be integrating sounds from Morgan into their dialect.

A researcher from St. Andrews University visited Loro Parque two weeks during 2012 to collect sounds and make observations on Morgan's dialect. Complimentarily, Loro Parque Fundación shared all the identified sounds of Morgan that were registered from the on-going bioacoustics research since she arrived to Loro Parque, in order to provide a broader set of data. A complete description of the group's dialect previous to Morgan's incorporation was also provided by Loro Parque, so the research team could verify if any eventual new call in Morgan's dialect derived from there.

The preliminary analysis of Morgan's dialect during 2012 did not show any incorporation of sounds from the Orca Ocean group. Given that by the end of 2012 the audiogram of Morgan confirmed that she had a significative hearing deficit (that could even be total deafness) the experiment can not be considered valid, and the hypothesis of the lifetime evolution of vocal dialect in *Orcinus orca* can not be rejected.

Alternatively, the research team from St. Andrews University submitted a continuation proposal to test the lifetime evolution of vocal dialects in *Orcinus orca* during 2013 using a different approach. The objective is to analyze the individual dialects of the six orcas at Loro Parque except for Morgan. As the auditory capacity of five animals has been verified, the analysis of the evolution of their dialects after the introduction of Morgan into the group can be used to test the hypothesis. During 2013 researchers from the University of St. Andrews will be visiting Loro Parque to collect sounds and make observations of the dialect of every individual except Morgan. The recordings will be analyzed to find incorporations of Morgan's calls into the individual dialects of the rest of the group. In order to provide the research team with a broader data set to analyze, Loro Parque will make available to the researchers all the identified calls registered and archived as a result of its in-house bioacoustic research activities.



**Figure 6:** Voluntary blood extraction of an orca at Loro Parque

### 3.3 Biometric information derived from bioacoustic analysis of *Orcinus orca* calls

This project is aimed to find the way to obtain biometric data from the bioacoustic signals of the orcas. This project is based on the technology developed by the University of La Laguna and Loro Parque Fundación in a previous 6 year project finished in 2011. Thanks to this technology, an individual repertoire for each of the seven orcas at Loro Parque is being established.

The research team is working in the parametrization of the calls as the stage prior to obtaining information related to the biometry of the animal producing the sound. The animals are also carefully measured and weighed on a regular basis to collect the accurate biometric information. The bioacoustic records of the group (including Morgan) have been made available to researchers when required, after presentation of an adequate scientific proposal.

The hydrophone system at Orca Ocean is being improved to digitalize the sound signals before any amplification. That will provide the top quality

measurements needed to parameterize the signals and, subsequently find biometric characteristics from the bioacoustic signals. If this methodology finally works, it would mean a very positive outcome in the medium range non-invasive assessment of the body shape of the wild killer whales, which could be helpful in the conservation of the species.

### 3.4 Behavioral audiogram of *Orcinus orca*

As the Auditory Evoked Potential (AEP) methodology was revealed to be inappropriate to detect the lower hearing threshold of *Orcinus orca*, a new proposal to perform a behavioural audiogram of the same animals during 2013 was submitted to the Advisory Board Meeting of Loro Parque Fundación.

In this new research the hearing capacity of Morgan and other four or five individuals will be accurately established. This methodology implies an intensive training of the animals to make them respond consistently to a discriminative stimulus (sd). The stimulus will initially be visual and acoustic and gradually shifted to only acoustic. Once the animals respond consistently to the acoustic sd,

long and detailed testing will be initiated by playing different sound intensities at multiple frequencies in order to determine the hearing threshold of every individual. If Morgan fails to shift from the visual/ acoustic discriminative stimulus to the acoustic sd, that would be an indication of total deafness.

The information resulting from this study will mean a major improvement in the actual knowledge of the auditory capacity of the species that comes from a study on a subadult male 40 years ago, and one more recent with two females over 20 years old. All the animals tested in this experiment are under 17 years old, which will give a better approach to the auditory capacity of young specimens of *Orcinus orca*. A detailed description of the auditory capacity of the species is a critical knowledge to be achieved in order to better protect the wild populations from the noise pollution.

### 3.5 Bioacoustical characterization of different behavioural situations in *Orcinus orca*

The Advisory Board Meeting of Loro Parque Fundación approved a proposal from the University of La Laguna and the Free University of Berlin to study the prevalence of different types of calls related with behaviour in *Orcinus orca*. It has been suggested that there is a relation between the different proportional call type usage by the same pods and their behaviour, despite that few studies have focused on the characterization of call usage and behaviour.

The goal of this proposal is to perform an ethological observation of the group of orcas at Loro Parque, while simultaneously recording the sounds that they produce underwater. A random focal observation schedule has been set up, and

focal individuals are randomly selected and video recorded for 15 minutes. Complementarily, a video recording covering the whole main pool (See Anex I) will be also made to provide further ad libitum or focal observations. The ethologists from both universities will analyse the video and audio recordings in order to determine if there are significant variations in the proportional call type usage in different behavioural situations.

