ONR
Marine Mammal & Biology Program Review

20-24 March 2017

Abstract Book

AGENDA

Day 1: Monday 20 March 2017						
Time	**Program Thrust**	**Last Name**	**Performer**	**Award Number**	**Project Title**	**Attendance Restrictions**
830		Weise	ONR		ONR MMB Program Update	
900	EoS-BRS	Miller	SMRU	N000141410390 / N000141512533	Towards relevant dose-response relationships for real-world naval sonar activities	Distribution A
930	EoS-BRS	Southall / Hazen	SEA	N000141512127 / N000141512647	The influence of prey on blue whale (*Balaenoptera musculus*) baseline foraging ecology and sonar playback experiments	Distribution A
1000	EoS-BRS	Visser	Kelp	N000141512341	Cetacean social behavioral response to sonar: biological relevance and severity of sonar responses	Distribution A
1030	Break					
1100	EoS-BRS	Jenson / Tyack	WHOI	N000141410410	Social ecology and group cohesion in pilot whales and their responses to playback of anthropogenic and natural sounds	Distribution A
1130	EoS-BRS	Calombokidis / Kellar	CRC	N000141310772 / N0001415IP00058	Behavioral and physiological response of baleen whales to ships and ship noise	Distribution A
1200	EoS-BRS	Discussion				
1230	Lunch					
130	EoS-Stress	Houser	NMMF	N000141512230	Variability of hormonal stress markers collected from a managed dolphin population	Distribution A
200	EoS-Stress	Champagne	NMMF	N000141310770	Stress hormones and their regulation in a captive dolphin population	Distribution A
230	EoS-Stress	Bechshoeft	AU	N000141310771 / N000141512187	Validating the novel method of measuring cortisol levels in cetacean skin by use of an ACTH challenge in bottlenose dolphins / Measuring and validating levels of steroid hormones in the skin of bottlenose dolphins (Tursiops truncatus)	Distribution A
300	Break					
330	EoS-Stress	Romano	SRF	N000141410411	Investigation of the molecular response in blood and skin of belugas in response to "stressors" to aid in assessing the impact of environmental and anthropogenic challenges on health	Distribution A
400	EoS-Stress	Atkinson	PSU-ARL	N000141410425 / N000141613016	Development and validation of a techniques for the detection of pregnancy and stress in large whales	Distribution A
430	EoS-Stress	Kellar / Claridge	NOAA SWFSC	N0001415IP00057 / N000141110433	Assessing beaked whale reproduction and stress response relative to sonar activity at the Atlantic Undersea Test and Evaluation Center (AUTEC)	Distribution A

Day 2: Tuesday 21 March 2017						
Time	**Program Thrust**	**Last Name**	**Performer**	**Award Number**	**Project Title**	**Attendance Restrictions**
830	EoS-Stress	Crocker	SSU	N000141410393	Variability of hormonal stress markers and stress responses in a large cross-sectional sample of elephant seals / Physiological impacts of variation in hormonal stress markers and stress responses in a large cross-sectional sample of elephant seals	Distribution A
900	EoS-Stress	Boonstra	UT	N000141512214	Quantifying stress in marine mammals: Measuring biologically active cortisol in cetaceans and pinnipeds	Distribution A
930	EoS-Stress	Trumble	BU	N000141410415	Determining baseline stress-related hormone values in large cetaceans	Distribution A
1000	EoS-Stress	Discussion				
1030	Break					
1100	Eos_Phys	Holt / Noren / Williams	NOAA - NWFSC	N0001416IP00023 / N000141410460 / N0001415IP00039 / N0001414IP20045	Comparative and cumulative energetic costs of odontocete responses to anthropogenic disturbance	Distribution A
1130	EoS-Hearing	Cranford	QMC	N000141612049	Building a virtual model of a baleen whale: Phase 3 - the cranial vibroacoustic antenna	Distribution A
1200	EoS-PCoD	Moretti / Falcone / Thomas	NUWC - Newport	N0001416WX00455 / N000141512899 / N000141512191	A population consequence of acoustic disturbance model for Cuvier's beaked whale (Ziphius cavirostris) in southern California	Distribution A
1230	Lunch					
130	EoS-PCoD	Booth	SMRU	N000141410406	PCoD Lite - Using an interim PCoD protocol to assess the effects of disturbance associated with US Navy exercises on marine mammal populations	Distribution A
200	EoS-PCoD	Lusseau	UA	N000141512377	The health black box in PCoD: Understanding the onset of health impacts caused by disturbance	Distribution A
230	EoS-PCoD	Moore / Barlow	NOAA SWFSC	N0001415IP00088	A power analysis and recommended study design to directly detect population-level consequences of acoustic disturbance	Distribution A
245	EoS-PCoD	Claridge / Durban	BMMRO	N000141512748	Photogrammetry with an unmanned aerial system to assess body condition and growth of Blainville's beaked whales	Distribution A
300	Break					
330	EoS-PCoD	Christiansen		N000141713018	Developing a bioenergetic model for baleen whales to assess population consequences of disturbance - Phase 1	Distribution A
345	EoS-PCoD	Discussion				
400	IER	Benoit-Bird	OSU	N000141512204	Linking deep-water prey fields with odontocete population structure and behavior	Distribution A
430	IER	West	HPU	N000141410412	The diet composition of beaked whales and melon-headed whales from the North Pacific	Distribution A
515	Adjourn					

Day 3: Wednesday 22 March 2017						
Time	**Program Thrust**	**Last Name**	**Performer**	**Award Number**	**Project Title**	**Attendance Restrictions**
830	IER	Tyack	UStA	N000141512553	Comparing the foraging efficiency of beaked whales on and off naval ranges	Distribution A
900	IER	Au	UH	N000141410673	Foraging behavior of beaked and other deep diving odontocetes in the Kona coast of Hawaii Island	Distribution A
930	IER-S&TD	Shorter / Hurst	UM	N000141410417 / N000141410391	Finalizing the DTAG: Implementation and testing of design improvements for reliability and availability	Distribution A
1000	IER-S&TD	Zerbini	CRC	N000141310653	Improving large cetacean implantable satellite tag designs to maximize tag robustness and minimize health effects to individual animals	Distribution A
1030	Break					
1100	IER-S&TD	Teilmann	AU	N000141410424	Acquisition of oceanographic measurements from baleen whales: Field deployments of tags developed under grant ONR (N00014-13-1-0854)	Distribution A
1130	IER-S&TD	Ware	UNH	N000141410395	Trackplot enhancements: Support for multiple animal tracks and gyros	Distribution A
1145	IER-S&TD	Discussion				
1215	Lunch					
130	Mon-Det	Wilcock	UW	N000141410423	An investigation of fin and blue whales in the NE Pacific Ocean using data from Cascadia Initiative ocean bottom seismometers	Distribution A
200	Mon-Det	Baumgartner	WHOI	N000141310807	Cetacean community ecology in the waters of Sri Lanka and the Bay of Bengal	Distribution A
230	Mon-Det	Oswald	Biowaves	N000141410413	Development of automated whistle and click classifiers for odontocete species in the western Atlantic Ocean and Temperate Pacific and the waters surrounding the Hawaiin Islands	Distribution A
300	Break					
330	Mon-Det	Lammers	OSI	N000141512373	Does depth matter? Examining factors that could influence the acoustic identification of odontocete species on bottom-moored recorders	Distribution A
400	Mon-Det	Roch	SDSU	N000141512299	Unsupervised learning (clustering) of odontocete echolocation clicks	Distribution A
430	Mon-Det	Shaffer / Thomas / Claridge	NUWC - Newport	N0001416WX00450 / N000141512648 / N000141512649	Beaked whale group deep dive behavior from passive acoustic monitoring	Distribution A
500	Adjourn					
515 - 715	Poster Session					

Day 4: Thursday 23 March 2017						
Time	**Program Thrust**	**Last Name**	**Performer**	**Award Number**	**Project Title**	**Attendance Restrictions**
830	Mon-Det	Miksis-Olds / Thomas	UNH	N000141410397 / N000141410394 / N000141612860 / N000141612364	Large scale density estimation of blue and fin whales	Distribution A
900	Mon-Det	Sirovic / Calambokidis	SIO	N000141410414	Behavioral context of blue and fin whale calling for density estimation	Distribution A
930	Mon-Det	Thomas	UStA - CREEM	N000141512142	A framework for cetacean density estimation using slow moving autonomous ocean vehicles	Distribution A
1000	Mon-Det	Siderius	PSU	N000141310769	Application of density estimation methods to datasets collected from a glider	Distribution A
1030	Break					
1100	Mon-Det	San Filippo / Angliss	Dahlgren / NOAA AFSC	N0001416WX00449	Comparing manned and unmanned aerial surveys of cetaceans in the Arctic: Preliminary results	Distribution A
1130	Mon-Det	Zitterbart / Boebel	AWIPMR	N000141310856	Exploring the limits of thermal automatic whale detection (ETAW)	Distribution A
1200	Mon-Det	Baker	OSU	N000141512297	eDNA barcoding: Using next-generation sequencing of environmental DNA for detection and identification of cetacean species	Distribution A
1230	Lunch					
130	Mon-Det	Klinck / Luby	UW	N000141612214	Low power, two-channel marine mammal recorder	Distribution A
200	Mon-Det	Baumgartner	WHOI	N000141410392	The DMON2: A commercially available broadband acoustic monitoring instrument	Distribution A
230	Mon-Det	Flagg	Desert Star	N0001415P5133	The development of advanced passive acoustic monitoring systems using microMARS technology	Distribution A
300	Break					
330	Mon-Det	Klinck	CU	N000141512240	Field testing and performance evaluation of the Long-term Acoustic Real-time Sensor for Polar Areas (LARA)	Distribution A
400	Mon-Det	Discussion				
430	Adjourn					

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Day 1: Monday March 20, 2017

2017 ONR
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Program Review

Towards relevant dose-response relationships for real-world naval sonar activities

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Background

The 3S international cooperative behavioral response study (BRS) research program investigates behavioral reactions of cetaceans to naval sonar, and the sound exposures associated with responses, in order to establish safety limits for sonar operations. 3S project efforts have been focused upon informing the 'dose-response' component of a risk-assessment framework. The 3S team has published quantitative received-level (RL) dose-response functions for behavioral changes in killer, sperm, long-finned pilot, and humpback whales – and described the RL associated with behavioral response of a beaked whale. Here, we report progress on development of state-of-the-art methods to improve the applicability and relevance of both 'dose' and 'response'.

A key challenge is to specify the biological relevance of responses and to develop criteria of what 'responses' to include in exposure-effect assessments. Behavioral effects of noise exposure may be shaped by anti-predator adaptations and natural selection will have operated substantially on the behavioral choices that underlie reactions to anthropogenic noise. Considerable recent research has focused on evaluating how behavioral effects might have longer-term consequences for individuals and populations of cetaceans. Modelling efforts have demonstrated the potential for behavioral effects to influence vital rates via energy balances [1]. Therefore, it is a priority to consider how behavioral response to sonar might alter energetics via changes in behavioral time budgets and increased metabolic rates associated with responses to sonar.

