Electronic Supplementary Materials

For the manuscript:

Title: First direct measurements of behavioural responses by Cuvier's beaked whales to mid-frequency active (MFA) sonar [Short Title: *Ziphius* response to MFA sonar]

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Tag Data

We used data from 15 DTAGs deployed on Cuvier's beaked whales (*Ziphius cavirostris*). Two datasets, tags zc10_272a and zc11_267a, were collected as part of the Southern California Behavioral Response Study (SOCAL BRS). Those two whales underwent controlled exposure experiments, in which the whales were exposed to simulated naval mid-frequency active sonar (MFA) sounds. The second whale of the SOCAL BRS whales, zc11_267a, was also incidentally exposed to MFA sonar from a distant naval exercise. To better characterize the baseline behavior of Cuvier's beaked whales, we also used data from 13 other Cuvier's beaked whale DTAG datasets collected in the Mediterranean Sea between 2003-2012.

We also included data from 2 mk9 time-depth-recorder tags (Wildlife Computers, Redmond, WA) deployed on *Ziphius* in Hawai'i in 2004 and 2006. Almost all of these tag datasets have been described in previous publications [1, 2, 3].

Details of all the tag deployments are included in Table 1 in the main article text.

Acoustic Data Analysis

For both the controlled and incidental MFA sonar exposures, we calculated the received level on the DTAG tag recording of each MFA sonar transmission. The details of the tag electronics are published elsewhere [4]; in summary, the tag recorded 16-bit acoustic data at 192 (2010) or 480 (2011) kHz; the tag acoustic recording system was calibrated and had a peak clip level of 176 (2010) or 178 (2011) dB re 1 μ Pa. The main received level metric we report is the rootmean-squared (rms) level in a $\frac{1}{3}$ octave frequency band centred at the average fundamental frequency of the MFA signal. Rms levels were calculated in 200 msec windows sliding over the duration of the signal, and the reported level was the highest observed in any one 200 msec window, following the method of Tyack and colleagues [5]. We chose to use rms values in a carefully defined window corresponding approximately to the time over which a whale likely integrates received sound intensity for loudness perception [6, 7]. Similarly, we chose to use a $\frac{1}{3}$ octave frequency band for analysis because it corresponds approximately to mammalian auditory filter bandwidths [8]; this choice is also consistent with previous work [5]. The CEEs used one simulated MFA signal type, while the incidental exposure included five different MFA sonar signal types; for each signal type, we used a bandpass filter centered on the signal's mean fundamental frequency. Signal and filter characteristics are detailed in Supplemental Table 1, and the CEE signals and the CEE sound source are described in detail elsewhere [9]. We note that the signal durations are approximate for the incidental exposure signals, since reverberation and time-spreading made it difficult to pinpoint the exact start and end times of a single arrival of the signal.

For completeness and for possible comparisons with other studies, we also present several other metrics of received level here. First, we include the noise level (rms) so that the reader can assess the signal-to-noise ratio (SNR) of each recorded transmission. Noise level was determined for each individual MFA sonar transmission using a 1-second sample immediately preceding the transmission. Within this sample, the level was determined exactly as described above for the MFA sonar transmissions, using a 200 msec averaging window and taking the largest resulting level observed in any one window [5]. We also report the sound exposure level (SEL, in dB re 1 $\mu Pa^2 * s$), a measure of the acoustic energy in a single transmission, as well as the cumulative SEL, a measure of the total acoustic energy to which the whale has been exposed from the start of the exposure until a given point in time. SEL values were calculated over the full duration of the signal; in this case, we defined signal duration objectively as the period during which the SNR was at least 6 dB [7]. Finally, we present broadband rms levels, calculated as above but replacing the $\frac{1}{3}$ octave filter with a bandpass filter spanning 3-8 kHz (CEEs) or 2-8 kHz (incidental exposures in 2011, some of which included signals at 2-3 kHz). These broadband filters span the fundamental and the first harmonic of the MFA sonar signals, and are presented to indicate that our use of narrower filters for the bulk of our analysis did not lead to general underestimation of the received levels. (One might argue that the $\frac{1}{3}$ octave filter slightly underestimated RLs for the 5th signal type in the uncontrolled exposure, which was quite low in frequency and thus had a certain amount of energy outside the $\frac{1}{3}$ octave filter band (Supplemental Table 1); however, the whale showed no response to that exposure, so this possible bias did not affect any conclusions. In the case of a more detailed analysis of a signal clearly spanning multiple $\frac{1}{3}$ octave bands, it would be better to make and integrate measurements in several bands [5], but for the sake of simplicity we have not done so here.) All received level results are summarized in Supplemental Figure 1. We did not apply any frequency-weighting in calculation of any of these level metrics; however, the analysis bands were within the range over which the mid-frequency cetacean M-weighting function has a value of 0, so applying the M_{mf} weighting would not have changed any of the results reported here [6].

