Assessment of basic audiometric functions in killer whales (*Orcinus orca*) at Loro Parque, Tenerife, Spain

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Summary

In November 2012, electrophysiological measurements of hearing sensitivity were conducted on Morgan and five other killer whales at Loro Parque, Tenerife (Spain). The goal of the study was to establish audiometric baseline information on Morgan relative to other whales held at Loro Parque and to determine whether Morgan suffered from hearing impairment. The six killer whales were between three months and 17 years of age. Animals of both sexes are held at this facility in four interconnected pools.

The electrophysiological measurements involved playing different types of sound stimuli to the whales and measuring the resulting auditory evoked potentials (AEPs). Comparisons of hearing sensitivity across whales consist of AEPs collected following the transmission of clicks either with the whales voluntarily beached or rested at the side of the pool with the electrodes out of the water.

While click-evoked AEPs were measured in all of the other killer whales tested, no AEP responses were found in Morgan, even at the highest click level that could be tested (134 dB dB re 1 μPa peak-to-peak equivalent (ppe)). A click-evoked response by Morgan would be expected based on the age and size of the whale and the success at recording click-evoked AEPs in the other whales..

The lack of a click-evoked response in Morgan suggests that this killer whale suffers from a hearing deficit. The magnitude and frequency range over which the hearing deficit occurs cannot be specified with the techniques used here as the click stimulus lacks the frequency specificity necessary for frequency-specific threshold measures. Nevertheless, it can be concluded that Morgan's hearing ability is at least 20–30 dB worse than the hearing sensitivity of the other whales tested. Morgan potentially suffers from a profound hearing deficit, or even complete loss of hearing, but this cannot be determined through currently employed electrophysiological means.

1. Introduction

In June 2010 a single female killer whale (*Orcinus orca*) was found in shallow waters of the Dutch Wadden Sea near the island of Schiermonnikoog. Since the animal was emaciated and considered to be in eminent danger of stranding, it was manually caught, lifted onto a vessel, and transported to the Dolfinarium in Harderwijk, The Netherlands, where it was subsequently rehabilitated over a period of more than a year. The animal, named Morgan, was probably 2-3 years old and weighed 350 kg upon arrival at the Dolfinarium. In November 2011 the animal was transported to Loro Parque, Tenerife, Spain, to provide her with a more suitable environment and with conspecifics.

2. Assignment

In November 2012, Klaus Lucke, James Finneran, and Dorian Houser travelled to Loro Parque to conduct electrophysiological measurements of hearing sensitivity on Morgan and the other whales at the facility. Behavioural observations by the trainers at Loro Parque indicated that Morgan did not show any reaction to purely acoustic cues. Only when paired with visual cues by the trainers or visually observable reactions/movements of other animals would Morgan show a behavioural reaction. This suggested that her ability to utilize auditory cues was compromised. The goal of the study reported here was to establish audiometric baseline information on Morgan relative to other killer whales held at Loro Parque and to determine whether Morgan suffered from hearing impairment.

3. Materials and Methods

The auditory measurements were conducted at the pools of Loro Parque, Tenerife (Spain) between 5 and 9 November 2012. Seven killer whales between three months and 17 years of age and of both sexes are held at this facility in four interconnected pools. The pools are formed together in an ellipsoid with a maximum extent of 120 m and a maximum depth of 12 m. Six of the animals were born in captivity with only Morgan coming from the wild. She had been introduced into the group about one year prior to testing and interacted with all other killer whales (except for the calf which was held separately in the medical pool) since. The auditory measurements were attempted in six of the seven animals. All animals were healthy at the time of the auditory tests.

The electrophysiological measurements involved playing sound stimuli to the whales and measuring the resulting *auditory evoked potentials* (AEPs), which are small neuronal signals produced by the brain in response to hearing a sound. Three specific tests were attempted that differed by the type of acoustic stimulus employed:

Clicks. Click sound stimuli consisted of a single rectangular pulse with a duration of 50 µs. Clicks were presented at a repetition rate of approximately 30 stimuli/s and the polarity of the click was reversed on each successive click to prevent the recording of stimulus artefacts. Of the signal types, clicks provided the greatest bandwidth and would be expected to produce the most robust AEP.