The dose term seeks to quantify aspects of the signals that drive responses, to enable prediction of the extent of habitat over which negative outcomes occur. RL of sonar quantifies the intensity of sonar exposure and is relatively easily calculated. However, one large systematic difference between BRS vs operational sonar exposure is the relatively low source level used in BRS exposures. The source-whale distance over which an operational sonar would exceed a given RL is much greater than was used in a BRS. Thus, it is a high priority to consider how source-whale distance itself might modulate how cetaceans respond to sonar.

Objectives

Use BRS data collected by the 3S project to:
1. Quantify how sonar exposure influenced functional time budgets of cetaceans;
2. Use respiration behavior and underwater activity recorded by Dtags. Consider possible impacts of sonar on energy expenditure;
3. Use observed responses during playback of killer whale sounds as a 'yardstick' to evaluate the biological significance of behavioral changes during sonar presentations.

Separately: 4. Conduct field trials to establish methods and collect pilot data on how source-whale distance affects how beaked whales far from regular sonar activities respond to sonar.

Methods

1. Use state-based statistical methods to identify biologically-relevant behavioral states; calculate if/how time budgets changed due to sonar.
2. Create an oxygen-store model to evaluate how breath-breath variation in oxygen uptake can be better used to estimate energetic costs.
3. Include killer whale sound exposures as a positive-control in quantitative analyses of sonar effects. Compare and contrast characteristics and magnitude of behavioral responses during exposure to sonar versus killer whale sounds.
4. Carry out fieldwork in Jan Mayen with northern bottlenose whales employing multi-scale observation techniques (archival Dtags, Argos Splash tags, acoustic recorders) to collect pilot data and design a study of how distance affects behavioral responses in a location far from ongoing sonar activity.

Results

State-based modelling has been successfully accomplished for sperm and long-finned pilot whales, with preliminary results obtained for humpback whales. Sperm whale behavior sequences could be classified into 3 foraging states (descent, layer-restricted search, ascent), surface and underwater resting, and a 'non-foraging-active' state. We found a statistically-significant reduction of feeding with an increase in 'non-foraging-active' behavior during 1-2kHz sonar and killer whale sound playbacks, though other sonar exposures did not lead to consistent changes in time budgets [2]. Long-finned pilot whale behavior is more complex than that of the solitary sperm whales of Norway, so a dive-based approach was used. Near-surface behaviors including short breathing dives were first pre-classified, and remaining dives were then classified into four functional dive classes. Time spent in foraging dives was reduced during the first sonar exposure, but not during playback of killer whale sounds (Isojunno poster). Effects on time-budgets can be used to better quantify the biologically-relevant effects of sonar.

An oxygen store model revealed that uptake of O_2 per breath by killer whales is predicted to vary substantially [3]. Breathing rates indicated no effect of a high-speed avoidance response to sonar exposure on metabolic rate (Fig 1, left), but predicted oxygen uptake from a store model identified the expected metabolic costs of high-speed avoidance (Fig 1, right). Therefore, breathing rate alone may not be an effective indicator of metabolic rate. Including information on the timing of breaths and breathing patterns linked to underwater activity enables more accurate predictions of oxygen uptake.

Analysis of sperm whale movement, feeding and social behaviors demonstrated that responses fell on a continuum with the strongest effects occurring to playback of killer whale sounds, followed by sonar stimuli, and control treatment with the least effects [4]. Quantitative analyses of responses to playback of killer whales sound vs sonar have shown similar effects for sperm whales and humpbacks whales [5,6], but differing responses for long-finned pilot whales. Responses to predator stimuli can serve as an effective biological-significance yard-stick, but species-species variation in predation risk needs to be considered when applying this theory.

Fieldwork was accomplished off Jan Mayen 2013-2016 during which we improved capabilities to tag and track whales using a custom Dtag ('Mixed-Dtag') attached using the ARTS pneumatic tagging system. Data from 12 SPLASH tags revealed fine-scale movements near Jan Mayen and large-scale movements to the Azores. Four sonar exposures have been conducted, including an exposure at 17km range from the Dtagged whale. Clear and strong avoidance and diving responses were observed using multi-scale observations of Dtag, SPLASH tag, and bottom mounted buoys (Wensveen poster). These data indicate that source-whale distance effects may be less substantial in this pristine habitat than has been indicated for beaked whales near a navy range [7]. Animals living near navy ranges may have more opportunities to learn how distant sonar signals affect them (or not) than animals in areas far from regular sonar activity.

Notes:

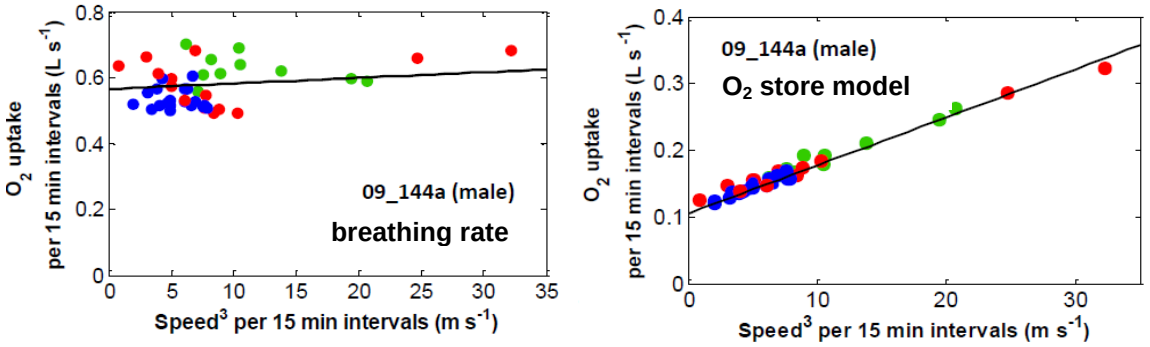

Figure 1. O_2 uptake versus speed^3 using breathing rate (left) and an oxygen store model with breath-breath variation (right), showing pre-exposure (green), during exposure (red) and post-exposure (blue).

[1] NRC 2016. Approaches to Understanding the Cumulative Effects of Stressors on Marine Mammals. NAS Press, 250 pp. **[2**] Isojunno S, et al. 2016. Sperm whales reduce foraging effort during exposure to 1-2 kHz sonar and killer whale sounds, Ecological Applications, 26 (1), 77-93. **[3]** Roos MMH, Wu G-M, Miller PJO. 2016. The significance of respiration timing in the energetics estimates of free-ranging killer whales (*Orcinus orca*). J. Exp. Biol. 219, 2066-2077. **[4]** Curé C, et al. 2016. Biological significance of sperm whale responses to sonar: comparison with anti-predator responses. End. Spec. Res., 30, 89-102. **[5]** Curé C et al. 2015. Predator sound playbacks reveal strong avoidance responses in a fight strategist baleen whale MEPS, 526, 267-282. **[6]** Sivle LD, et al. 2016. Naval sonar disrupts foraging in humpback whales. MEPS 562, 211-220. **[7]** DeRuiter SL, et al. (2013) First direct measurements of behaviour responses by Cuvier's beaked whales to mid-frequency active sonar. Biology Letters 9, doi: 10.1098/rsbl.2013.0223.

The influence of prey on blue whale (*Balaenoptera musculus*) baseline foraging ecology and sonar playback experiments

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Background

Blue whales (*Balaenoptera musculus*) are a mobile predator in the California current system that target a single prey resource, krill (*Euphausia pacifica, Thysanoessa spinifera*), and travel broadly to exploit ephemeral patches. Long term tags have been used to describe broad scale blue whale movement and behavioral patterns at the basin scale. However, less is known about fine-scale relationships foraging behavior and krill. As the largest predators and prey specialists, blue whales show substantial behavioral plasticity to satisfy extreme energetic demands. This study contributes to a better contextualizion of the impacts that anthropogenic threats (e.g. climate change, noise) have on their ecology and life history.

Objectives

As an integrated element of the LMR-funded SOCAL-BRS project, we were able to assess how prey influenced both baseline ecology as well as blue whale responses to sound in controlled exposure experiments.

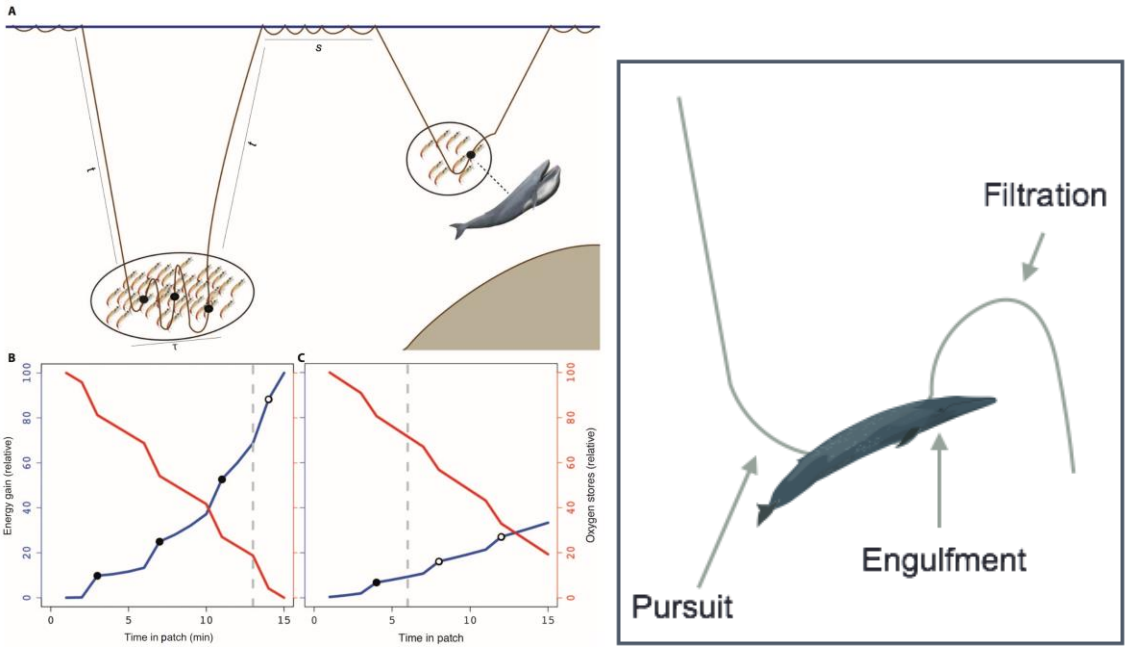
Methods

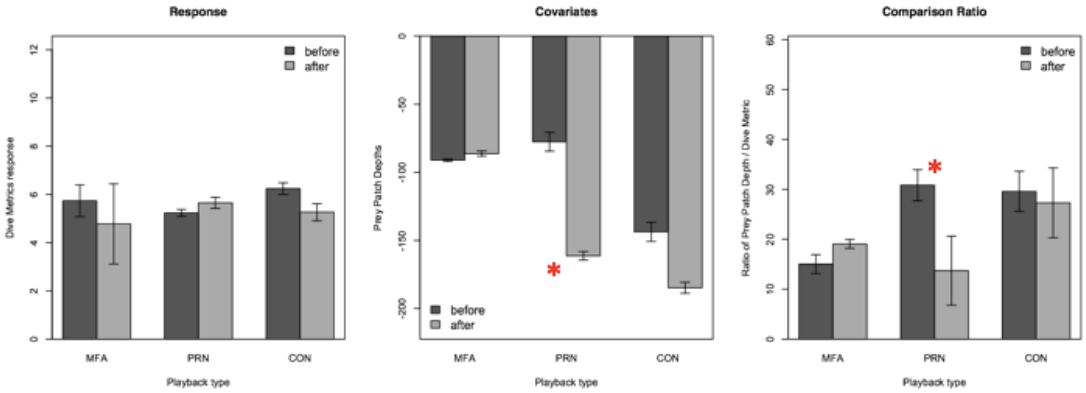
We used multi-sensor archival tags to define fine scale dive parameters and lunge kinematics of blue whales and fisheries acoustics to determine how krill dynamics affect foraging behavior in space and time.