Tag Sensor Data Analysis

For all DTAG datasets, we calculated 13 dive and movement parameters (Supplemental Figure 2, Supplemental Table 2). Each parameter was measured or averaged over the period of one dive, from fluke-out to surfacing, although the original tag data were sampled much more finely (movement data at 5 Hz or greater, and acoustic data at 192 kHz or greater). If a value could not be determined (for example, surface duration after dive cannot be calculated for the last dive in a dive record), we substituted the mean value of all available control dives (dives by baseline animals that did not undergo any CEEs). These substitutions made up 2% of the data points, and making them allowed us to avoid systematically excluding from analysis the last dive and the last deep dive from each tag record.



Supplemental Figure 1: Received Levels of all MFA sonar transmissions recorded on the DTAGs. Reported metrics include: rms level in a $\frac{1}{3}$ octave band centered on the signal center frequency (solid black lines); broadband rms level (dotted black lines); SEL (green lines); cumulative SEL (dotted green lines); and rms level of background noise in the in $\frac{1}{3}$ octave band (solid grey lines). Data are shown for the 2010 CEE (top), 2011 CEE (middle), and 2011 incidental exposure (bottom).

To the extent possible, we calculated the same dive and movement parameters for the mk9 datasets; however, since the mk9 tag recorded dive-depth information



Supplemental Figure 2: Ziphius dive profiles and dive parameters for 272 dives from 15 DTAG records and 50 from 2 mk9 records. Panel A shows dive profiles of deep foraging dives. Dives without sound exposure are plotted in grey; precontrolled-exposure, no-incidental-exposure dives by exposed whales in green; controlled exposures to simulated MFA sonar in red (triangles, 2010; circles, 2011); incidental exposures to naval MFA sonar in blue (squares, 2011); and ship noise in magenta [3]. Panel B shows dive profiles for shallower, silent dives, with color-coding as in A. The 2011 controlled exposure dive is included in all panels to facilitate comparison with both dive types. Panel C contains box-plots of dive parameters for the control foraging dives from A, with * preceding parameters that include mk9 tag data (for plots only, not statistical modelling). Black boxes span 25th-75th percentiles, black horizontal lines mark medians, error bars span 1.5 interquartile ranges, and + symbols indicate more extreme values. Exposed dive parameters were excluded from box-plot calculations but plotted individually, with symbol and color-coding as in A (except that pre-exposure dives, shown in green in the upper panels, are here included in the box-plots). Panel D shows box plots as in C, but for the dives plotted in grey (and green) in B. Abbreviations: Dur., duration; T., time; ODBA, overall dynamic body acceleration.

only, it was not possible to calculate any of the metrics based on accelerometer or magnetometer data. The parameters calculated for the mk9 data are shown in Supplemental Figure 2 and indicated in Supplemental Table 2. The mk9 datasets were excluded from subsequent statistical analysis (since including them would have limited us to considering only the dive parameters obtainable from both DTAG and mk9 tag types). They are included only in Figure 1, to provide a more complete picture of the baseline behavior of the species in the absence of MFA sonar exposure.

