FINAL REPORT
Tagging and Playback Studies to Toothed Whales

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LONG-TERM GOALS

While atypical mass strandings of beaked whales have been linked to naval exercises using mid-frequency sonar, the causal chain of events from sound exposure to stranding has not been elucidated. We now know that beaked whales react strongly to sonar, killer whale, and bandlimited noise by ceasing echolocation and completing an unusually slow, directional ascent, in many cases leaving area entirely (Pirotta et al., 2012; Tyack et al., 2011; DeRuiter et al. 2013). Less is known about potential risks to other species of odontocetes that are exposed to sonar during navy exercises. This project is part of a collaborative research program with the goals to:

a) Increase our understanding of the baseline ecology and natural variation in behavior for a different deep-diving species, long-finned pilot whales (*Globicephala melas*).
b) Compare responses of beaked whales vs other odontocetes to playbacks of mid-frequency sonar sounds vs other anthropogenic signals.
c) Conduct combined visual and acoustic surveys for beaked whales and other cetaceans along with collecting oceanographic data for input into models to predict beaked whale distributions based upon characteristics of their habitats.

The ultimate goals are to predict the distribution of species at risk from sonar, to define dose: response curves for risk to beaked and other whales for exposure to naval sonars, and to suggest improvements for monitoring and mitigation.

OBJECTIVES

The objectives of this study, which involved tagging and playbacks to pilot whales in the Alboran Sea, were to:

- Increase the sample size of baseline data on pilot whales
- Conduct playback experiments to tagged pilot whales

- Increase the number of simultaneously tagged pilot whales to study social communication
- Refine methods to visually locate several animals simultaneously

All of these objectives were met.

APPROACH

This study included a 6 week research cruise in 2009 on the NATO RV Alliance in the western Mediterranean Sea, a research cruise in 2010 (Med10) and a research cruise in 2011 (Med11) using smaller vessels to study pilot whales in the Alboran Sea using digital acoustic recording tags (DTAGs). We had already conducted survey and tagging research (including tagging a *Ziphius*) in 2008 at this site, in collaboration with the NATO Undersea Research Centre, using their research vessel Alliance and staff. We collaborated in the fieldwork with Ana Cañadas and other biologists from the Alnilam Marine Research Center from Madrid Spain.

WORK COMPLETED

All three planned research cruises were successfully completed, providing a total of 36 tags placed on long-finned pilot whales (Table 1). During these expeditions, our team has worked out field methods for successfully tagging multiple animals simultaneously and achieving relatively long tag attachment durations. In several cases, we achieved multiple simultaneous tag-ons within the same social group, allowing us to establish a more complete picture of pilot whale baseline behavior and vocalization rates in different social contexts, as well as calculating more exact individual and group vocal rates as a function of time. In 2010, Cuvier's beaked whales were encountered during two days of fieldwork, but despite focal follows and attempts at tagging these animals, no tags were successfully deployed. In 2011, playbacks of both mammal-eating killer whale calls and pseudorandom noise were conducted to two groups of pilot whales, each group containing two tagged individuals. We fine-tuned the behavioral protocols developed by the earlier BRS Med09 and 3S projects (Canadas et al. 2009, Visser et al. 2009, submitted), adapting them for use on a smaller observation vessel and for simultaneous deployment of multiple tags on the same group, emphasizing behaviors related to cohesion. These protocols were used when conducting focal follows of tagged animals in 2010 and 2011.