Tone pips. Tone pips are very short duration tones. Each tone pip was 9 cycles in duration, with 2 cycle rise and fall times and 5 cycles at the maximum amplitude. Tone pips provide more frequency specificity compared to clicks, but still produce relatively robust AEPs.

Sinusoidal amplitude modulated (SAM) tones. SAM tones are pure tones (carrier frequencies) whose amplitude is modulating using a sinusoidal waveform. SAM tones are commonly used to elicit AEP because they produce a steady state response, called the auditory steady state response, that can be objectively, statistically analysed. SAM tones were used with different amplitude modulation rates and carrier frequencies. The signal provides the narrowest acoustic bandwidth (greatest frequency specificity) but also produces the least robust AEP.

Three electrodes (inverting, non-inverting, and ground) were positioned along the dorsal midline of the animals during the measurements to record the AEPs. The non-inverting electrode was placed approximately 17 cm behind the animals' blowhole, slightly lateral to the dorsal midline. The inverting electrode was placed approximately midway between the non-inverting electrode and the dorsal fin. The ground electrode was placed near the dorsal fin. The electrodes consisted of 10 mm diameter gold cup-electrodes embedded in a suction cup made of silicon rubber. The AEPs were differentially amplified and bandpass filtered between 100 Hz and 1 kHz before being digitized and recorded on a ruggedized laptop computer. Stimulus transmission and AEP recording were performed using EVREST software designed by J. Finneran (Finneran, 2008; Finneran, 2009). During the measurements, the pumps of the filtration system were switched off to reduce the ambient noise level in the pools and to avoid electromagnetic fields that interfered with the electrophysiological measurements.

In preparation for the tests, three animals (Keto: male, 17 yr., Skyla: female, 8 yr. and Morgan, female, ca. 4-5 yr.) were trained to station themselves with their rostrum at an underwater target. The target consisted of a thin stainless steel plate (15 cm diameter), which was positioned at a depth of one meter and one meter in front of a gate connecting the test pool with the adjacent medical pool. The sound projector was located so that it was approximately 1.5 m away from the lower jaw of the stationed whale. The stationing allowed AEP measurements to be attempted with the animal completely submerged and in the direct field of the acoustic stimulus. This positioning is relevant with respect to how the animals received sound underwater when completely submerged. Recordings were also conducted with the same killer whales while they rested at the surface alongside the test pool. During this procedure, the animals were still in the direct field of the acoustic stimulus, but the electrodes were located above the water surface. This permitted a better AEP signal to noise ratio since the AEP recording suffers from electrical leakage to the surrounding water when animals are completely submerged. A similar procedure was completed with a female calf at the facility (Victoria, 3 months old) while it was suspended in a stretcher, but only the click stimulus was used for the test and the test was constrained to less than 10 minutes time.

A third set of auditory measurements were conducted while whales voluntarily beached on a concrete platform in the main pool, a behaviour which is trained for husbandry purposes. This procedure required the use of a sound projector embedded in silicone rubber, a "jawphone," to be placed on the whale's lower jaw. This procedure has been previously used in odontocete cetaceans (Finneran, 2006). In-air testing permitted data collection from a fourth whale (Tekoa, male, 12 yr). Testing of the female killer whale Kohana (10 yr) was briefly attempted, but she did not beach herself for a sufficient time period to permit AEP measurements.

Table 1. Various auditory tests performed and the whales that participated in each.

Name	Sex	Age	Underwater	In air (jawphone)
Morgan	F	4-5	x	x
Keto	M	17	x	x
Skyla	F	8	x	x
Tekoa	M	12		x
Victoria	F	<1	x	
Kohanna	F	10		(no data collection)

Acoustic stimuli presented to animals while submerged or resting at the side of the pool were projected through an underwater transducer, type ITC 1001. Transmitted signals were calibrated at the location of the whale's ears while on station but without the whale present. Additional measurements were made at varying spatial scales around the calibration point to characterize the variation in the acoustic field. The projector utilized in the jawphone was an ITC 1042. The jawphone was not calibrated against a killer whale and received sound pressure levels should only be considered estimates at this time. The best position in terms of signal presentation (i.e. resulting in the highest AEP amplitude) was identified by presenting a constant level click stimulus and moving the jawphone to different positions along the lower jaw. When AEPs were successfully recorded, acoustic stimuli were presented to the animals in a series of attenuating amplitudes, starting at levels that clearly produced an AEP and reducing it to the point that the AEP was no longer visible.