Most of the parameters were calculated from the tag data in a straightforward manner, as indicated in Supplemental Table 2, with the exception of the acoustic range. This range was calculated only during the controlled exposure, as it was based on the tag recordings of the CEE sounds. The interval between MFA signal start times on the tag recordings was determined by visual inspection of spectrograms to mark each individual start time (20 second window, fast Fourier transform block size 2048, Hamming window with 50% overlap). The same procedure was applied to a recording from a monitor hydrophone less than 20 m from the sound source. The inter-transmission intervals on the tag recordings were slightly longer than those on the monitor hydrophone recording, because of the additional time required for the sound to propagate the additional distance to the tag. We calculated the relative source-whale range at the time of each transmission by subtracting the monitor hydrophone inter-transmission intervals from the corresponding tag intervals, subtracting the minimum such difference (which corresponds to the closest point of approach (CPA) between whale and source), and multiplying the resulting delay by 1500 m \sec^{-1} to convert from a delay in seconds to a relative range in meters. Position data from surface visual observations were combined with the relative ranges and dead-reckoned track data from the tag to convert to absolute ranges. The range data were also used to calculate whale swim speed relative to the source during the playback, by simply dividing the range difference between sonar transmissions by the inter-transmission interval. We calculated these speed estimates for each transmission, then averaged them over the playback period to obtain a mean swim speed relative to the source during playback. The acoustic range calculations were carried out independently by both WMXZ and SDR. The resulting range values were consistent, with a mean absolute difference of only 17.1 meters, demonstrating that potential human error in marking the transmission start times did not adversely affect the results.

Supplemental Table 1: MFA signal characteristics (duration in seconds and fundamental frequency range in kHz) and filter center frequencies (F_c , Hz)

Signal	Frequency Range	Duration	$\frac{1}{3}$ Octave Filter F _c
simulated MFA sonar	3.5-4.1	1.6	3729
incidental MFA sonar 1	2.5-3.5	1.5	2970
incidental MFA sonar 2	2.2-3	1.5	2610
incidental MFA sonar 3	3.2-4.5	1.9	3920
incidental MFA sonar 4	2.3-4.3	4.5	3280
incidental MFA sonar 5	2.2-3	3.5	2630

Cluster Analysis

The objective of this analysis was to collapse the multivariate time-series of dive parameters into a univariate time-series, with one value to characterize each dive. This "distance metric" (to be described in detail later on) was intended to be a measure of how different a given dive is from the "average" dive by this species. Most marine mammals perform several different types of dives. For example, beaked whales undertake at least two: deep foraging dives and shallow non-foraging dives. Since one would expect the average dive parameters to vary between dive types, it is important to classify dives by dive type before calculating the distance metric.

We chose to do this classification by applying k-means clustering analysis to data from control whales only. Each parameter was normalized prior to clustering by subtracting the mean value and then dividing by the standard deviation. The optimal number of clusters to use – in this case, two – was determined using silhouette analysis [12]. In this case, the choice to use two clusters agrees with our *a priori* assumption that beaked whales have two main dive types. Supplemental Figure 3 shows the results of the clustering analysis.

Mahalanobis Distance Calculation

We then calculated the Mahalanobis distance [13] of each point (that is, each dive) from the center of the cluster to which it belonged, assigning dives by exposed whales to clusters such that the resulting distance was minimized. The Mahalanobis distance is a measure of distance in multi-dimensional space. It is scale-invariant and takes into account correlations between dimensions. Mahalanobis distance is calculated according to:

