A Study to Interpret the Biological Significance of Behavior Associated with 3S Experimental Sonar Exposures

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LONG-TERM GOALS

The long term goal of the 3S international cooperative research program is to investigate behavioral reactions of cetaceans to naval sonar and various control sounds, and the sound exposures required to elicit responses, in order to establish safety limits for sonar operations. 3S project efforts have been focused upon informing the 'exposure-effect assessment' component of a full risk-assessment framework (Fig. 1), which has been identified as a crucial missing component of the risk-assessment need (Boyd et al., 2008).

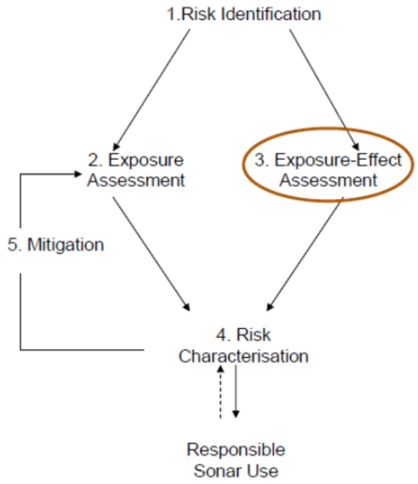

Figure 1. A risk-assessment framework. To characterize risk, both assessment of the amount of exposure in the environment and assessment of the relationship between exposure and effect (circled) are required.

A significant challenge to establishing safety limits for sonar operations in in specifying what types of 'effects' need to be considered in the exposure-effect assessment. Under the US Marine Mammal Protection Act, both direct harm and harassment of marine mammals is regulated, so effects of concern cover a wide span of possible effects. Under new guidelines specified in the European Marine Strategy Directive, nations are required to specify 'healthy state of the marine environment', which could potentially include evaluation of a large array of potential effects from underwater noise emissions. Considerable recent focus has been shifted to understanding how behavioral effects might have longer-term consequences for individuals and populations of cetaceans (NRC, 2005). Modelling efforts under the PCAD working group have demonstrated the potential for behavioral effects to influence vital rates via energy balances (New et al., 2013). Behavioral effects of noise exposure may be shaped by anti-predator adaptations (Frid & Dill, 2002), and it is likely that natural selection will have operated substantially on the behavioral choices that underlie reactions to anthropogenic noise. This study is relevant to the US Navy to advance research in the area of determining methods of assessing 'effects' that have the potential to influence population vital traits by affecting growth, survival and reproduction of individual whales.

OBJECTIVES

The specific objectives of this project are: 1.) use of state-based modelling (e.g. hidden Markov models) to assess how sonar exposure might affect functional behavioral time budgets across 3S species; 2.) quantitative comparison of behavior, and behavioral changes, during sonar presentation and playback of killer whale sounds across the 3S species; and 3.) quantification of the possible impacts of sonar exposure on energy expenditure by linking respiration behavior and underwater activity recorded by Dtags.

APPROACH

The analyses in this study take advantage of baseline data available for each target species, as well as the full range of experimental exposures conducted, including silent approaches as negative control and killer whale playback as positive control stimuli (Table 1).

Table 1. The 3S data-set collected to date.

Species	# tagged	# with CEE	# of sonar exposures	# of silent controls	# of KW playbacks
Killer whale (*O. orca*)	10	4	8	1	2
LF pilot whale (*G. melas*)	30	8	14	4	8
Sperm whale (*P. Macrocephalus*)	10	4	10	2	5
Humpback whale (*M. novaeangliae*)	28	11	20	11	8
Minke whale (*B. acutorostrata*)	1	1	1	1	0
N bottlenose whale (*H. ampullatus*)	6	1	1	0	0
TOTAL	80	29	54	19	23

Technical approach for objective 1: Behavioral state modeling to derive time budgets

The goal of this task is to classify behavioral time series into functional behavioral states, enabling quantification of time budgets and proxies for costs and benefits of behavior within each functional state. Internal and external drivers of behavior may be combined by considering behavioral time series to arise from discrete functional units ('functional states') that are associated with the fulfillment of a particular proximate or ultimate goal or set of goals based on a priori hypotheses (Nathan et al., 2008). States may be classified directly from data ('behavioral state'), such as area-restricted search, or refer to an underlying motivation that drives observed behavior ('motivational state', Bindra, 1978), such as hunger level. With advances in statistical computing, there is increasing scope to estimate these states within more realistic hidden process models that distinguish the observation and underlying ('hidden') process explicitly (Patterson et al., 2008; Schick et al., 2008) and integrate multivariate and multi-scale descriptors of behavior (McClintock *et al.*, 2013).