4. Results

Efforts to collect sufficient data for comparing the frequency-specific auditory sensitivity of the killer whales was not achieved using SAM tones or tone pips. The reason for this is most likely the reduced amplitude of the AEP to these narrower bandwidth acoustic stimuli and the attenuation of the evoked response at the surface due to the large body mass of the killer whale (i.e. the farther the signal travels from the brain to the skin surface, the more the voltage is attenuated). Furthermore, no useful results were obtained with the whales completely submerged, likely because of the above mentioned issues and the added problem of signal leakage to the surrounding water. As a result, comparisons of hearing sensitivity across whales consist of AEPs collected following the transmission of clicks either with the whales voluntarily beached or resting at the side of the pool with the electrodes out of the water. Clicks have the greatest bandwidth and produce the most robust evoked response, but they lack the frequency specificity required for comparisons at particular frequencies.

Figure 1 shows the click-evoked response for Keto during sessions in which he rested at the surface near the side of the pool. At the maximum click level produced, the peak-to-peak (p-p) amplitudes of the evoked response were greater than 500 nV. The amplitude of the AEP declined with decreasing stimulus level and is not observed at 94 dB re 1 μPa peak-to-peak equivalent (ppe) sound pressure level. Figure 2 shows a similar series for the smaller female whale, Skyla. The AEP amplitude was slightly larger than that of Keto for the same stimulus level, probably due to the smaller size of Skyla. In some instances, only a single AEP measurement at a particular click level was obtained for Skyla; however, the trend of decreasing AEP amplitude with click level is apparent.

Figure 3 compares the AEP produced in response to a click stimulus across four different whales. Each of the whales was resting at the surface with the suction cup electrodes positioned out of the water. The peak-peak equivalent rms (ppe) level of the click stimulus was approximately 134 dB re 1 μPa for Morgan, Keto, and Skyla, and 114–124 dB re 1 μPa for Victoria. The highest amplitude AEP to this stimulus was observed in the smallest and youngest whale, Victoria, even though her stimulus levels were 10–20 dB lower than that used for the other whales.

The amplitude of the AEP exceeded 1.5 μV (p-p), which is consistent with the small size of Victoria and the expectation of good hearing ability in a young animal. The amplitude of the AEP to the same click stimulus was substantially smaller for Keto and Skyla, which is likely due to the larger size of the adult whales and possibly compounded by age related reductions in hearing sensitivity. Of considerable note is the fact that no click-evoked AEP was found in Morgan, even though it would be reasonably expected that the click-evoked response would occur due to her age and size. Figure 4 shows a similar comparison for animals that were tested while voluntarily beaching themselves out of the water. This test condition provided the greatest signal to noise ratio for the AEPs, yet no click-evoked AEP was observed in Morgan for the highest click level tested. Click-evoked responses were observed in each of the other killer whales tested using the same or lower sound levels.