Results

We deployed 38 tags on blue whales in 2011 through 2016 off the southern California coast as a part of the SOCAL-BRS, collecting over 80h of concurrent prey and predator data. We used several multivariate statistics (e.g. principle component, canonical correlation analyses, and generalized additive mixed models) to examine lunge kinematics relative to measurements of prey. We also calculated blue whale foraging efficiency as a function of prey density and depth. Finally, we also tested whether dive behavior changed as a function of SONAR exposure and prey patch metrics. Blue whale feeding rates and stereotyped feeding behaviors (e.g. 360° rolls) were directly related to prey patch metrics (e.g. depth, density, shape). Blue whales also changed foraging strategies, diving longer and lunging more frequently when prey was deeper and more dense. Data on prey and bottom depth dramatically increased variance explained in behavioral models from 0.17 to 0.72, revealing how whale responses to anthropogenic sound may be shaped by key environmental covariates. These results provide critical new insights into contextual covariates that must be considered in evaluating response probability for Navy sonar and other sounds.

Notes:





A.S. Friedlaender, E.L. Hazen, J.A. Goldbogen, A.K. Stimpert, J. Calambokidis, B.L. Southall, 2016. Prey-mediated behavioral responses of feeding blue whales in controlled sound exposure experiments. *Ecological Applications*, *doi: 10.1002/15-0783*

E.L. Hazen, A.S. Friedlaender, J.A. Goldbogen, 2015. Blue whales (Balaenoptera musculus) optimize foraging efficiency by balancing oxygen use and energy gain as a function of prey density. *Science Advances,* 1:9*, DOI: 10.1126/sciadv.1500469.*

J. Goldbogen, E.L. Hazen, A. Friedlaender, J. Calambokidis, S. DeRuiter, A. Stimpert, B. Southall, 2015. Prey density and distribution drive the three-dimensional foraging kinematics of the largest filter feeder. *Functional Ecology,* 29: 951–961. doi: 10.1111/1365-2435.12395

A.S. Friedlaender, J.G. Goldbogen, E.L. Hazen, J. Calambokidis, B.L. Southall. 2014. Feeding performance by sympatric blue and fin whales exploiting a common prey resource. *Marine Mammal Science,* 31:345-354. doi: 10.1111/mms.12134

J.A. Goldbogen, B.L. Southall, S.L. DeRuiter, J. Calambokidis, A.S. Friedlaender, E.L. Hazen, E.A. Falcone, G.S. Schorr, A. Douglass, D. Moretti, C. Kyburg, M.F. McKenna, P.L.Tyack. 2013. Blue whales respond to simulated mid-frequency military sonar. *Proceedings of the Royal Society B.* 280: 20130657

Cetacean Social Behavioral Response to Sonar: *biological relevance and severity of sonar responses*

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Background

Targeted research efforts investigating effects of naval sonar on marine mammals (Behavioral Response Studies (BRSs) have made apparent that changes in behavior resulting from sound exposure are likely to translate to a negative effect at the population-level. In response, BRS research efforts have become increasingly focused on these behavioral changes. Often, exposure context plays an essential role in the quality and severity of the response. One such important context is sociality. The behavior of individual social animals is influenced by, and influences, the behavior of their group members (Marshall et al. 2012). Social cetaceans coordinate vital activities, such as foraging and defense against predators (Visser et al. 2014, 2016). Hence, it is highly likely that individual behavioral responses of social cetaceans to sonar and other stimuli will be mediated by social context and social behavior. Long-finned pilot whales (*Globicephala melas*), for example, being strongly reliant on group members, may anticipate against a potential loss of social cohesion (by grouping at the surface) already at low levels of disturbance (Visser *et al.* 2016).

Objectives

This project forms the continuation of the effort to investigate the function and importance of social behavior in shaping the behavioral response to sonar in social cetaceans. We investigate: 1) the nature and magnitude of social behavioral responses to sonar and other potential disturbances of cetaceans with different social organizations; 2) the natural and disturbed social behavior in two separate populations of a social cetacean (*Risso's dolphin*). In addition, the project PI maintains a specific role as member of and liaison between BRS studies.

Methods

We analyzed data collected during controlled exposure experiments off Norway (3S Project) and California (SOCAL-BRS Project) between 2008 and 2016 and collected new data on Risso's dolphin behavior and behavioral response at the Azores (Azores-Baseline Project, 2011-2016). In these projects, social behavior data collection constituted an integrated part of multi-sensor data recording (Dtags, visual tracking, acoustic monitoring), using a visual observation protocol developed in the first part of this study. Controlled exposure experiments consisted of several phases, including baseline, tagging and experimental exposure (naval sonar, killer whale sound playback or control). Response type and magnitude were investigated by comparison of behaviors before, during and after exposure and by comparison of responses between exposure types and with behavioral changes observed during baseline behavior.

Results

We discovered that social cetaceans with different social organizations employ different social tactics in response to naval sonar and other potential disturbances, such as tagging and perceived presence of killer whales (*Orcinus orca*). A unifying characteristic of the response was a high or increased degree of social cohesion. Such social response was not observed in a species that does not form stable groups, but may aggregate and interact socially with conspecifics – the humpback whale (*Megaptera novaeangliae*). This indicates a dual importance of sociality in shaping behavioral response: 1) the necessity to maintain a high degree of cohesion may drive the nature and onset of response in species that form stable groups; 2) individuals that form long-term stable associations can employ group-defense strategies, but strategy choice is disturbance-specific (*e.g.* predator presence initiates group defense, but naval sonar exposure does not). Risso's dolphins at the Azores and off California were found to have comparable use of social calls, used to mediate coordination and cohesion. This work in progress provides preliminary evidence that social behaviors, and behavioral responses may be consistent across populations.

Notes:

References

Marshall HH, Carter AJ, Rowcliffe JM, Cowlishaw G (2012) Linking social foraging behaviour with individual time budgets and emergent group-level phenomena. Animal Behaviour 84: 1295-1305

Visser F, Miller PJO, Antunes RN, Oudejans MG, Mackenzie ML, Aoki K, Lam FPA, Kvadsheim PH, Huisman J, Tyack PL (2014) The social context of individual foraging behaviour in long-finned pilot whales (Globicephala melas). Behaviour 151:1453-1477

Visser F, Curé C, Kvadsheim PH, Lam FPA, Tyack PL, Miller PJO (2016) Disturbance-specific social responses in long-finned pilot whales, Globicephala melas. Scientific Reports 6:28641. doi: 10.1038/srep28641

Social ecology and group cohesion in pilot whales and their responses to playback of anthropogenic and natural sounds

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Background

This project investigates the social ecology and cohesion of long-finned pilot whales as part of a broad multi-investigator research program that seeks to understand how cetaceans are affected by mid-frequency sonar and other sources of anthropogenic noise. The study of how noise affects large delphinids such as pilot whales is important since these species have different social systems and different responses to sound sources such as sonar and killer whales (Curé et al., 2012) compared to beaked whales (DeRuiter et al., 2013; Tyack et al., 2011). However, the baseline behavior of pelagic delphinids is much less well understood than that of beaked whales, making design and interpretation of controlled exposure experiments difficult (Miller et al., 2012). For gregarious species that rely on social strategies to defend against predators or competitors, the size, composition and cohesion of their group as well as the dive activity of group members likely plays an important role in shaping the decision processes of individuals to determine how to respond to a potential threat.

Our goal here is to study the social dynamics and effects of anthropogenic noise on group-living delphinids. This project aims to gather data needed to design, conduct and interpret controlled exposure experiments to social delphinids such as pilot whales, with the ultimate goal of understanding responses to naval sonar and improving Navy environmental analyses.

Objectives

The objectives of this research project are:
1) To collect baseline data on pilot whale behavior within a social context
2) To improve our understanding of social coordination and group cohesion in delphinids, and how these processes might influence responses to anthropogenic sound
3) To tag individuals across years to understand stability of social bonds and stereotyped calls
4) To develop and test a stereo camera geocoding system to quantify surface group cohesion, speed and orientation of delphinids
5) To perform sound playbacks to a subset of tagged animals to evaluate behavioral response patterns to natural and anthropogenic sounds

Methods

This project is a continuation of a long-term tagging project, supporting a 1-month field expedition investigating the resident population of long-finned pilot whales in the Strait of Gibraltar, Spain. To understand how acoustic communication mediates delphinid social behavior, we simultaneously instrumented multiple pilot whales within the same social group with sound and movement recording DTAGs (Johnson and Tyack, 2003). These tags sample a pair of hydrophones, a depth sensor, and 3-axis accelerometers and magnetometers, thus collecting acoustic and movement data on multiple group members simultaneously.

Following successful tagging, we tracked animals visually and using radio beacons on tags while collecting focal-follow data. When conditions such as weather and vessel traffic allowed, we performed controlled playback experiments of natural and anthropogenic signals to tagged animals when the group was in a travelling mode at the surface. Finally, to augment the fine-scale tag data and collect fine-scale data on surface responses of untagged individuals, we developed a stereo camera system to measure the position and orientation of individuals, making it possible to quantify surface cohesion over time.

Results

The team tagged a total of 85 long-finned pilot

whales in the Strait of Gibraltar, including 9 groups of 3 to 5 simultaneously tagged animals, providing an unprecedented glimpse into within-group interactions. Long-finned pilot whales exhibit a relatively tight, long-term social structure that may modulate responses to disturbance. Our work in the Strait of Gibraltar has revealed a social foraging strategy where individuals within small, compact subgroups synchronize individual foraging dives, going on dives to the bottom and collecting some 5-10 prey items per dive. Other subgroups that are outside visual range of one another decrease this synchrony but still synchronize foraging bouts and thus activity state. Tightly coordinated subgroups spread out during the descent and bottom phases, separating by more than twice their biosonar search range, but at distances where clicks from conspecifics are still audible.