The framework we will apply will include analysis of a broad suite of recorded behaviors, and specifies the use of functional 'currencies' (e.g. feeding rates, locomotion costs) in conjunction with state-modelling to measure benefits and costs associated with a functional state (see Fig. 1 in Isojunno & Miller, 2015). If functional currencies can be estimated given a state, fitness consequences can be evaluated with or without evidence of a specific behavioral response. This 'functional state approach' helps to frame the cross-disciplinary links between the motivating currency, proximate constraints and ultimate consequences of behavior, and encourages the view that behavioral context is a signal that could potentially fill in knowledge and data gaps of individual-based approaches to population consequence, rather than noise adding unexplained complexity to behavioral records.

The statistical analysis methodology for this objective will utilize state modelling (hidden or non-hidden) to quantitatively classify behavior into functional states determined for 3S study species (Jonsen et al., 2013). The time-series of functional behavioral state, and the quantitative indicators of benefits and costs within the state (feeding indicators, energy expenditure, social investment), are then used to test for systematic variations across different exposure conditions (tagging periods, baseline data periods, sonar-exposure sessions, killer-whale playbacks).

Technical approach for objective 2: Contrasting response to sonar exposures and predator playbacks: In this task, additional data and statistical analyses will be conducted to explicitly contrast the behavior of whale subjects in the 3S dataset during sonar exposure and playback of killer whale sounds, with the goal to evaluate the risk-disturbance hypothesis (Frid & Dill, 2002). Focused analysis of behavior changes following playback of killer whale sounds to sperm whale (Curé et al., 2013), long-finned pilot whale (Curé et al., 2012), and humpback whale have been published (Curé et al., 2015). Linking analyses of responses to killer whale playback and sonar exposure (Miller et al., 2012) provide a qualitative basis for contrasting behavioral changes during acoustic exposures. We will therefore contrast both the intensity and direction of behavioral effects of these exposure types.

Technical approach for objective 3: Quantifying potential energetic impacts of sonar exposure: The goal of this task is to evaluate possible energetic-expenditure changes due to sonar exposure, based upon linkages of underwater activity and breathing events recorded by Dtag sensors. We will use the fine-scale kinematic data recorded by Dtag to evaluate energetics and related breathing patterns in 3S study species. The aim will be to analyze: **1)** the strength of correlations between respiration rates and underwater activity levels (Williams et al., 2004; Williams and Noren, 2009); **2)** to what extent respiration timing (in addition to rate) alters the predicted oxygen taken up by each breath and therefore creates a more accurate predictor of metabolic requirements; and **3)** the impact of disturbance from naval sonar transmissions or playback of killer whales sounds on energetic requirements. Energetic requirements might increase due to behavioral changes such as increased swimming speed associated with avoidance (Miller et al., 2012). This analysis will enable direct energy expenditure contrasts by exposure condition, and will also provide useful methods to quantify the 'cost' component of behavior within different functional states (Task 1).

WORK COMPLETED

In the past fiscal year, work on this project has progressed on all three objectives.

For objective 1, post-doctoral investigator Isojunno has developed new state-based models for baseline and exposure dataset of the long-finned pilot whale (*Globicephala melas*). A hidden Markov model (HMM) approach was developed to quantify behavioral states of long-finned pilot whales, in collaboration with the MOCHA project (see related programs, below). The HMM framework is desirable as it can accept data collected at varying time resolutions, acknowledges the time series nature of the data, and is robust to missing data values. The completed HMM does not attempt to include covariates of exposures to sonar or killer whale playback. Next steps in this research will be to modify the procedure to quantitatively compare behavioral state budgets across different exposure conditions, and to develop a similar model for humpback whale dive states.