Table 1: DTAG deployments and playbacks during Med09-Med11

Med09	**Tag deployments**	**Playbacks**
Globicephala melas	1	0
Med10	**Tag deployments**	**Playbacks**
Globicephala melas	16	0
Ziphius cavirostris	*Two days of attempts*	0
Med11	**Tag deployments**	**Playbacks**
Globicephala melas	19	8 (4 animals, KW + PRN)

RESULTS

Over the past three years, we have developed a powerful method for studying the vocal repertoire and use of acoustic signals to maintain cohesion between individual pilot whales with differing social bonds. A key problem has been to correctly ascribe vocalizations to tagged and non-tagged animals. Carefully tagging closely associated individuals, where acoustic signals would be difficult to discern from each other, provides us with the information needed to make these classifications, and opens up the potential for a much more detailed look at social communication in cetaceans. The data collected under this project have made us realize how closely many of these pilot whale groups coordinate their diving behavior, and it has allowed us to identify different types of acoustic signals that are used for social communication during simultaneous and asynchronous foraging dives of individual whales. Correctly identifying the source of calls allows for much more accurate estimates of individual vocalization rates for individuals in different life stages, including dependent juveniles, independent subadults, and adult animals (Fig. 1) and to compare call rates to different behavioral contexts, including the vocalization rates observed during strandings or during aggressive interactions with a sperm whale in 2009 (Fig. 1).

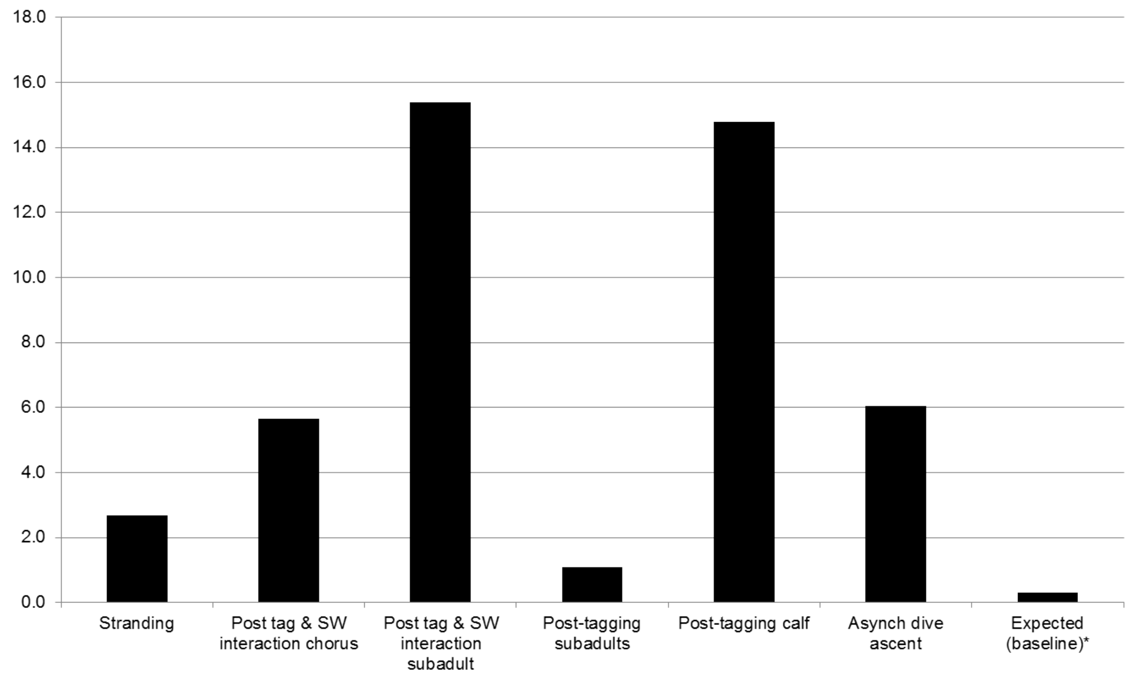

Fig. 1: Individual vocal rates (calls per minute) recorded from long-finned pilot whales in different contexts and for different age classes. These include stranded animals, aggressive interactions with a sperm whale (SW), repetitious call behaviour during the ascent from asynchronous dives, overall vocal rate of a subadult and a juvenile calf (likely 3-4 years old), and a baseline of average vocal rate from adult animals at the surface, excluding foraging and socializing. The first 30 minutes of tag time has been excluded to decrease the effects of tagging.