$$D_M(x) = \sqrt{(x-\mu)^T S^{-1}(x-\mu)},$$

Description	Details	Which Tags	
Descent Duration (min-	Time from last surfacing to first	DTAG, mk9	
utes)	depth exceeding 90% of the maxi-		
	mum depth of the current dive		
Ascent Duration (minutes)	Time from the last time the animal	DTAG, mk9	
	was at or below 90% of the maxi-		
	mum depth until surfacing		
Descent Rate (m sec ^{-1})	Mean descent rate during the whole	DTAG, mk9	
1	descent (as defined above)		
Ascent Rate (m sec ^{-1})	Mean ascent rate during the whole	DTAG, mk9	
	ascent (as defined above)		
Dive Duration (hours)		DTAG, mk9	
Maximum Dive Depth (m)		DTAG, mk9	
Surface Interval (minutes)	Time from surfacing at the end of	DTAG, mk9	
	the current dive until the start of the		
	next dive exceeding 50 m		
Time to Next Click (hours)	Time from surfacing at end of cur-	DTAG	
	rent dive until next start of echolo-		
	cation clicking		
Clicking Duration (hours)	Time from first to last echolocation	DTAG	
	click produced during the current		
	dive		
Average Fluke Rate (Hz)	Fluke-stroke rate was determined as	DTAG	
	in [1], using a pitch threshold of 3		
	degrees and a period of 0.3-4 s		
Average Overall Dynamic	Calculated as in [10], using an aver-	DTAG	
Body Acceleration (odba, -2)	aging period of 5 s		
m sec ⁻²)			
Average Variance of Head-	Calculated (for circular variable) as	DTAG	
ing	in [11], using a 1-minute window		
	sliding forward at sensor sampling		
	rate	DTAC	
Acoustic Range	Range from CEE sound source to	DTAG	
	whale		

Supplemental Table 2: Variables Measured for Each Dive



Supplemental Figure 3: Results of k-means cluster analysis to produce 2 clusters based on mean-normalized beaked whale dive data. The clustering results are indicated by the symbols (triangles and circles), with each cluster enclosed by an ellipse. The results are plotted in 2-dimensional component space using the first two components from a Principal Components Analysis (PCA), although the PCA output was not used by the clustering algorithm.

where x is a vector of dive parameters 1 - N for an individual dive,

$$x = (x_1, x_2, x_3, \dots, x_N)^T$$

 μ is the vector of coordinates of the center point of the cluster of dives from control whales, in other words, a vector of mean values for each parameter for all control dives in a cluster,

$$\mu = (\mu_1, \mu_2, \mu_3, ..., \mu_N)$$

and S is the covariance matrix for all control dives in the cluster. The distance values were normalized by dividing by the standard deviation of the distance for control whales in each cluster. This adjustment was intended to correct for possible different shapes or spatial extents of the two clusters. Supplemental Figure 4 shows the results of the Mahalanobis distance calculations for beaked whale dives.



Supplemental Figure 4: Empirical distribution of Mahalanobis distances for beaked whale shallow dives (cluster 1) and deep dives (cluster 2). The black curves show the proportion of control observations at each distance (specifically, they are kernel density estimates with Gaussian kernels and bandwidths satisfying Silverman's 'rule of thumb' [14]). At the bottom of the plot, along the x-axis, the black tick marks indicate observed distances for individual dives by control whales, and red ticks the same for exposed whales.

Mahalanobis Distance as a Measure of Response Intensity

Supplemental Figure 5 shows the Mahalanobis distances for the two whales that were exposed to MFA sounds, plotted as time series, with sonar exposure dives

indicated in colour. In both cases, there was a large peak in the distance metric at the time of the exposure. The distance also remained elevated for a time after the exposure, slowly decaying back to baseline values after a number of dives. Similar patterns were not prominent in the data from control whales (Supplemental Figure 6).



Supplemental Figure 5: Time series of Mahalanobis distances for MFA-exposed beaked whales. Dives during which experimental MFA exposures occurred are shown in red. Dive during which the whale was incidentally exposed to distant, incidental Naval MFA sonar are shown in blue.

To check for temporal autocorrelation in the time-series of Mahalanobis distances for control whales, we plotted the Mahalanobis distance results (Supplemental Figure 6). We also calculated the autocorrelation function for these control datasets, and we did not find strong evidence of autocorrelation in the data.