For objective 2, collaborative research with Dr. C. Curé has focused on a descriptive contrast of how sperm whales responded to sonar versus playback of killer whale sounds. It is intended that state-based modelling of behavior from objective 1 will link to completion of objective 2 in the next fiscal year, as state-modelling methods are applied to directly contrast behavior related to sonar exposures versus playback of killer whale sounds.

For objective 3, a model of oxygen uptake from breathing events of killer whales has been completed, using both fluke stroke rate and speed-through-the-water as underwater activity metrics (Roos, 2015). The key novel ingredient of both models was the inclusion of an oxygen uptake curve, rather than the assumption that an equivalent amount of oxygen is taken up for each breath (Williams and Noren, 2009). A key outcome from fitting oxygen uptake against speed was the derivation of a cost-of-transport (COT) curve with a clear minimum, a novel result which was not obtained using breathing rates alone. A publication from this analysis is currently being prepared.

RESULTS

Objective 1. Hidden Markov models (HMM) of long-finned pilot whale baseline data.
The goal of this analysis was to quantify behavioral state transitions of long-finned pilot whales using hidden Markov modelling. This analysis made use of the baseline data periods of 22 pilot whale Dtag and visual records. Depth, pitch and roll records from Dtags were used to mark breath times, using established methods (Miller et al., 2010). Fluke-strokes were extracted using oscillations in the accelerometer record. For each 'dive' defined as the time period between two breaths, duration (time to next breath), maximum depth, total vertical displacement, number of fluke strokes, mean vertical speed and dispersion of pitch, roll and heading were used as input tag sensor data. Acoustic data from the tags included the proportion of time clicks, buzzes, and social sounds were recorded in each dive. Mean horizontal speed for each dive was derived from visual tracks.

Quantitative pre-classification of behavior was required to reduce the total number of parameters estimated in the hidden Markov model. First, 'breathing' dives were pre-classified using 2-state mixture models. Most inter-breath intervals were highly repetitive stereotype shallow movements ('breath dives'), which accounted for 51.4% of the total dive time (75 hr) and 73.4% of total dives. Breathing dives had a mean (SD) duration of 20.1s (9.5s) and mean vertical displacement of 6.8m (2.8m).

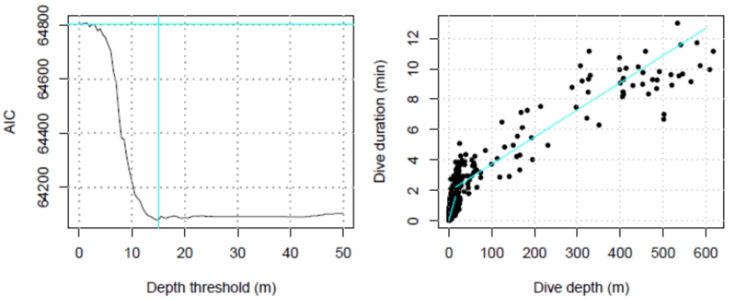

Figure 2. Identification of a break-point in dive depth versus duration relationship. The break point separates two classes of behavior: shallow dives that can have a wide range of durations (steep slope), and deeper dives for which dive duration is more limiting (shallower slope).

After removal of 'breathing dives', deep foraging dives were pre-classified from the remaining dives first by determining a break-point depth in the depth versus duration relationship, and then by 2-state mixture models of dive shape, acoustic activity and movement effort. A break-point depth of 15m was identified by finding the minimum AIC point to divide dive depth versus duration relationships (Fig. 2). 50.8% of dives greater than 15m in depth were classified as foraging dives, and had a greater likelihood of clicking, greater depth, and greater variation in orientation parameters than dives classified as 'non-foraging dives' which never exceeded 59m in depth.