The data collected during these expeditions have revealed how long-finned pilot whales coordinate the timing of much of their diving behavior. The degree to which animals coordinate their foraging behavior varies between groups and individuals and may depend on the strength of the association between animals. Figure 2 shows an example dive pattern from a highly associated pair of animals tagged during the Med10 field expedition. These animals were observed during visual observations to be each others' nearest neighbour 97% of the focal follow period. Their diving behavior mirrors this close association, both in how 7 out of 8 deep foraging dives are closely timed, but also in very tight synchrony in the shallow diving behavior (not shown). Closely coordinated swimming and breathing behavior for pilot whales has been hypothesized to function as an affiliative or anti-predatory behavior, where animals might benefit from a rapid, coordinated response from closely associated individuals to a potential predator (Senigaglia et al., 2012).

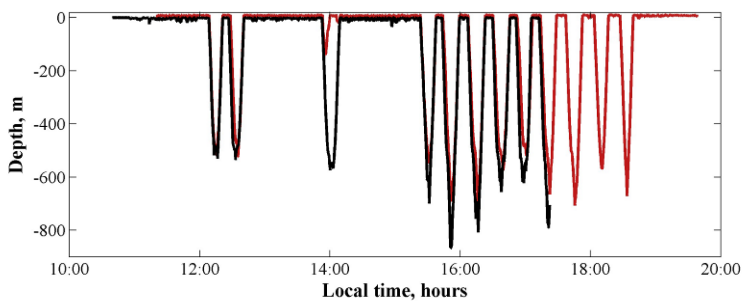

Fig. 2: Simultaneous dive profiles (depth versus time) of two tightly associated pilot whales (black and red) tagged during the August 2010 field expedition. These two whales performed 8 simultaneous deep foraging dives and one non-simultaneous dive where one of the whales broke off the dive early and returned to surface.

The tight temporal synchronization observed between pilot whales gave rise to the question of how movement was coordinated between animals. During simultaneous foraging dives, animals often separated out during the initial part of the dive, then reunited during ascent to surface together. For animals at foraging depths, where light is likely of little importance, this could be achieved through active echolocation or mediated through communication signals. A systematic pattern for sound signals used by terrestrial animals that need to relocate each other is the exchange of sound signals when animals reunite. Pilot whales have a highly diverse sound repertoire, and seem to use different sound signals to facilitate changes in cohesion over varying spatial scales. Fig. 3 demonstrates how rapid sequences of pulsed sounds, termed rasps (Aguilar Soto et al., 2012) are abundant during many simultaneous foraging dives, and how they can be exchanged between close associates (in this case with both animals tagged). Ongoing investigations will test whether these signals are preferentially used for short-range coordination.