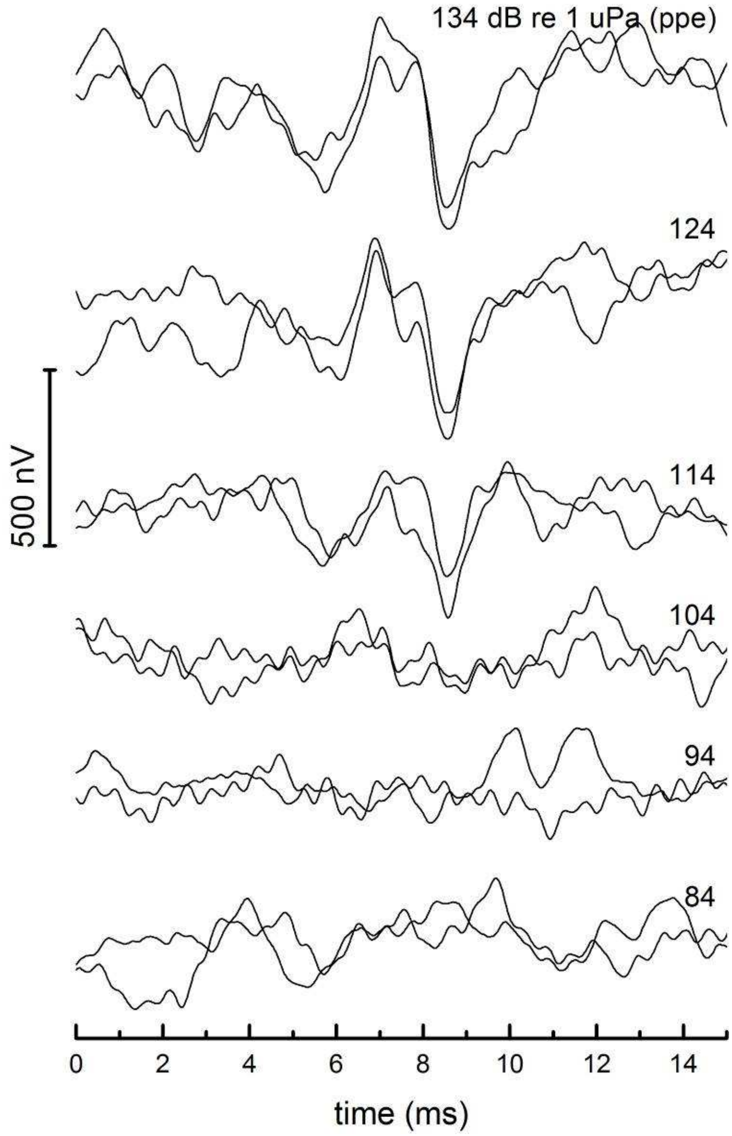

Figure 1. Click evoked potentials measured in an adult male killer whale (Keto) at Loro Parque while it rested at the side of the pool. Clicks were presented across a range of amplitudes, which were characterized according to the peak-to-peak equivalent (ppe) sound pressure level. The amplitudes are presented above and to the right of each AEP waveform. Multiple waveforms represent repeated AEP measurements collected at the same click level (with a varying number of measurements at different levels).

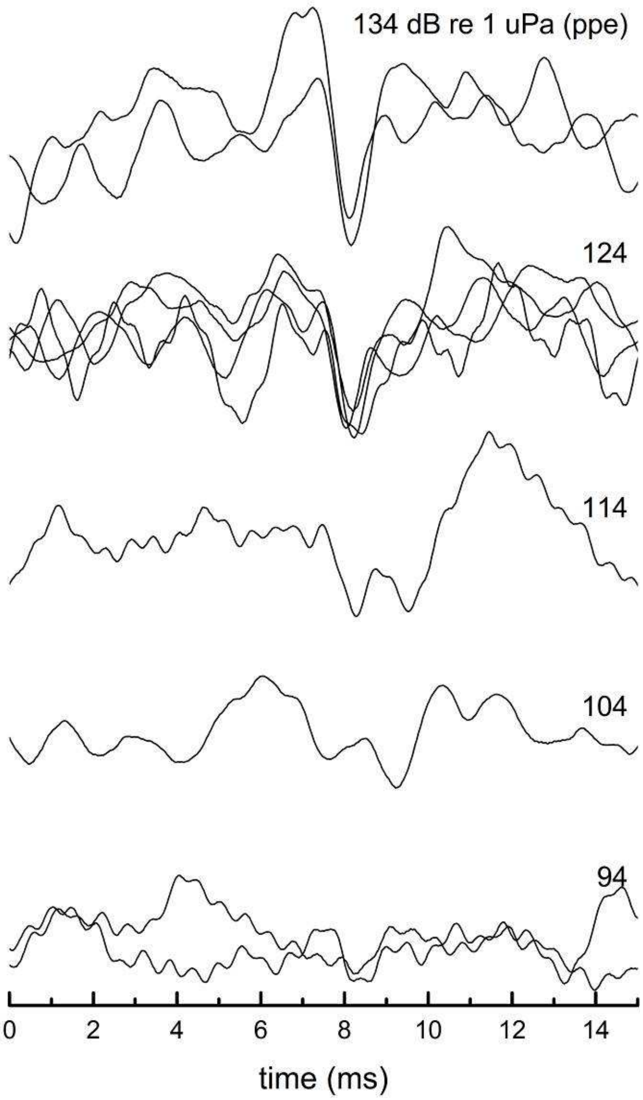

Figure 2. Click evoked potentials measured in an adult female killer whale (Skyla) at Loro Parque while it rested at the side of the pool. Clicks were presented across a range of amplitudes, which are located to the right and above each AEP waveform. Multiple waveforms represent repeated AEP measurements collected at the same click level (with a varying number of measurements at different levels).