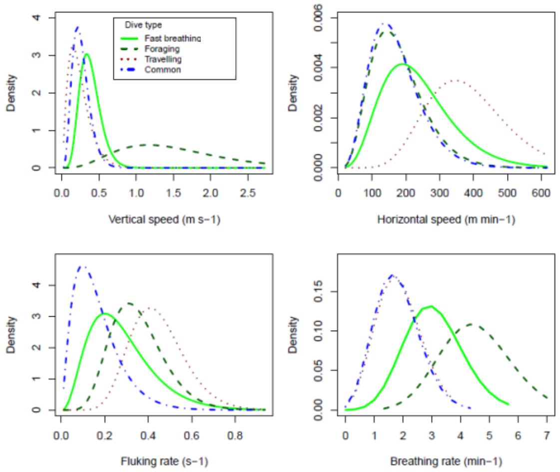

Figure 3. State-dependent data distributions for the four dive states included in the model. 'Foraging', 'Fast-breathing', Travelling' and 'Common' dives based upon their characteristics. 'Breathing rate' refers to the number of breaths in the 3-min period following the particular dive type.

The HMM model was then fit to the remaining non-breathing dives. AIC and BIC supported at least four functional dive types in addition to stereotyped breathing (Fig. 3). All dive types, except for foraging dives, were most likely to be followed by the same dive type. Foraging dives were most commonly followed by 'fast breathing' dives, indicating a need to uptake O_2 following energetically-costly deep foraging dives. In conclusion, this baseline model of functional state of dives appears to effectively capture the diversity of behaviors observed in long-finned pilot whales. Next steps in this area will be to apply these dive-state classifications to the 3S exposure and post-exposure data, and evaluate any exposure effects on the diving time budget as well as energetic proxy currencies within the states. An analogous state-based modeling approach is being developed for humpback whales.

Objective 2. Contrasting response to sonar exposures and predator playbacks
Under this objective, a new analysis contrasting the type and magnitude of responses of sperm whales to sonar and playback of mammal-eating killer whale sounds has been completed. The analysis focused on horizontal movement, foraging and resting, and social behaviors – all of which have functional interpretations and are expected to be modulated in an anti-predator response. The entire 3S sperm whale data-set was used in the analysis, requiring additional severity scoring of killer whale playbacks which were not included in Miller et al. (2012) and quantitative analysis of acoustic behavior, including coda and slow-click production (Table 2).

Table 2. Summary table illustrating the type and magnitude of behavioral responses of sperm whales to sonar transmissions and killer whale playbacks. Within each behavioral response type we scored the presence, severity, and consistency of responses. From Curé et al., submitted.

Type of behavioural response	**Parameters assessing magnitude of the disturbance response**	**LFAS**	**LFAS -DS**	**MFAS**	**no-sonar CTRL**	**KW**	**noise CTRL**
Changes in horizontal movement	i) Consistency (%)	75	100	50	50	80	100
	ii) Highest severity score	6	4	6	1	7	3
	iii) Avoidance response (yes/no)	yes	yes	yes	no	yes	no
Alteration of foraging/resting activities	i) Consistency (%)	100	50	0	0	100	0
	ii) Highest severity score	6	6	0	0	6	0
	iii) Cessation of feeding (yes/no)	yes	yes	no	no	yes	no
Social response	i) Consistency (%)	75	50	75	50	100	100
	ii) Highest severity score	4	0	4	0	6	4
	iii) Production of codas (yes/no)	yes	no	yes	no	yes	no
	iii) Grouping behaviour (yes/no)	no	no	no	no	yes	no

In summary, an index of response magnitude for the three behavioral classes was generated (Fig. 4). This systematic contrast indicates that responses to predation risk simulated by killer whale playbacks generated the highest-magnitude responses, and that responses to LFAS (1-2 kHz) upsweep sonar were similar in type and magnitude – except for a lack of grouping behavior which was only observed in response to playback of killer whale sounds. In contrast, responses other sonar signals and control presentations (no-sonar control, noise-control) led to fewer responses that were not as severe (Table II, Fig. 4). Anti-predator reactions are expected to be costly if those costs are outweighed by increased survival by avoiding predation. Because of their similarity to anti-predator reactions, responses to the LFAS sonar appear to have the greatest potential to lead to effects on vital rates, but such effects would only occur if exposure to sonar was sufficiently frequent (see Fig. 1).