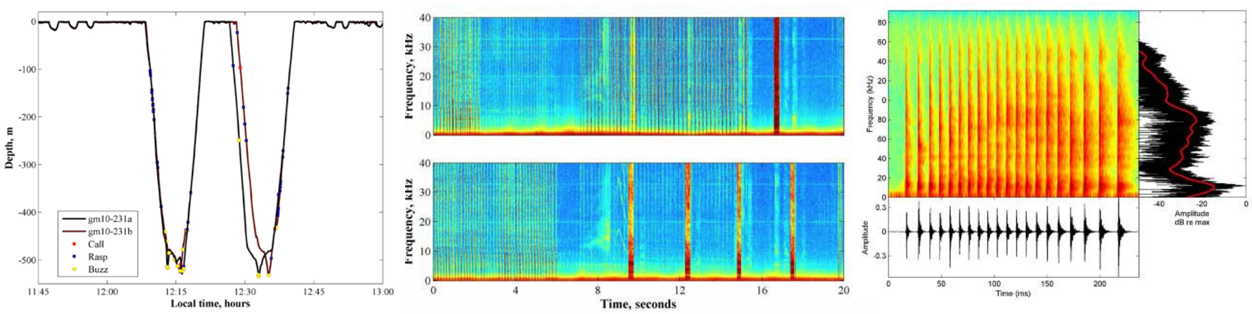

Fig. 3 Left: Dive profiles showing the first two simultaneous foraging dives of gm10_231a (black) and gm10_231b (red). Yellow circles indicate foraging buzzes indicative of potential prey capture, blue circles indicate rasps. Center: Synchronized spectro-grams of part of the ascent of the second dive, showing an exchange of rasps between the two animals, with 4 rasps being produced by gm10_231b around 9, 12, 15 and 17s, and one rasp produced by gm10_231a around 16s. Right: Sound analysis of an example rasp, showing a spectrogram with corresponding waveform below and Welch power spectrum to the right. Note the pulsed structure.

Non-simultaneous dives, on the other hand, represent periods where we know that the diving animal has left its close associates to forage by itself. Figure 4 illustrates an example of such a non-simultaneous dive, where one animal breaks off the dive while the other tagged animal completes it. In this example, the foraging animal produces a few rasps early in the ascent, but then switches to producing frequent calls with a mean individual call rate of 24 calls per minute (Fig. 4 left). The animal at the surface soon starts producing calls in a similar signaling exchange as seen with rasps, but over much greater distance (Fig. 4 center) and call exchanges continue until the animals reunite at the surface. The stereotyped nature of the initial calls is illustrated in Fig 6 (right) by traces of the two independently modulated time-frequency contours in five consequent calls, and an example spectrogram shows the multiple harmonics of both contours as recorded on the source animal. Similar stereotyped calls have been recorded in other contexts, including strandings and during aggressive interspecies encounters (Sayigh et al., 2011) suggesting that they are important for mediating social cohesion within groups of animals, especially during stress or excitement.

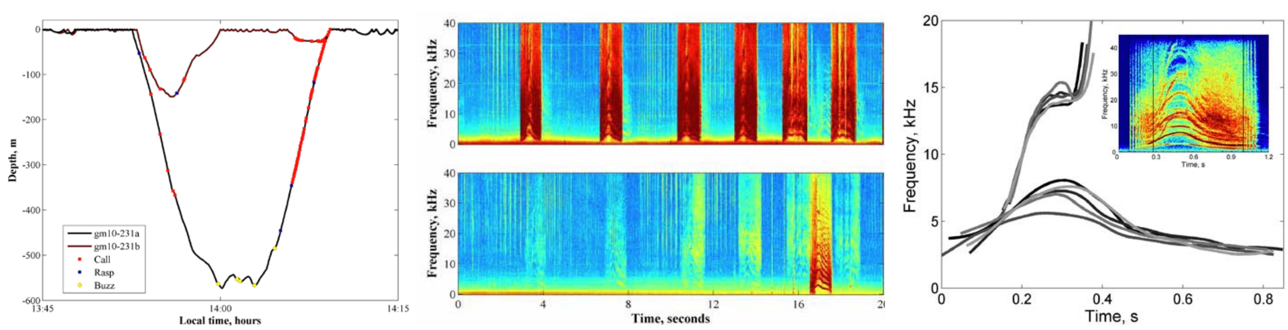

Fig. 4 Left: Dive profiles showing the third (asynchronous) foraging dive of gm10_231a (black) where gm10_231b (red) returns early to the surface. Note especially the frequent stereotyped call pattern during the ascent phase (red circles). Center: Synchronized spectro-grams during the ascent phase of the individual dive performed by gm10_231a (top). During ascent from this dive, high-amplitude, stereotyped calls are repeated frequently during most of the ascent until animals meet at surface. Right: Stereotyped calls consist of two independently modulated frequency contours similar to killer whale calls. Low and high frequency contours are here shown by traces of 5 consequent calls. Insert shows spectrogram.