Model for Response Intensity as a Function of Sonar Dose

We took Mahalanobis distance (as calculated earlier) as a proxy for behavioural Response Intensity (RI). In modelling this response intensity as a function of sonar exposure intensity, we considered two possible metrics for exposure intensity or "dose": sound level and distance between the whale and the sound source.



Supplemental Figure 6: Time series of Mahalanobis distances for all beaked whales. Sub-plot titles indicate whale ID, with the IDs for whales that were exposed to MFA sonar printed in red.

Sound exposure levels were determined from the DTAG acoustic recordings according to the method described in [5]. Briefly, after filtering with a $\frac{1}{3}$ octave filter spanning the CEE sound frequencies (512-point FIR filter, 3300-4158 Hz) and processing to remove loud transients such as animal clicks or other impulsive noise, the root-mean-squared sound pressure level (in dB re 1 μ Pa) was calculated

in 200 msec windows spanning the duration of the signal. Signal duration was defined as the time when the signal to noise ratio was at least 6 dB. The reported level for an individual MFA transmission was the highest level measured in any one 200 msec window. The level used for response intensity modelling was the highest observed for any transmission that occurred during the dive in question. It is important to note that expert inspection of the controlled exposure data indicated that the animals began to react to the sound near the start of the exposure, when the received sound level was much lower than the maximum level during the same dive.

The distance from the sound source to the whale is more difficult to estimate with currently available data, since the exact positions of the beaked whales during the sound exposures are unknown. For the controlled, experimental MFA exposures, source-whale range at the start of the MFA playback was determined by combining data from the DTAG movement record, animal sighting positions from before the exposure, and acoustically-based estimates of relative source-whale range during the exposure (described earlier). For the incidental exposure of whale zc11_267a to U.S. Navy MFA sonar from a distant naval exercise, an approximate position and range of the sound sources relative to the tagged whale was obtained from the U.S. Navy (by D. Moretti). Supplemental Table 3 shows current range and level estimates for dives during which beaked whales were exposed to MFA.

Using these data on range and level for each dive, we proceeded to model response intensity (expected value of Mahalanobis distance, RI = E(D)) as

$$RI = E(D) = \beta_0 + \sum_{i=1}^{n} x_i(t)$$
 (1)

$$x_i(t) = \begin{cases} \frac{\beta_1 L_{\tau_i} e^{\beta_2(\tau_i - t))}}{(1 + \beta_3 R_i)} & \text{if } t \ge \tau_i \\ 0 & \text{otherwise} \end{cases},$$
(2)

where RI is the expected value of the Mahalanobis distance, t = 1, 2, 3...T is the dive number, n is the number of exposures, L_{τ_i} is the received sound level during exposure dive τ_i , R_i is the source-whale range at the start of exposure i, τ_i is the dive number of exposure dive i, and $\vec{\beta} = [\beta_0, \beta_1, \beta_2, \beta_3]$ are parameters to be estimated. β_0 is the expected distance when there is no exposure, β_1 scales the initial response intensity as a function of received sound level, L, β_2 is the decay rate of the response, and β_3 scales the response intensity as a function of range, R. We modelled the observed Mahalanobis distance using a Gamma distribution. The Gamma distribution for a random variable x with shape parameter k and scale parameter θ has probability density function

$$\Gamma(x,k,\theta) = \frac{1}{\theta^k} \frac{1}{\Gamma(k)} x^{k-1} e^{-\frac{x}{\theta}},$$
(3)

expectation $\frac{k}{\theta}$, and variance $k\theta^2$. To fit the model, we expressed the parameters of the gamma distribution in terms of the RI (that is, the expected value of Mahalanobis distance $(E(D(\vec{\beta})))$) and the new parameter ω defined as

$$\omega = \frac{(E(D(\vec{\beta})))^2}{k} = E(D(\vec{\beta}))\theta \tag{4}$$

or

$$k = \frac{(E(D(\vec{\beta})))^2}{\omega}; \theta = \frac{\omega}{(E(D(\vec{\beta})))}$$
(5)

Assuming that each observed Mahalanobis distance is independently distributed, given the above model, the log-likelihood \mathcal{L} is given by

$$\log \mathcal{L} = \sum_{t=1}^{T} \log \mathcal{L}_t = \sum_{t=1}^{T} -k \log \theta - \log(\Gamma(k)) + (k-1)\log(D_t) - \frac{D_t}{\theta}$$
(6)

We estimated the parameters ω and $\vec{\beta}$ by maximizing this likelihood in the statistical software R (http://www.r-project.org/).