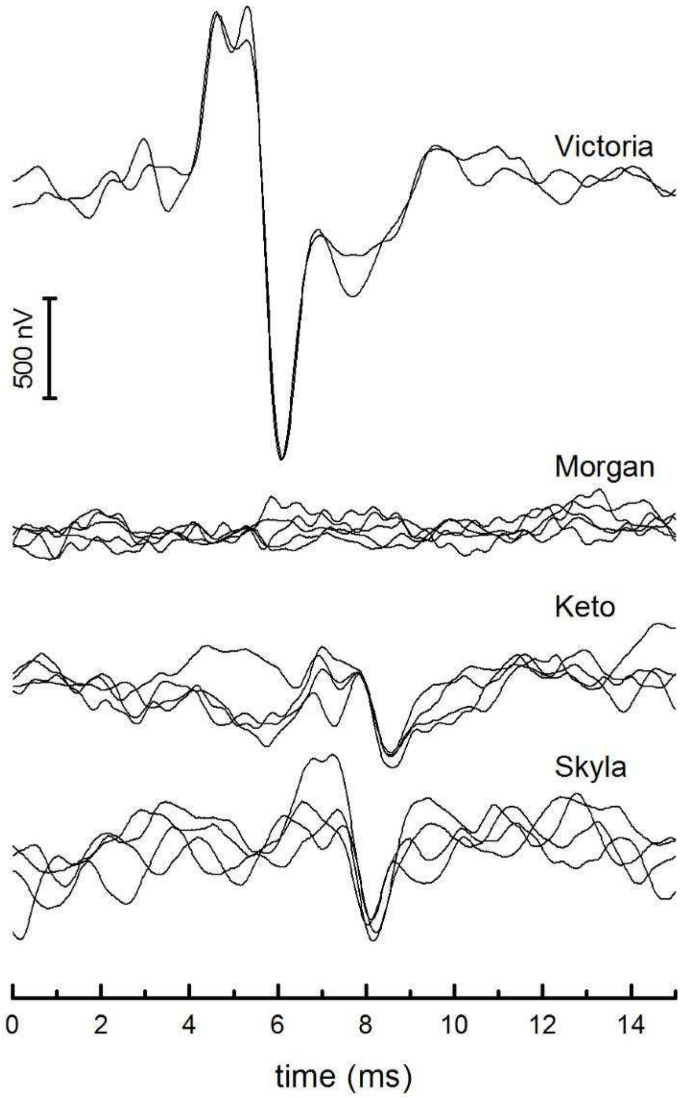

Figure 3. Click evoked potentials measured in four killer whales while resting at the side of the pool or suspended in a stretcher (Victoria). Click levels were 134 dB re 1 µPa (ppe) for Morgan, Keto, and Skyla, and 114–124 dB re 1 µPa (ppe) for Victoria. No click evoked response was observed in Morgan with the 134 dB re 1 µPa (ppe) stimulus, which was the highest that could be generated. Multiple waveforms represent repeated measurements in the same subject under constant measurement conditions.