Our team conducted two sets of playbacks to groups of tagged pilot whales. Playback stimuli were composed of either mammal-eating killer whale calls or pseudo-random noise with identical amplitude and frequency spectrum identical to the matched killer whale sequence. Figure 5 shows the dive profiles and playback timing for two tagged whales in the same group. These whales were separated during playback of killer whale sounds by at least 800m, but were together at the surface during playback of control sounds. During a different playback trial to two other tagged animals, both tagged animals were at the surface and reacted strongly to the killer whale playback by both approaching speaker and by producing repeated, stereotyped calls.

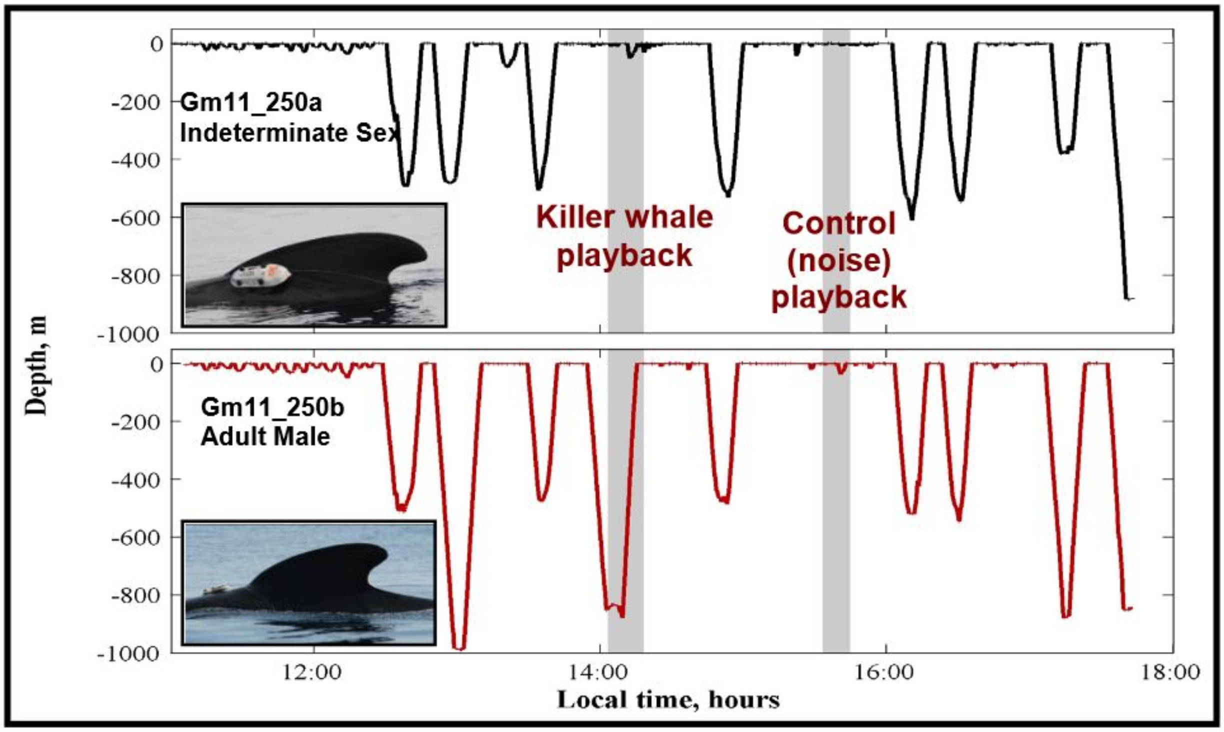

Fig. 5. The dive profiles and playback timing for two tagged whales in the same group.

In addition to the tagging efforts, our team investigated photogrammetric methods for measuring the location of animals in the field. The system that was successfully developed in this project combined a GPS and compass accurate to several degrees with a digital commercial camera and two parallel lasers at a known separation distance of 10 cm. However, due to issues with identifying the laser dots on animals far away from the boat, this configuration was abandoned in favor of a new stereo camera design developed during 2012 and 2013.