We fit the model to each whale individually. The parameters ω , β_0 , β_1 and β_3 can only take positive values, so they were log-transformed for the maximization only, to allow fitting over all real values. Results are presented in the original (untransformed) parameter space; we calculated standard errors on the parameters using the Hessian matrix, obtaining standard error estimates for the transformed parameters in the original parameter space using the delta method [15]. For whale $zc10_272a$, there was only one exposure at a single range and level, so it was not possible to extract information about the effects of both range and level on response; we thus excluded the range term β_3 for the 2010 whale. We also fit a set of models with fewer parameters (see Supplemental Table 4). For each fitted model, we calculated Akaike's Information Criterion (AIC), and selected for inference the model with lowest AIC. Supplemental Table 4 shows the AIC values; the ΔAIC values between the full models and the next-best reduced models were ≥ 28.5 , giving very strong support for the full models in both years. The resulting parameter estimates (ω , β) (and their standard errors), for the full models only,

Supplemental Table 3: Received Levels and Ranges for Beaked Whale MFA Exposures

Whale ID	Dive Number	Maximum Received Level	Source-Whale Range
		(dB re 1 μ Pa rms)	(start of playback, km)
zc10_272a	9	138	4.0
zc11_267a	1	106	118
zc11_267a	2	94	118
zc11_267a	3	91	118
zc11_267a	4	94	118
zc11_267a	5	96	118
zc11_267a	13	144	4.9
zc11_267a	27	97	118

Supplemental Table 4: Akaike's Information Criterion for all fitted models

Year	Parameters	Number of Parameters	Model Description	AIC	ΔAIC
2010	$\omega, \beta_0, \beta_1, \beta_2$	4	Full Model	101.9	0
2010	ω, β_0, β_2	3	Intercept, Time Decay	130.4	28.5
2010	ω, β_0, β_1	3	Intercept, RL	135	33.1
2010	ω, eta_0	2	Intercept Only	136.4	34.5
2011	$\omega, \beta_0, \beta_1, \beta_2, \beta_3$	5	Full Model	104.5	0
2011	$\omega, \beta_0, \beta_2, \beta_3$	4	Intercept, Time Decay, Range	149.8	45.3
2011	$\omega, \beta_0, \beta_1, \beta_3$	4	Intercept, RL, Range	158.3	53.8
2011	$\omega, \beta_0, \beta_1, \beta_2$	4	Intercept, RL, Time Decay	159.7	55.2
2011	ω, β_0, β_3	3	Intercept, Range	156.5	52
2011	ω, β_0, β_2	3	Intercept, Time Decay	150.6	46.1
2011	ω, β_0, β_1	3	Intercept, RL	157.3	52.8
2011	ω, eta_0	2	Intercept Only	188.9	84.4

were [1.05 (0.27), 3.6 (0.21), 0.063 (0.0068), 0.43 (0.088)] for 2010, and [0.68 (0.16), 1.9 (0.6), 0.074 (0.024), 0.11 (0.023), 0.12 (0.093)] for 2011. Supplemental Figures 7-8 show the data, the fitted models, the residuals, and the residual autocorrelation functions.



Supplemental Figure 7: Model results for whale zc10_272a. Upper panel: data with fitted model (black line); lower left panel: internally Studentized residuals; and lower right panel: residual autocorrelation function.



Supplemental Figure 8: Model results for whale zc10_267a. Upper panel: data with fitted model (black line); lower left panel: internally Studentized residuals; and lower right panel: residual autocorrelation function.

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