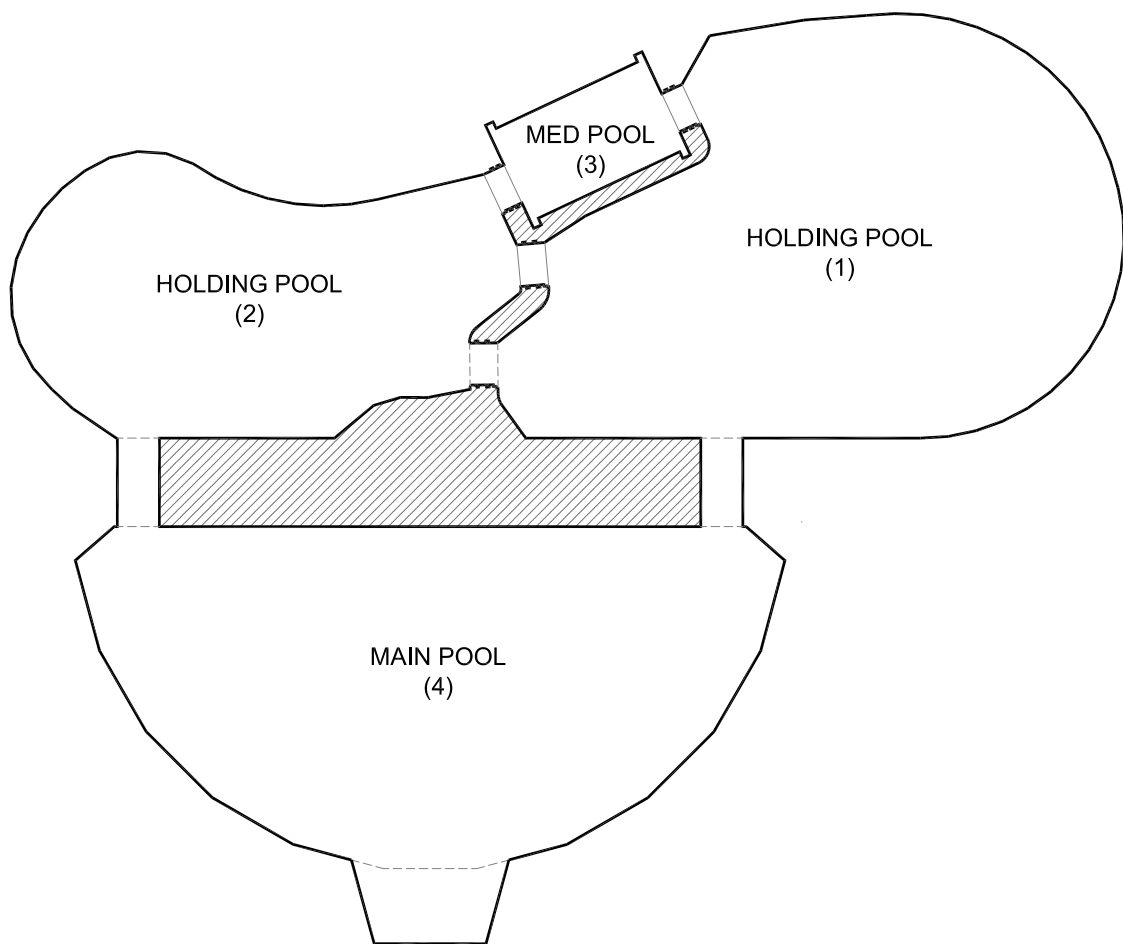
### 3.6 Biometrics proposal

In the beginning of 2013 Loro Parque received a proposal from the University of St. Andrews to perform a research project to measure the dimensions of the killer whales in Loro Parque using a high-frequency scanning sonar. Each whale will be scanned by the sonar as it swims past, and measurements taken from the sonar image will be related to hand-measurements taken from that individual. The sonar uses sound frequencies centered at 375 and 610 kHz. The results should lead to a peer-reviewed publication on the feasibility of this method to remotely measure dimensions of wild whales. This methodology would mean a very positive outcome in the non-invasive assessment of the body shape of the wild killer whales, which could help in the management of its endangered subpopulations and, thus in the conservation of the species.

Loro Parque has expressed its willingness to cooperate in this research as long as it does not compromise the welfare of the animals. Currently Loro Parque is gathering advice from different experts in acoustics and cetacean hearing to ensure that the sonar will not affect the animals. If the welfare of the animals can be guaranteed, the experiments will be performed during the last quarter of 2013.



KILLER WHALE EXHIBIT AND REPRODUCTION AREA



TOTAL VOLUME: 21,157,000 LITERS

15 SAND FILTERS, CPS CHAMBER (3No.PROTEIN SKIMMERS, FLUIDISED CARBON CHAMBER, VENTURI CHAMBER) WITH A TURN OVER OF 210 MINUTES.  
DESINFECTION BY CHLORINE AND OZONE.

	<u>MAX. DEPTH</u>	<u>MAX. HORIZONTAL WIDTH</u>	<u>MAX. HORIZONTAL LENGTH</u>
MAIN POOL	12m.	24.5m.	50.8m.
MED POOL	4.2m.	7.1m.	12.4m.
HOLDING POOL 1	8.1m.	30.5m.	44.8m.
HOLDING POOL 2	8.1m.	20.5m.	36.5m.