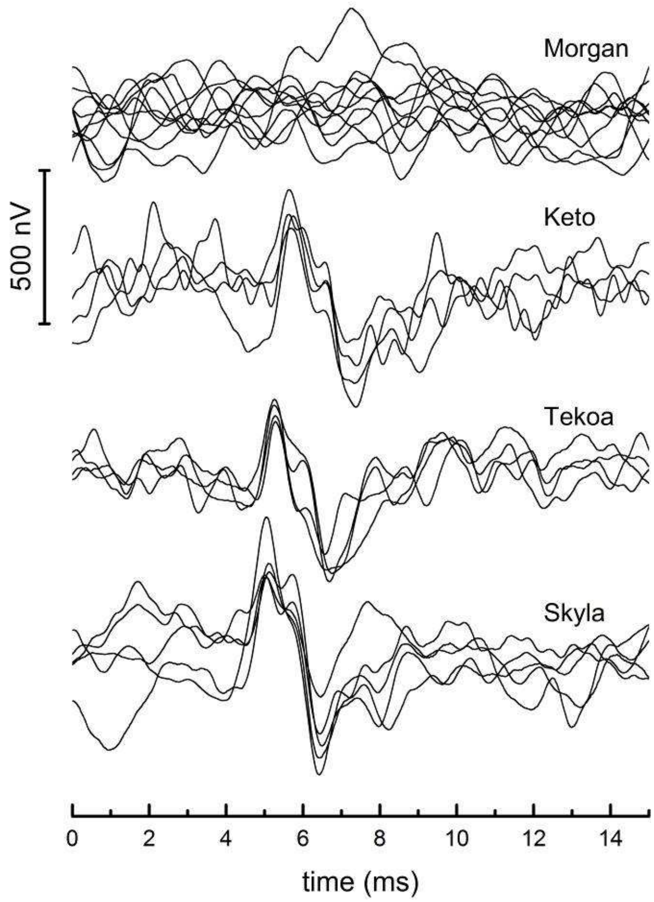

Figure 4. Waveforms showing the click evoked potentials measured in four different killer whales that voluntarily beached themselves for testing. The maximum possible click levels were used for Morgan, Keto, and Tekoa; click levels for Skyla were 10 dB lower. Multiple waveforms represent repeated measurements in the same subject under constant measurement conditions. Note that no click-evoked AEP is observed in the killer whale, Morgan.

5. Conclusions

The click-evoked AEPs measured in most of the killer whales at Loro Parque resemble the expected pattern of evoked responses to this stimulus type previously reported for this species (Szymanski et al. 1999). The lack of a click-evoked response in the killer whale, Morgan, suggests that this animal suffers from a hearing deficit. The magnitude and frequency range over which the hearing deficit occurs cannot be specified with the techniques used here as the click stimulus lacks the frequency specificity necessary for frequency-specific threshold measures. Nevertheless, it can be concluded based upon the recording of click-evoked responses at varying click levels in other whales that Morgan's hearing ability is at least 20–30 dB worse than the hearing sensitivity of the other whales tested. It is possible that Morgan suffers from a profound hearing deficit, or even complete loss of hearing, but the frequency range and magnitude of the loss cannot be determined through currently employed electrophysiological means. The failure to observe a click-evoked AEP is consistent with behavioural observations by the trainers at Loro Parque, who indicated that Morgan did not show any reaction to purely acoustic cues and would often not respond to hand slaps on the water surface immediately behind her. Only when paired with visual cues by the trainers or visually observable reactions/movements of other animals would Morgan show a behavioural reaction. The specific cause of Morgan's hearing impairment is unknown. Acoustic recordings made to characterize the ambient noise levels in the pools at Loro Parque, as well as the successful AEP measurements in the other whales, indicate that noise at the facility is unlikely to be the cause for her hearing impairment.

Future studies involving Morgan should test her hearing capabilities using psychoacoustic procedures. If Morgan can be trained to react to the presence of an acoustic stimulus, it might be possible to assess the extent of her hearing impairment. Testing should be performed in other whales so that comparisons between individuals can be made. The effort would not only provide a better understanding of the hearing deficit experienced by Morgan, but would contribute to the broader knowledge base on the demographic variation in hearing sensitivity among killer whales. Although AEP measurements allow a quick assessment of hearing capabilities, due to the size-dependent loss of the electrophysiological signal in these large mammals, hearing thresholds at any particular frequency are unlikely to be quickly obtained with AEP methods. Additional work would need to be conducted to determine optimal hardware configurations and sampling paradigms. Given the timeframe over which this effort would need to occur, a behavioural approach to audiometry would likely be a more expedient method to determining differences in frequency-specific thresholds.

6. Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V.

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Justification

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The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

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Date: 6 March 2013

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Date: 28 March 2013