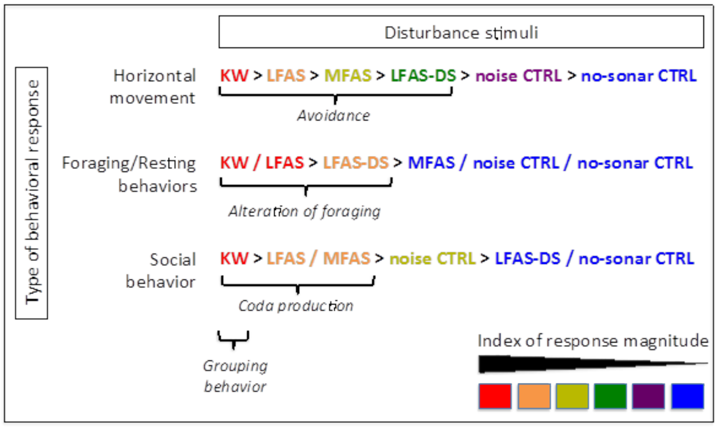

Figure 4. schematic representation of the behavioral disturbance magnitude, based upon presence, consistency and severity of behavioral responses within each category. From Curé et al., submitted.

Objective 3. Quantifying potential energetic impacts of sonar exposure.
In the past year, in successfully completing her MPhil thesis (Roos, 2015), graduate student Marjoleine Roos expanded the oxygen store model to enable estimation of metabolic costs due to swimming speed, building on the previous model that used fluke stroke rate as an underwater activity indicator.

As was previously found using fluke stroke rate as an underwater activity indicator, much stronger correlations were found between predicted metabolic rate and activity levels when an oxygen uptake curve was used (Fig. 5, left bottom) than assuming a constant amount of oxygen uptake per breath (Fig. 5, left top). Assuming constant oxygen per breath led to a very similar pattern for breathing rates and COT estimates as has been observed in previous studies (contrast Fig 5 top panels with Figs 2 and 4 from Williams and Noren, 2009). In contrast, the expected U-shaped COT curve was obtained when oxygen uptake varied per breath (Fig 5, bottom right). We conclude that accounting for breath-by-breath variation in oxygen uptake appears to be critical when attempting to use breathing events to estimate metabolic rates of cetaceans at sea (Roos, 2015). However, accurate estimates of field metabolic rates using these approaches will require additional measurements of breath-by-breath variation in oxygen uptake.

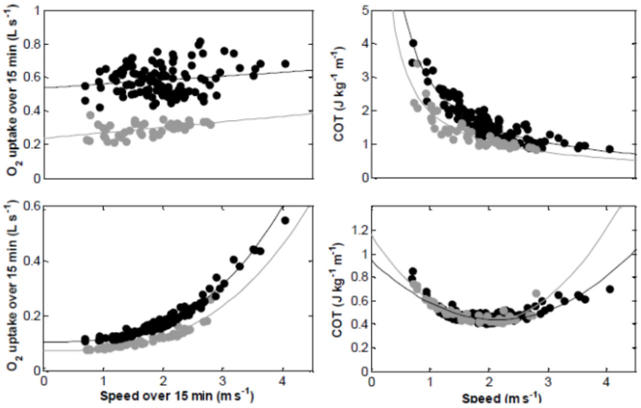

Figure 5. Oxygen uptake (left) and cost-of-transport (COT; right) versus speed. Top panels show results assuming a constant amount of oxygen is absorbed per breath, while the bottom panels use an oxygen uptake function in which uptake depends upon the oxygen store at the time of the breath. Note that use of the uptake function led to a clear minimum in the COT curve.

RELATED PROJECTS

This study builds upon data collected during two previous projects "Cetaceans and naval sonar: behavioral response as a function of sonar frequency" award number N00014-08-1-0984, which expired in 2011 and "3S^2: Behavioral response studies of cetaceans to navy sonar signals in Norwegian waters" which expired in 2014. Statistical support and collaboration is ongoing with the MOCHA project award N00014-12-1-0204. Collaborative research is pursued with Kelp Marine PI Fleur Visser under award N00014-11-1-0298. Additional support for this project is provided by French Ministry of Defence.

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PUBLICATIONS

Curé, C., Isojunno, S., Visser, F., Wensveen, P. J., Sivle, L. D., Kvadsheim, P. H., Lam, F. P. A., and Miller, P. J. O. (submitted). Biological significance of sperm whale responses to sonar: comparison with anti-predator responses. Submitted to Endangered Species Research.