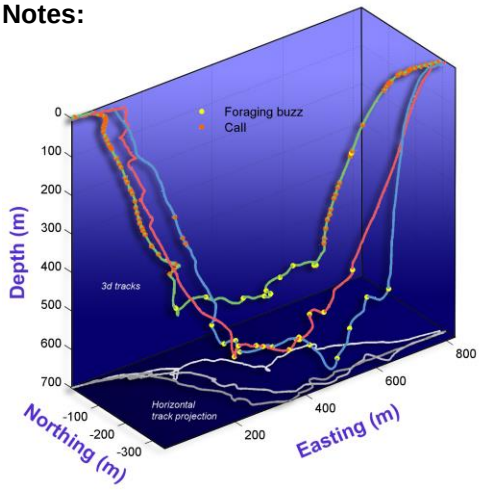
To explore the consequences of this foraging strategy, we built an agent-based model of biosonar-based foraging informed by empirically collected tag data. This model shows that distributed foraging decreases the amount of search zone overlap between close conspecifics, and consequently reduces the amount of competition between conspecifics, but with decreasing benefit for increasing social group size. Furthermore, decreasing synchrony of foraging dives leads to lower foraging rates for lagging group members, essentially reflecting an increasing uncertainty about where conspecifics have been feeding. These foraging driven changes in the spread and cohesion of social groups are likely to modulate the response to anthropogenic and natural signals and should be taken into account when designing controlled response studies.

Another goal has been to elucidate how these animals use acoustic signals to coordinate movement and behavior. Pilot whales use a complex, acoustic repertoire; we have found different categories of acoustic signals that function in different phases of the dive, including low-amplitude, click-based signals that seem to coordinate timing of descent and in joining together after a dive. At the surface, high-amplitude tonal calls are more common, with stereotyped calls especially important for mother-calf pairs. These calls are stable over years and may be important for group identification. These findings on baseline acoustic behavior will facilitate analyses of acoustic responses to anthropogenic disturbances or controlled playbacks as measured using tags or acoustic monitoring.

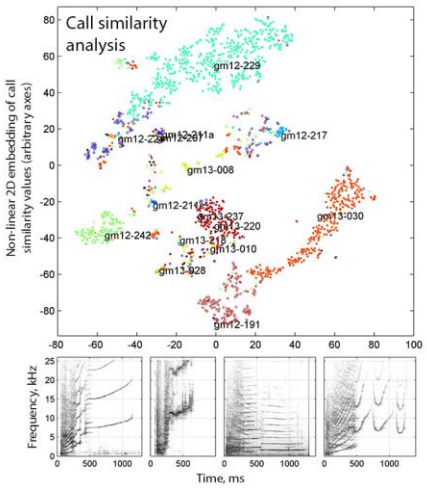
We have completed playbacks of pilot and killer whale sounds to a subset of seven tagged individuals. While these data are sparse, they do support previous findings from 3S project that pilot whales seem to exhibit a social defense strategy against killer whale predation.

Finally, we have developed a handheld stereo camera geocoding system for tracking animals at sea and for quantifying movement and cohesion with high temporal and spatial accuracy during behavioral response experiments. This system provides reasonable localization accuracy up to 100m away and without the battery restrictions of commercial drones.

Notes:



Left: *Long-finned pilot whales coordinate foraging dives closely in time and employ a variety of acoustic signals to achieve a high level of coordination.*



Right: *Call similarity analysis based on dynamic time warp distances (plotted using*

Behavioral and physiological response of baleen whales to ships and ship noise

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Background

Ship noise has been identified as the major source of anthropogenic noise in the oceans especially in areas of high vessel traffic. Ship strikes are also a growing concern especially for several species including blue and right whales that appear to be particularly susceptible. Initial research demonstrated the feasibility of documenting whale response to opportunistic close approaches of ships in areas of high levels of ship traffic particularly near known high concentrations of whales off California. This juxtaposition has resulted in high levels of ship strikes (Berman-Kowalewski et al. 2010) as well as potential impacts of ship noise on blue whales (Melcon et al. 2012) and other species. In this study we continue research on behavioral response of baleen whales to ship close approaches and specifically examine how this varies with ship speed; one strategy proposed to mitigate ship strikes. We also test the response of blue whales to controlled playback of ship noise to determine the cues blue whales respond to and also to allow comparison between the response to ship noise and other anthropogenic sounds like mid-frequency sonar. To gain insight into whether ship noise and frequent passages of ships might be causing a stress response, the study includes collaboration with SWFSC to compare stress hormone levels in blue whales feeding for extended periods in areas of high ship traffic with those feeding away from shipping lanes.

Objectives

Our objectives include:

- Determine behavioral response (avoidance and changes in dive behavior) of blue whales and other large mysticetes to exposure to close approaches by ships.
- Examine the stimulus that appears to trigger the response to ships and whether this is a response to ship noise or the presence of the ship.
- Examine how reaction varies with differences in ship speed and approach.

- Determine sound exposure of a whale directly in the path of a ship.
- Examine whether chronic exposure to ship noise causes a noticeable change in stress hormones (Kellar et al. 2006, 2009).

Methods

Our overall approach involved:

• Conduct additional deployments of multi-sensor GPS tags on whales to examine responses to ships moving at different speeds and additional species of whales. Focus would include work with full speed ships in the Santa Barbara Channel and slower ships near the entrances to Los Angeles/Long Beach Harbors.
• Conduct controlled exposure experiments (CEE) with playback of ship noise to blue whales with a J15-3 projector using identical methodologies to the SOCAL BRS (Southall et al. 2012, 2013) to allow direct comparison of blue whale response to ship noise to that from other anthropogenic sources like mid-frequency sonar (MFA). Response to playback may or may not be similar to the response to close approaches of real ships being conducted opportunistically. This will also allow comparison to the response of right whales to ship noise from distant ships and playback (Nowacek et al. 2004) using somewhat similar methodologies.
• Collect and examine stress hormone levels in biopsy samples from blue whales feeding in areas of high ship traffic such as off LA/Long Beach Harbor, especially over multiple days, compared to those from blue whales feeding farther from shipping lanes. Dr. Nick Kellar at SWFSC is testing of stress hormones for this project from the >100 samples collected.

Results

We deployed 66 tags on blue, fin, and humpback whales gathering over 2,500 hours of data on whales in the shipping lanes from 2014-2016. The successful development and use of medium duration archival tags that recorded up to three weeks of high resolution multi-sensor data dramatically improved our ability to look at interactions between whales and ships.

Controlled Exposure Experiments (CEEs) with playback of ship noise from the J15-3 were conducted and completed on five occasions involving with seven whales in 2016 (and one from 2015). Even though we achieved the hoped for source levels, higher than previous CEEs with ship noise on right whales (Nowacek et al. 2004), the ship noise playback was often difficult to detect on the tags, due to flow noise and the degree to which the whales were exposed to much higher levels of sound than what we generating from passing real ships or on some occasions our own research vessels.

Data on whale response to proximity or received level during ship close approaches was obtained using ship AIS positions and where tags included acoustic sensors, from the received sound level on the tags. One tag deployed on a whale off San Francisco in 2016 (see below) documented 13 close passes of ships in a 10-day period where the sound of the ship passage was well above the flow noise on the tag.

Notes:

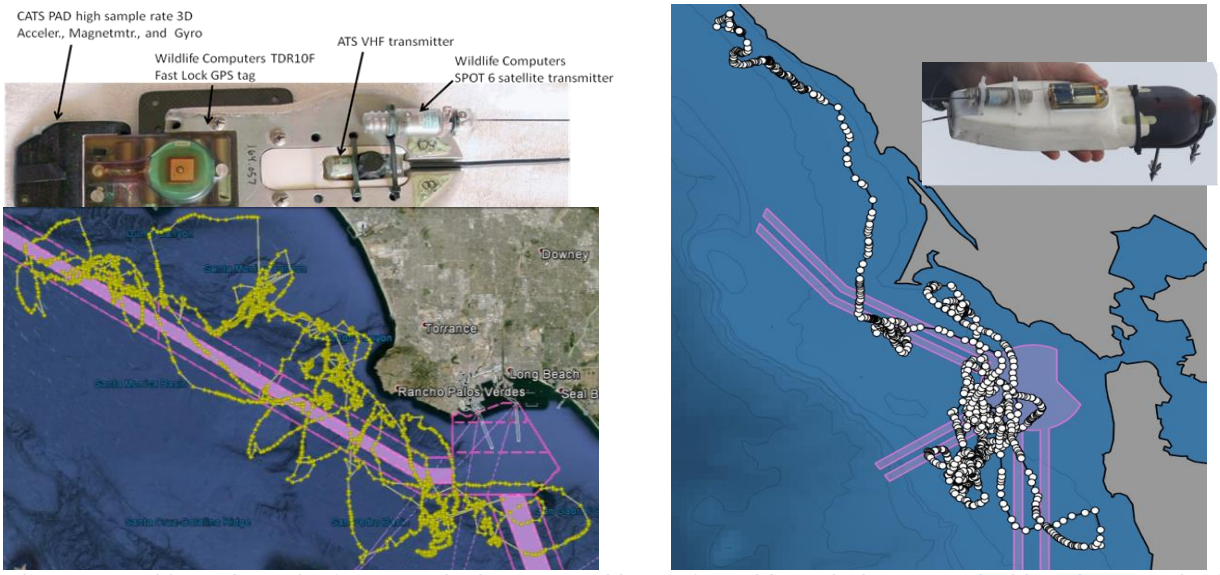

Figure 1. Multi-week tracks from Fastlock GPS positions of two blue whales tagged with a dart attached archival tags, left from a TDR-10F tag from in 2014 off LA//Long Beach and on right a dart-attached Acousonde with added floatation, GPS, and satellite transmitter off San Francisco in 2016.

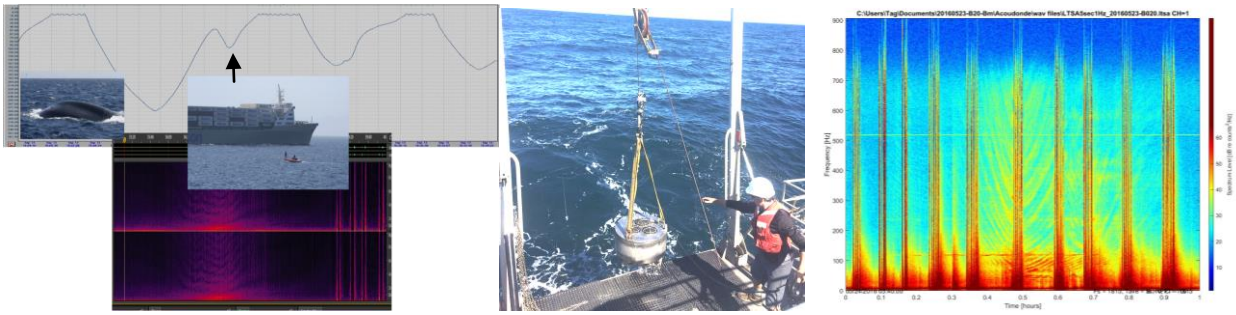

Figure 2. Left shows close encounter between a whale and ship off LA/Long Beach on 13 September 2014 showing response/avoidance to a near collision (Photos of tagged whale, passing ship, dive record with abort of surfacing at time of ship closest approach, and sound spectrum of ship noise at close approach). Middle shows J15-3 being deployed from Shearwater for ship noise playback. Right shows ship noise from tag which was 1 of 13 of such close ship approaches documented from a single 10-day deployment of a new dart-attached acoustic tag (Acousonde with GPS) near shipping lanes.

McKenna, M.F., J. Calambokidis, E.M. Oleson, D.W. Laist, and J.A. Goldbogen. 2015. Simultaneous tracking of blue whales and large ships demonstrates limited behavioral responses for avoiding collision. Endangered Species Research 27 (3):219-232 doi:10.3354/esr00666

Szesciorka AR, J Calambokidis, JT Harvey. 2016. Testing tag attachments to increase the attachment duration of archival tags on baleen whales. *Animal Biotel.* 4(1):1-12. doi: 10.1186/s40317-016-0110-y.