Our team also developed software for processing data from multiple simultaneously tagged animals. This software visualization tool employs acoustic cues such as intensity and angle-of-arrival to the two hydrophones in each tag to identify the source of individual signals. This multi-tag comparative approach allows us to identify the source of signals produced during foraging dives, and to identify the source of most, if not all, of the social communication signals produced at the surface where the animals may be moving closely together.

IMPACT/APPLICATIONS

The field methods developed here for tagging entire subgroups of long-finned pilot whales, in combination with the analytical tools for processing the large amounts of data in a multi-tag framework, provide a powerful tool for studying the behavior and acoustics of social odontocetes. It allows for: 1) Reliable identification of the source of communication signals, a non-trivial challenge for toothed whales in a tight social group. 2) Estimation of both acoustic and kinematic changes in response to different acoustic signals from known individuals. 3) Estimation of separation distance and identification of periods where some individuals have left the social group.

The stereo camera system under development allows for accurate estimates of the GPS position, speed, and heading of animals within the field-of-view. This system has a large potential as a method of quantifying changes in movement and social cohesion during disturbances. In combination with simultaneous tagging of multiple whales, it also allows for simultaneous tracking of multiple whales and evaluating how different types of acoustic signals modulate the spatial configuration of groups of animals with different individual-specific social relationships. Earlier methods have focused on the problems that observers have in tracking more than one individual at a time, so they have focused on single focal individuals or have dropped individual observations in order to record behavioral states of groups (Mann, 1999). Neither method is well suited to studying how communication mediates individual-specific relationships.

More playbacks of sonar have been conducted to large delphinid pilot whales (*Globicephala sp.*) than to any other genus of cetacean. Interpretation of the effect of sonar has been hampered by our lack of understanding of baseline behavior and reasons for behavioral transitions and variability in behavior such as calling rate. Information from simultaneously tagged whales has demonstrated that social variables, such as the spatial separation of the group, and the foraging behavior of conspecifics, may have a large effect on the acoustic and movement behavior of these highly gregarious animals. These investigations have already helped us identify separate acoustic mechanisms that likely mediate social cohesion of the group across different spatial scales. In particular, results showing how pilot whales repeat stereotyped calls to re-establish contact with their social group following separations may prove valuable for testing whether monitoring for stereotyped calls (Sayigh et al. 2012) might be an alternative acoustic measure for evaluating social responses to disturbance. In general, these results should help us to interpret variation in calling behavior observed during previous controlled exposure experiments transmitting sonar sounds to pilot whales. We have contributed data to the 3S and MOCHA projects and will continue to make our developing results available to BRS collaborators and the broader scientific community. We expect that this project will contribute significantly to the long term goal of the BRS research program to understand whether and how delphinids may use strategies for social defense against threats, and whether this provides a lower risk for stranding for this taxon. Such results will provide a critical scientific basis for acoustic criteria of harassment and risk of stranding.

RELATED PROJECTS

The study “Understanding the patterns and causes of variability in distribution, habitat use, abundance, survival, and reproductive rates of three species of cetacean in the Alboran Sea, western Mediterranean,” award number N000141110196 to Ana Canadas of Alnilam, uses some of the data from the research cruises funded under this project for her own analyses. The data obtained in this study on responses of pilot whales to playback of killer whale sounds were made available to Charlotte

Cure of the 3S2 project “3S2 - Behavioral response studies of cetaceans to naval sonar signals in Norwegian waters”, award number N000140810661, so that they could boost their sample size of playbacks of killer whale sounds to pilot whales, and to add playbacks that used sounds recorded from mammal-eating killer whales. We are also making data from this project available to the MOCHA project – “Multi-study ocean acoustics human effects analysis,” award number N000141210204, so that they can use the data for innovative statistical analyses, and can pool the data with those from other studies in integrative analyses. The study “Baseline behavior of pilot whales and their responses to playback of anthropogenic and natural sounds”, award number N000141210417, is a continuation of this project exploring the use of a new field site, the Strait of Gibraltar, for repeated tagging of well-known pilot whales across field seasons.

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