Variability of hormonal stress markers collected from a managed dolphin population

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Background

Quantifying physiological indicators of stress in wild marine mammals and the interrelationships between different stress markers can be used to estimate the impact of anthropogenic stressors on marine mammal populations. The United States Navy, as part of its environmental stewardship, can utilize stress markers to assess the acute and chronic impacts that its actions might have on marine mammals. This approach would permit better mitigation of potential impacts and ensure that Navy activities do not come at a deleterious cost to marine mammal populations.

Objectives

The objectives of this effort are to: 1) determine the variation in corticosteroid hormones, thyroid hormones, and catecholamines within a dolphin population relative to season, time of day, gender, age and reproductive state; 2) assess relationships between serum corticosteroid levels and levels found in other matrices (i.e. biological samples), specifically feces and blubber; 3) and to perform stress tests and hormone stimulations to characterize the activation of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-thyroid (HPT) axes across multiple matrices.

Methods

Regular sampling of blood and feces were collected from the U.S. Navy Marine Mammal Program (MMP) dolphin population over the course of two years (n=32). To avoid the effects of handling stress, blood and fecal samples were collected only through voluntary dolphin participation. Blood samples were collected biweekly in the first year and only in the morning. To address diurnal variability, samples were collected monthly during the second year in the morning, at midday, and in the evening. Fecal samples were collected the day following blood samples on the assumption that fecal hormone metabolites reflected serum hormone levels the day prior.

The response of hormonal axes to simulated and acute stressors was accomplished via a) the administration of adrenocorticotropic hormone (ACTH), b) a physical stress test (beaching and manual restraint), c) the chronic pharmacological elevation of serum cortisol, d) and the administration of thyroid stimulating hormone (TSH). Serum samples were collected in the days prior to the tests (baseline), repeatedly throughout the tests, and in the days following the test (recovery). Fecal samples were collected daily throughout the test periods. For the physical stress tests and pharmacological elevation of serum cortisol, blubber samples were collected during the course of the tests to characterize the incorporation of cortisol into blubber.

Serum hormones were processed by enzyme- or radio-immunoassay (EIA and RIA, respectively) or high-performance liquid chromatography (HPLC). Hormone metabolites were extracted from fecal samples and measured via RIA (Wasser *et al.*, 2010). A multi-step biphasic organic solvent extraction was used to isolate the corticosteroids from the blubber tissue and then processed via EIA (Kellar *et al.*, 2015).

Results

1) Season and time of day exerted the greatest influence on hormones, with sex and age contributing to hormone level variability.
2) Cortisol and aldosterone demonstrated strong diurnal variation but in opposite directions; cortisol decreased through the day and aldosterone increased. Cortisol increased with age in males but was relatively consistent across age in females. Cortisol and ACTH were related, as expected, but aldosterone varied independently of ACTH suggesting primary control of aldosterone by the renin-angiotensin-aldosterone system.
3) All thyroid hormones showed a significant seasonal effect. Patterns were variable across hormones and no two thyroid hormones followed the same pattern. Free triiodothyronine (fT3) and reverse triiodothyronine (rT3) were positively related to free and total thyroxine (fT4 and tT4, respectively). Patterns and relationships were indicative of a complex interaction of thyroid hormones in the regulation of metabolism.

4) Epinephrine was significantly higher in the fall than other seasons, had higher levels in the evening than other times of day, and tended to increase with age. Norepinephrine was lowest in the summer and had highest diurnal levels in the morning. Epinephrine and norepinephrine were positively related to one another across seasonal and diurnal temporal scales.
5) Fecal glucocorticoid metabolites showed no relationships to sex, age or season, and a marginal relationship to serum cortisol across seasons. Similar findings were made with fecal aldosterone metabolites and serum aldosterone. Fecal T3 metabolites were higher in spring than in winter and generally declined with age. No relationship to serum T3 was found. Lack of relationships were potentially due to the timing of sampling (see 9)).
6) The response to ACTH administration was no different than the physical stress test and similar to that previously reported. Results suggest that dolphins are either unresponsive to ACTH administration or that dosages used are insufficient to mount a strong HPA-axis response.
7) Cortisol and aldosterone increased by as much as 3-fold and 10-fold, respectively, in response to the physical stress test. Mean cortisol increased ~100% within 15-minutes of the stress onset. Cortisol and aldosterone were strongly correlated to one another and to ACTH during the stress response, suggesting primary control of both hormones through the HPA axis when the dolphin was stressed.
8) Blubber cortisol increased with the progression of the stress test and correlated with rises in serum cortisol. The rate of incorporation was variable across individuals, but demonstrated that blubber has potential for use in monitoring cortisol levels in wild marine mammals. Similar findings were found in the cortisol feeding study, in which serum cortisol pharmacologically elevated over a week significantly increased levels of blubber cortisol (Champagne *et al.*, 2016).
9) Fecal glucocorticoid metabolites correlated with elevations in serum cortisol during stress tests. The time lag between serum increases and fecal spikes was ~2-3 hours following the stressor, which is significantly shorter than the time lag of ~24 hours associated with terrestrial mammals.
10) Chronic pharmacological elevations in serum cortisol resulted in the downregulation of ACTH, presumably through established feedback mechanisms, and suppression of free thyroid hormone, possibly related to metabolic suppression. However, fecal glucocorticoid metabolites did not increase, as anticipated, but declined. Reductions in fecal metabolites potentially occurred due to a change in the primary cortisol elimination pathway from fecal excretion to urinary excretion.
11) Free T3 and T4, as well as total T4, increased in response to TSH administration. Peaks in the response occurred ~24 hours post-administration. Free T3 and T4 showed a strong direct relationship during the TSH stimulation; however, T3/T4 declined with increasing T4. The finding is contrary to an expectation of T3/T4 constancy.

Notes:

Champagne, C., Kellar, N. M., Crocker, D., Wasser, S. K., Booth, R. K., Trego, M. L., and Houser, D. S. (2016). "Blubber cortisol qualitatively reflects circulating cortisol concentrations in bottlenose dolphins," Mar. Mammal Sci. 33, 134-153.

Kellar, N. M., Catelani, K. N., Robbins, M. N., Trego, M. L., Allen, C. D., Danil, K., and Chivers, S. J. (2015). "Blubber Cortisol: A Potential Tool for Assessing Stress Response in Free-Ranging Dolphins without Effects due to Sampling," PLoS ONE 10, e0115257.

Wasser, S. K., Azkarate, J. C., Booth, R. K., Hayward, L., Hunt, K., Ayres, K., Vynne, C., Gobush, K., Canales-Espinosa, D., and Rodríguez-Luna, E. (2010). "Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species," General and Comparative Endocrinology 168, 1-7.

Stress Hormones and their Regulation in a Captive Dolphin Population

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Background

The U.S. Navy requires an understanding of how putative markers of stress (e.g. glucocorticoid hormones) vary with time, life-history state (e.g. age, sex, reproductive status), and in response to disturbance. This study capitalized on an existing research project (*Variability of hormonal stress markers collected from a managed dolphin population*, N000141110436, hereafter referred to as the *Parent Project*) using the dolphin population managed by the U.S. Navy's Marine Mammal Program (MMP). The Parent Project characterized natural variation in corticosteroid, thyroid, and catecholamine hormones and assessed the sensitivity of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid (HPA and HPT, respectively) axes to stimulation. From this work, several additional opportunities became available to leverage from the existing study design.

Objectives

We had four objectives complementing those of the Parent Project: (1) Improve the quantification of low-levels of corticosteroids (cortisol and aldosterone) commonly found in dolphins residing in managed care facilities. (2) Evaluate corticosteroid-binding globulin (CBG) and its influence on free cortisol availability. (3) Measure reverse T_3 (rT_3) in response to induced stress. And (4) assess the metabolic consequences of stress on metabolism in two experimental manipulations: (i) stimulation of the HPA axis during an out-of-water stress event, and (ii) stimulation of the HPT axis by administering thyroid-stimulating hormone (TSH).

Methods

Task 1: We tested several extraction protocols to extract and concentrate corticosteroids from serum and commercially available enzyme- and radio-immunoassay (EIA and RIA, respectively) kits for ease and cost-effectiveness to detect low-levels of corticosteroids in dolphin serum. Task 2: We collaborated with Dr. Rudy Boonstra on a related effort (N000141512214) to measure CBG in dolphin serum using cell-harvester and dialysis techniques. Task 3: We validated a commercially available kit to measure rT3 in dolphins (Alpco, Inc. cat#3-RT3HU-R125). Task 4: In samples collected in before, during, and in recovery from HPA and HPT axes stimulation, we evaluated the metabolic response of stress treatment using a global metabolomics approach.

Results

(1) Several assay kits were suitable for modification to determine low corticosteroid concentrations in managed-care dolphin populations. RIAs are often robust to small changes in sample volume, thus increasing the serum volume used in RIA can frequently increase the sensitivity of the assay. If matrix effects are detected, or for using EIAs, corticosteroids can first be extracted into an organic solvent (e.g. 3:2 mixture of ethyl acetate:hexane) followed by drying and reconstitution in assay buffer before performing the assay.

(2) The binding capacity of CBG did show small, but statistically significant changes at the end of the out-of-water stress tests (Figure 1). For further detail on CBG variability, see the collaborative project N000141512214.

(3) Marine mammals commonly have high concentrations of rT3 and this was found in the MMP population as well (470 samples collected from 21 dolphins, mean 6.8 SD 1.9 nM). rT3 concentrations showed small but statistically significant variation in response to the out-of-water stressor, during both HPA axis stimulation (Figure 2, LMM, $p < 0.05$) and HPT axis stimulation (LMM, $p < 0.05$, data not shown).

(4) The out-of-water stress tests had broad but relatively short-term effects on metabolism. Metabolomics analysis identified 454 biochemical compounds in circulation, grouped into eight broad pathway categories. Of these, 251 compounds showed some significant variation among sample time points (LMM, $p < 0.05$) but most compounds returned to baseline levels within one to two days (Figure 3). The largest effect of the stress treatment was on lipid metabolism. Numerous free fatty-acids (FFA) increased during the treatment, including polyunsaturated medium and long-chain FFA. These increases suggest higher rates of lipid mobilization from adipose stores and use during stress. We failed to detect strong influences on amino acid metabolism during stress treatment; specifically, principal gluconeogenic amino acids (alanine and glutamine) were not altered during or following the treatment as might be expected in other mammals experiencing stress.

Notes—supporting figures

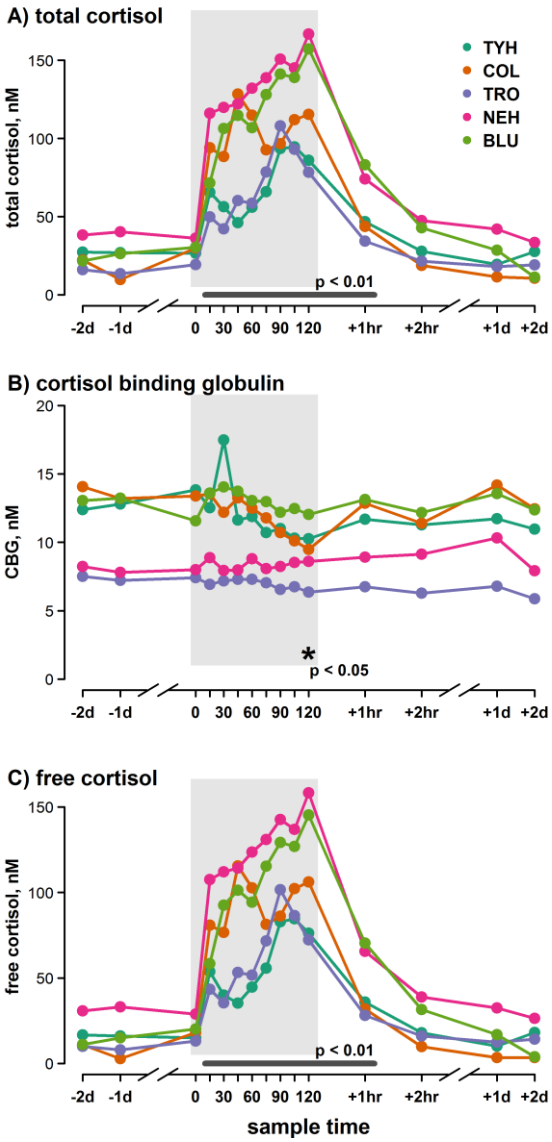

Figure 1. Cortisol response in each of five dolphins (dolphin ID at top right) to HPA axis stimulation by an out-of-water stress event. Samples were voluntarily collected 2 and 1 day prior to the stress test; then, during the out-of-water event (shown in gray shading) serial samples were collected at 15-30 min intervals; dolphins were returned to the water and recovery samples were collected +1 and +2 h later; and post-stress samples were collected +1 and +2 days later.

A) total serum cortisol levels increased rapidly and throughout the out-of-water period. B) Cortisol binding globin (CBG) levels, however, showed only minor changes (only the 120 minute sample differed from baseline conditions). Thus, C) free cortisol showed similar patterns as seen in total cortisol.

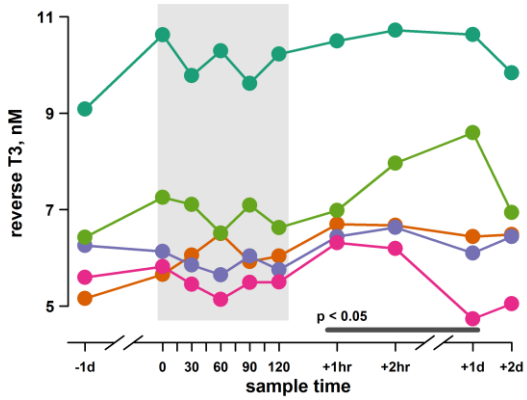

Figure 2. Reverse triiodothyronine (rT_3) showed a delayed response to the out-of-water stress event relative to cortisol. rT_3 concentration differed from the initial sample not during the stress test but in the recovery period: +1 and +2 h after returning to the water, and was still somewhat elevated the following day. By +2 days following the stress event, rT_3 concentration no longer differed from baseline.

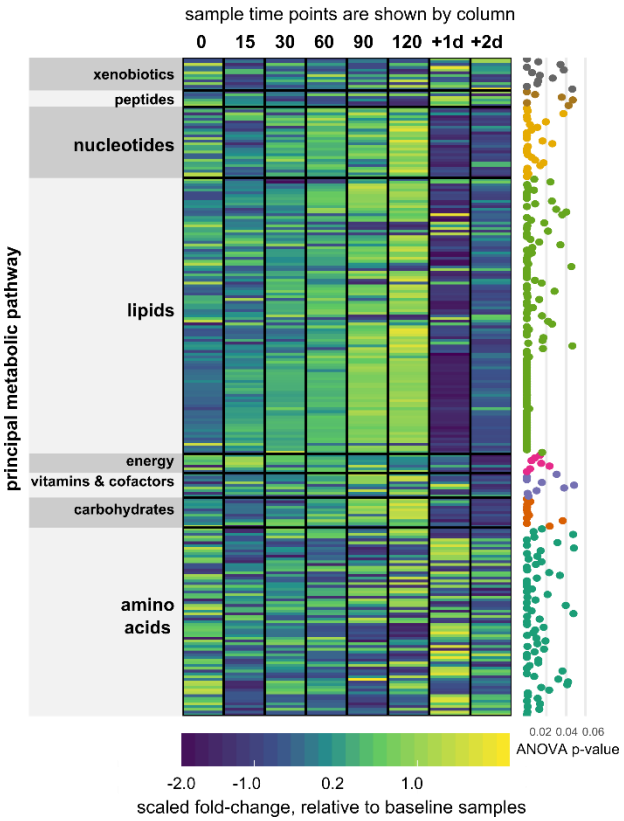

Figure 3. Relative changes in circulating biochemicals detected in association with an out-of-water stress event. Lipid metabolism was the principal metabolic pathway activated by stress.

Validating the novel method of measuring cortisol levels in cetacean skin by use of an ACTH challenge in bottlenose dolphins / Measuring and validating levels of steroid hormones in the skin of bottlenose dolphins (*Tursiops truncatus*)

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Background

Marine mammals face a wide range of anthropogenic stressors including pollution, underwater noise, and climate change (Durner et al. 2009; Wright 2009). The consequences of these disparate stressors are often difficult to assess. Levels of the hormone cortisol in the blood are typically used to assess stress levels in mammals. However, it is not always possible to obtain blood from free-ranging marine mammals and even when this can be done; the potential for sampling protocols to induce handling artifacts may complicate measurements (Cook et al. 2000; Eskesen et al. 2009). Thus, to better assess the impacts of a wide range of stressors on marine mammals, several alternative matrices are currently being explored for use in the detection of cortisol. Our recent Office of Naval Research project (award N000141210896) facilitated the development of a method to extract and quantify cortisol from cetacean skin samples (epidermis of harbor porpoises, *Phocoena phocoena*), which can itself be collected in various minimally-invasive ways (Bechshoft et al. 2015). Based on this, a second ONR project (award N000141310771) was initiated with the purpose of validating the cortisol values from skin samples obtained with the use of a stress test in a group of bottlenose dolphins (*Tursiops truncatus*). For this validation study, a number of dolphins were sampled as a supplemental part of an ongoing study of stress hormones conducted by Dr. Dorian Houser in collaboration with the U.S. Navy Marine Mammal Program (award N000141110436). Results from other ONR-funded projects that were presented at the most recent ONR Program Review meeting (May, 2014) made it clear that hormones other than cortisol are also important for assessing stress responses in cetaceans, including progestogens, oestrogens, androgens, and corticosteroids such as aldosterone. Aldosterone was discussed as being of particular importance at the ONR project review meeting, as it appears to be a more reliable indicator of an acute stress response in cetaceans than cortisol is. Due to this discussion another project (award N000141512187) was added onto the existing validation project, with the aim of expanding on the number of steroid hormones extracted from the bottlenose dolphin skin samples already collected by Dr. Houser. In addition to cortisol, the projects thus attempt to quantify aldosterone, corticosterone, pregnenolone, progesterone, DHEA, androstenedione, testosterone, dihydrotestosterone, estrone, 17α-estradiol and 17β-estradiol in the skin samples. The occurrence of most of these hormones in cetacean skin is unknown, but the method of extracting multiple hormones has previously been applied in other keratinous martices by our lab (Weisser et al. 2016).

Objectives

The aim at the conclusion of the project period was to have provided a greater understanding of the potential of cetacean skin as a matrix for steroid hormone assessment. When our two ongoing projects are concluded, the main objective is to have provided a validated method for assessing a range of skin steroid hormones related to stress responses in cetaceans. We also expect to have provided a greater understanding of:

- the relationship between cortisol and aldosterone concentrations in plasma and skin;
- how long it takes for an acute stress response to be measurable in the skin matrix ; and
- baseline steroid concentrations in skin, as well as an overview of inter- and intra-individual fluctuations.

Methods

Three types of skin samples were collected from five bottlenose dolphins (1 female, 4 male) in connection with the aforementioned stress test:

- prior the test (to establish baseline hormone concentrations during the weeks leading up to the test)
- at the time of the test (to establish T0)

- after the test (up to 18 weeks of sampling in order to ensure the documentation of any peaks in skin steroid hormone concentrations induced by the stress test [Hicks et al. 1985; St. Aubin et al. 1990]).

All skin samples were analyzed at the University of Copenhagen under Dr. Styrishave. Dr. Styrishave's lab has further refined the skin hormone extraction procedure since the publication of our first method paper (Bechshoft et al. 2015) and the required clean-up procedure is thus now only one step instead of the previous two. The protocol uses an integrated clean-up Pressurized Liquid Extraction (ic-PLE) technique. In this, the initial PLE extract was subjected to a clean-up procedure using amino-propyl solid phase extraction (SPE) cartridges. The resulting aliquot was then analyzed for the abovementioned steroid hormones using either gas chromatography-tandem mass spectrometry (GC-MS/MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS), depending on which hormone were being quantified.

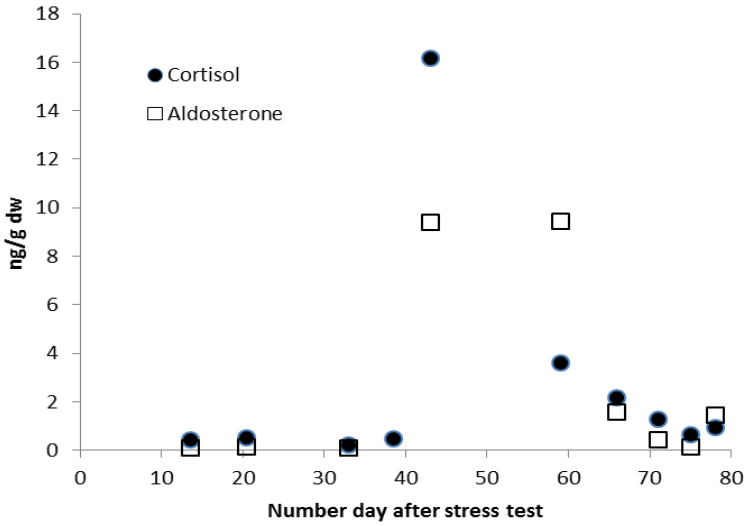
Results

The hormone analysis is completed. In some cases the size of a sample was too small to allow for analysis and so several had to be pooled before moving forward with the lab procedure. Using this approach, we successfully extracted and quantified the hormones cortisol, aldosterone, corticosterone, progesterone, testosterone, and DHEA from the skin samples. Data analysis is currently in progress, and results will be presented in full at the Program Review meeting this March.

Preliminary examination of the data shows significant variations between the five animals included in the study with regards to baseline hormone concentrations, but also with regards to the degree to which the impact of the acute stressor was reflected in the skin hormone concentrations. Substantial variation between individuals has also been observed when analyzing stress hormones in other mammal species (Bechshoft et al. 2015; Mislan et al. 2016). Furthermore, while pooling samples decreased the temporal resolution overall, there appears to be a relative spike in skin cortisol and aldosterone around 6-8 weeks after the stress test (Fig. 1 shows one such example).

Notes:

Fig 1



References:

Bechshoft T, Wright AJ et al 2015. Conservation Physiology, 3(1): cov016 / Cook CJ, Mellor DJ et al. 2000. CABI Publishing, p. 123-146 / Durner, GM, Douglas DC et al. 2009. Ecological Monographs, 79: 25-58 / Eskesen IG, Teilmann J et al. 2009. Journal of the Marine Biological Association of the UK, 89: 885-892 / Hicks BD, St. Aubin DJ et al. 1985. The Journal of Investigative Dermatology, 85: 60-63 / Mislan P, Derocher AE et al. 2016. Ecological Indicators, 71: 47-54 / St. Aubin DJ, Smith TG & Geraci JR. 1990. Canadian Journal of Zoology, 68: 359-367 / Weisser JJ, Hansen M et al. 2016. Journal of Chromatography B, 1017-1018:45-51 / Wright AJ (ed). 2009. Okeanos - Foundation for the Sea, Darmstadt. 67+iv p. Available from http://www.sound-in-the-sea.org/download/CIA2009_en.pdf

Investigation of the Molecular Response in Blood and Skin of Belugas in Response to "Stressors" to Aid in Assessing the Impact of Environmental and Anthropogenic Challenges on Health

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Background

There is increasing concern regarding anthropogenic and environmental impacts such as loud sound, pollutants and changing temperatures on the health and viability of marine mammals in our oceans. In order to determine impacts and cumulative effects a better understanding of stress physiology and the impact of stressors on the immune system of marine mammals is needed. Moreover, a minimal amount of sample and obtaining the samples in a non-invasive manner, particularly for free-ranging cetaceans is desired. Gene-based biomarkers have the potential to reveal information on the physiological response to stressors while utilizing a minimal amount of tissue sample. While blood is the gold standard, skin may be a potential tissue matrix that can be obtained from free-ranging whales via biopsy and may provide health information. This study screened for potential stress and immune markers (cytokines, acute-phase and stress-response proteins) in archived blood samples obtained from aquarium belugas that were subject to stressor events, and paired blood and skin samples obtained from capture-released and subsistence-hunted wild belugas. The use of skin as an alternate matrix to gain information about health and immune status in cetaceans was investigated.

Objectives

The overall goal of this study is to characterize the beluga stress and immune response at the molecular level utilizing blood and skin samples collected in association with different stressors. The specific objectives are:

1. To assess the quality and quantity of total RNA from blood and skin samples collected in the field from wild belugas, and compare with samples collected and archived from Aquarium belugas.
2. To utilize archived tissues collected outside the proposed study to validate previously published primers for use in blood and skin from belugas.
3. To apply molecular methods to describe and quantify changes in the expression of immunological "stress" markers in peripheral blood from Aquarium and wild belugas.
4. To apply molecular methods to describe and quantify the expression of immunological markers in skin.
5. To correlate the biomarkers in blood from the proposed study to the findings of the previous ONR funded project (N00014-11-1-0437).

Methods

Blood samples were collected from both live and subsistence hunted belugas via venipuncture of the fluke vessels in PAXgene® RNA tubes. Stressor samples included blood taken before, during and after out-of-water events and before and after transport in Aquarium whales as well as from wild belugas obtained after chase and capture and/or subsistence hunts. Pieces of skin (0.5-1.0 cm) were collected from subsistence hunted whales and live-capture released belugas in association with satellite tagging or skin biopsies and preserved in RNAlater™ solution. Total RNA was extracted from individual samples, and quantification of selected gene products was carried out utilizing real-time polymerase chain reaction (RT-PCR) with two-step SYBR®Green technology. Relative quantification of each target gene normalized to reference genes, and subsequent data analysis are carried out by GenEx 6.0.1 software. Non-parametric t-tests (Wilcoxon and Mann-Whitney) and analysis of variance (ANOVA) are used to evaluate the significant changes in the expression of genes in blood and skin. Hierarchical clustering was performed, and distance matrices and correlations were used to explore the relationship between the expression of genes in paired skin and blood samples from wild belugas.

Results

Objective 1. RNA extractions were successfully optimized to obtain sufficient quantities of high-quality RNA (A260/280 >2.0) from both blood and skin samples. The quality and quantity of total RNA from blood samples collected from wild belugas in the field did not significantly differ from those obtained from aquarium belugas.

Objective 2. Out of a total of 33 primer sets tested, 17 primer sets were validated for blood, and 14 sets were validated for skin, including two housekeeping genes. Their amplification efficiencies were also calculated to verify successful validation. Out of these, 12 primer sets were further utilized in subsequent analysis (including nine to ten target genes and two housekeeping genes).

Objective 3. The out-of-water examination (OWE) events produced variable responses in aquarium whales suggesting individual coping mechanisms. While all three whales showed a significant ($p<0.05$) up-regulation of pro-inflammatory interleukin-2 (IL2) message one hour post-OWE, whale-1 and whale-3 displayed similar profiles with most significant ($p<0.05$) changes occuring for up to 48 hours post-OWE. Whale-2 displayed a more prolonged response for these genes lasting as long as 96 hours post-OWE. The belugas that were transported showed significant ($p<0.05$) down-regulation for inflammatory cyokines interferon-gamma (IFNγ), interleukin-10 (IL10), as well as the heat-shock protein-70 (HSP70) genes between pre- and post-transport, suggesting immune suppression. Wild belugas in general showed significantly higher ($p<0.005$) levels of expression for inflammatory cytokines IFNγ, transforming growth factor-beta (TGFβ), and the stress/immune response markers aryl hydrocarbon receptor (AHR), HSP70 when compared to aquarium whales, suggesting their differentiated health status and continuous exposure to stressors in a natural environment. Among the two wild populations, subsistence-hunted whales showed a significant ($p<0.01$) down-regulation of IL10 when compared to live captured-released whales. For the discovery of co-regulated or functionally related genes, the hierarchical clustering of gene expression profiles indicated the highest correlation between TLR4 and IFNγ ($r=0.75$, $p=0$), followed by IL12 and IL1β ($r=0.62$, $p=2.4x10-7$).

Objective 4. Skin samples showed significantly ($p<0.05$) higher expression of cyclooxygenase-2 (COX2) and HSP70 when compared to paired blood samples for both populations. Besides, expression of AHR, glucocorticoid receptor (Nr3c1) and TGFβ genes were at comparable levels in paired blood and skin samples. Expression of IL10, IL12 and IFNγ were significantly lower in skin samples as compared to blood ($p<0.001$), indicating lower copy numbers. Pearson correlation analysis performed between paired blood and skin samples showed significant correlation for pre-blood and skin for IFNγ, COX2 and Nr3c1 genes ($p<0.02$). Correlations were significant for post-blood and skin for AHR ($p= 0.0179$) and Nr3c1 genes ($p<0.02$). For the subsisted hunted whales, Pearson correlation analysis performed between paired blood and skin samples showed significant correlation only for the IL12 gene ($p= 0.003$).

Objective 5. IFNγ blood levels from pre- and post- examination samples of live capture released whales are inversely correlated with serum cortisol levels ($r=-0.51$, $p=0.026$); and positively correlated with plasma ACTH levels ($r=0.57$, $p=0.010$) and absolute lymphocyte counts ($r=0.64$, $p=0.003$). HSP70 levels in transported whales are positively correlated with absolute lymphocyte counts ($r=0.63$, $p=0.036$) and white blood cell counts ($r=0.79$, $p=0.004$). The results obtained from the tested biomarkers are further being investigated for correlations with additional paired blood hematology and catecholamine measurements.

Overall, the gene expression profiles of the markers utilized in this study demonstrate promise as indicators of immune and health status in belugas and can be a useful tool in assessing impact of environmental and anthropogenic stressors.

Notes:

Development and Validation of Techniques for the Detection of Pregnancy and Stress in Large Whales

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Background and Objectives

Hormonal biomarkers are useful indicators of the reproductive and metabolic state in mammals. The purpose of this study was to validate analytical methods for endocrine biomarkers to define reproductive and stress physiology in humpback and blue whales. The developed techniques allow for analysis of newly collected samples, as well as archived samples, and facilitate the merging of historical sample data with that of the new samples. Combined with behavioral and resighting data, endocrine biomarkers provide a powerful tool in assessment of physiology and life history states.

Methods

Humpback whales: enzyme immunoassays (EIA) for both progesterone and testosterone in blubber were validated for use in humpback whales. These hormones were measured in samples from free-ranging animals (2002-2016; n = 252; 21 male, 231 female (17 juvenile)) and ship struck (2007-2012; n=6; 2 male, 4 female). In addition, high pressure liquid chromatography (HPLC) was used to analyze progesterone in both pregnant and non-pregnant females. Both EIAs were used to analyze concentration differences between different depths sampled from large blubber chunks taken from skin to muscle and progesterone concentrations between males and juvenile, lactating, and pregnant females.

Blue whales: EIAs for both progesterone and cortisol in blubber were validated for blue whales from the Gulf of California and the US West Coast. Blubber samples from 110 blue whales (n = 56 females, 54 males) with known sighting histories were collected between 2002 and 2013 and analyzed for these two hormones. Additionally, HPLC of a pooled female was used to determine elution characteristics relative to a ^{3}H-progesterone standard. The progesterone concentrations from blubber biopsies were used to determine pregnancy in blue whale and combined with sighting data (i.e., the presence of a calf in the subsequent year) to confirm pregnancy. An ongoing experiment is testing the feasibility of samples stored in dimethyl sulfoxide for hormone analysis.

Results

Humpback whales: both progesterone and testosterone assays proved to be valid for use in humpback whale blubber. For the progesterone assay, serial dilutions (1:32 to 1:512) of the female pool exhibited displacement parallel to that of the standard curve and proved accurate ($y=3.97 + 0.97$, $r^2 = 0.99$). The male pool also exhibited parallel displacement in the progesterone assay (neat to 1:32). Accuracy of the progesterone in males was $y= -9.63 +0.85$, $r^2 = 0.99$). For the testosterone EIA, the female pool exhibited extremely low mass, only binding at neat and 1:2 while serial dilutions of the male pool demonstrated displacement parallel to that of the standard curve across all dilutions. Determination of assay accuracy through regression analysis resulted in $y=1.36 + 0.97x$, $r^2=0.99$ and $y=3.4 +0.90x$, $r^2 = 0.66$ for female and male pools, respectively.

HPLC for a pregnant female exhibited an immunoreactive peak associated with radioactive ^{3}H-progesterone tracer (fractions 35-38) that represented 77.2% of the total mass recovered from the column. In contrast, HPLC of non-pregnant pool exhibited an immunoreactive peak that did non co-elute with the ^{3}H-progesterone. Although this peak represented 100% of the total mass recovered from the column, it eluted prior to the progesterone tracer at fraction number 32. In addition, total mass for the pregnant animal recovered from the column differed by two orders of magnitude from that of the non-pregnant animal.

Analysis of the female blubber depth study samples indicated that progesterone concentrations at 1cm below the skin were significantly higher than at any other sampling depth (P=0.001 for skin, P=0.004 for 4cm,

P=0.001 for 7cm, P=0.002 for 10cm, P=0.005 for 13cm, P=0.001 for muscle). Testosterone analysis for the male depth study samples indicated that testosterone concentrations for the skin samples were significantly higher than the other sampling depths (P=0.004 for 1cm, P=0.011 for 4cm, P=0.007 for 7cm, P=0.004 for muscle). A comparison of gender differences in progesterone concentrations indicated that average adult male progesterone concentrations (0.54ng/g $^{+}$- 0.09) were significantly lower than any group of female humpback whales tested: juvenile (0.88ng/g ± 0.12), lactating (11.80 ± 3.49), and pregnant (74.01 ± 24.45).

Blue whales: The EIA for both progesterone and cortisol proved valid for use in blue whale blubber. The use of extracted blubber from female blue whales in the progesterone assay resulted in parallel displacement of that standard curve. Likewise with the cortisol assay, parallel displacement of the standard curve occurred with the blubber extracted from both male and female blubber pools. The accuracy of the progesterone assay for the female pool was y=-2.57 + 1.00x, r^2 = 0.98. For cortisol, accuracy for the female pool was y=-7.36 + 1.02x, r^2=0.99 and y=13.21 + 0.94x, r^2=0.99, respectively.

The HPLC profile for a female blue whale blubber pool had two immunoreactive peaks; the large peak occurred in fractions 32-34 and represented 67,4% of the pooled sample mass. The smaller immunoreactive peak occurred in fractions 36-37 and represented 4.9% of the pooled sample mass. ^{3}H progesterone used as a standard eluted at fractions 34-37.

Blubber concentrations of progesterone were low and not significantly different in female blue whale calves and adults with a calf from the Gulf of California during the calving/breeding season. The progesterone concentrations in the adult female with a calf (2.4 ±1.42ng/g) were used as proof of a non-pregnant whale. Progesterone concentrations of the adult females with no calves were split into those with low and high progesterone, using the upper range of progesterone concentrations (5.83ng/g) of lactating whales as a cut-off. There was an order of magnitude difference between adult females with low progesterone concentrations (2.00 ± 1.60 ng/g) and those with high progesterone (25.73 ± 12.18 ng/g) and these were considered non-pregnant and pregnant, respectively. The pregnant females had significantly higher progesterone concentrations than all other female groups (P<0.001). Further, when the proportions of adult females with high progesterone were compared to the total number of adult females in the study, it equates to a 29.6% pregnancy rate. Further measurements of progesterone concentrations in blue whales from the US West Coast confirmed high progesterone as an indicator of pregnancy. (Cortisol concentrations differed significantly between male and female blue whales (p=0.049). Cortisol concentrations were extremely variable in the calves and there were no significant differences in the cortisol concentrations in any group of female blue whales.

This work has been summarized in two manuscripts, by species, which are currently under review.

Notes:

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Assessing beaked whale reproduction and stress response relative to sonar activity at the Atlantic Undersea Test and Evaluation Center (AUTEC)

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Background

There is increasing evidence that cetacean behavior is altered in response to a variety of acoustic perturbations, including the use of sonar at the U.S. Navy's Atlantic Undersea Test and Evaluation Center (AUTEC). However, little is known about sonar's potential impacts on 1) the physiology of individual cetaceans and 2) population-level vital rates.

Physiological responses are often associated with behavioral reactions to potential stressors, including sound. The most widely studied is the activation of the hypothalamic-pituitary-adrenal axis (HPA), in which glucocorticoid, especially cortisol production, is a hallmark. Prolonged activation of this pathway leads to numerous pathologies including those injurious to reproduction. Although glucocorticoids have been previously measured in cetaceans as a diagnostic of stress response, this has predominantly been done from blood samples. However, in these cases, the act of sampling (i.e. collection of blood) itself often elicits a response that elevates blood glucocorticoid concentrations such that it is difficult to discern whether the stressor of interest or the sampling event is responsible for the rise. It has been long thought that the dynamics of hormone accumulation in blubber tissue (which can be obtained rapidly from wild cetaceans via dart biopsy) are much less rapid than in the blood. Therefore, steroid hormone signals within blubber represent longer windows of physiological time and are not heavily influenced by the events that take place in the minutes immediately prior to sampling. To investigate this process, a number of projects were initiated to develop protocols to measure blubber cortisol (BC), validate that BC levels respond to known stressors, and then begin applications in areas with sonar use to evaluate the relationship with BC levels and sonar activity.

Objectives

For this set of studies, five major goals were delineated. Three were focused on method development and two on application to wild populations. The goals were:

1) develop a method for measuring cortisol from skin/blubber biopsies
2) validate and compare BC dynamics to those in the blood
3) validate BC with respect to a known stressor: restraint time of the animal
4) measure BC in biopsies of free-ranging target species with a focus on Blainville's beaked whales (*Mesoplodon densirostris*) at AUTEC where sonar is regularly used and compare to those from the reference area of minimal sonar use, Abaco Island
5) assess population-level pregnancy (via blubber progesterone) and reproductive success rates in Blainville's beaked whales at AUTEC and Abaco Island

Methods

For the development and validation objectives, goals 1-3, blubber samples were taken primarily from common and bottlenose dolphins (wild and managed care animals). Samples were collected as follows:

Objective 1: blubber samples were taken from a) dead-stranded and b) fishery-bycaught common and bottlenose dolphins.
Objective 2: hydrocortisone was orally administered in managed-care bottlenose dolphins for 5 days; blood and blubber samples were taken at days 1, 3, and 5.
Objective 3: during capture-release events of wild bottlenose dolphins, serial blubber biopsies were collected during 1-3 hour restraint periods.

For the application objectives 4 and 5, dart biopsy blubber samples were collected from sperm whales and Blainville's and Gervais' beaked whales around AUTEC and Abaco

Island. Photo-identification data were collected from sampled whales to document successful calving events, to compare with hormone-derived pregnancy rates.

Cortisol and progesterone were isolated using serial organic solvent extractions from blubber tissue samples and then measured via enzyme immunoassays. Lipid content was measured gravimetrically.

Results

In general, BC shows suitable QA/QC characteristics in the laboratory and strong diagnostic characteristics as an indicator of previous stress response activity over a period of many tens of minutes to many tens of hours. Relatively brief stressors before sampling, even traumatic ones such as lethal net-entanglements, are not represented in the blubber, indicating that sampling activities themselves do not substantially impact its cortisol levels. Blubber cortisol levels tightly track blood cortisol levels given sufficient magnitude of elevation over a period of 3-5 days (Fig. 1). We found that when wild animals were restrained, initial estimated doubling times of cortisol concentrations in blood (3-10 min) were much faster than in the blubber (40-50 min) (Fig. 2). At max levels, we estimate that the increasing levels in the blubber lag behind those in the blood by 4-24 hours, suggesting the maximum temporal window of the blubber cortisol signal is between 8 and 48 hours.

Knowing this window of signal integration is important when interpreting the blubber cortisol values obtained from Blainville's beaked whales of AUTEC and Abaco Island. We found no significant differences in blubber cortisol levels between these areas of differing levels of sonar use (Fig. 3, note: east and south Abaco were broken down into 2 strata, both with low sonar use). Unfortunately, because we did not yet understand the relatively rapid blubber dynamics, and safety constraints on field activities during exercises, the majority of our sampling at AUTEC was conducted immediately *before* multi-ship sonar exercises. This effectively minimized our likelihood of capturing any potential blubber signal due to sonar use. However, this work has provided strong baseline characterization of blubber cortisol levels at AUTEC and adjacent reference areas, which we can use in future comparisons.

In addition, we began the process to document the reproductive success rates of Blainville's beaked whales in both areas. To our knowledge, this has never been documented before in any beaked whale population. For AUTEC, we found 1 pregnant mother yielding 0 viable calves. For Abaco, we found 3 pregnant mothers yielding 2 viable calves, but more work is needed to assess suitable population-level estimates. However, this effort does show that this information can be obtained from these populations. Given additional focus on biopsy sampling and aerial imaging to identify pregnant animals, we believe suitable estimates could be made.

Notes:

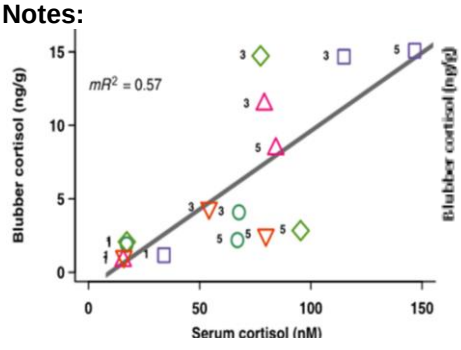

Figure 1. Relationship between serum and blubber cortisol concentrations in hydrocortisone-fed dolphins. Different colors represent different animals, and numbers represent day sample was taken.

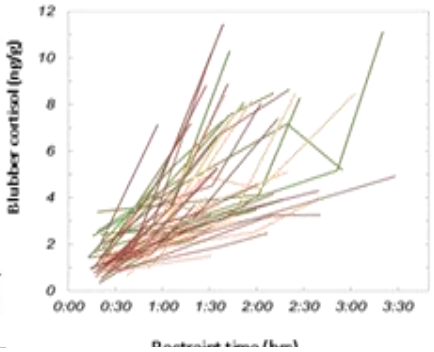

Figure 2. Individual temporal cortisol profiles in the blubber of wild bottlenose dolphins (n = 48) restrained for health assessments. Colors represent different stocks.

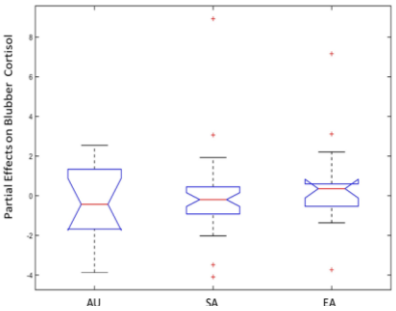

Figure 3. Box plot characterizing the distributions of partial effects of sampling area on blubber cortisol values in Blainville's beaked whales with respect to three different areas: AUTEC (AU), South Abaco (SA), and East Abaco (EA).

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Day 2: Tuesday March 21, 2017

2017 ONR
Marine Mammal & Biology
Program Review

Variability of hormonal stress markers and stress responses in a large cross-sectional sample of elephant seals / Physiological impacts of variation in hormonal stress markers and stress responses in a large cross-sectional sample of elephant seals

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Background

The NEPA, the MMPA and the ESA require Federal agencies to address potential impacts of their major activities in the ocean on marine mammals. This assessment is hindered by a lack of knowledge of the behavioral and physiological responses to these activities and whether potential impacts represent biologically significant events that affect reproduction or survival. One approach to evaluating the significance of a response is through characterization of hormones associated with the generalized stress response. There are no large cross-sectional datasets of stress markers in free ranging marine mammal populations. This study provides critical information for interpreting samples from free-ranging marine mammals and a direct model for assessing physiologcal effects of stress on threatened pinnipeds. It is critical to the US Navy that these factors be understood to adequately assess the physiological condition of animals in the wild and the resulting individual and population-level consequences, particularly in regions regularly used for Navy acoustic activities.

Objectives

The objectives of this effort are to: 1) determine the variation in cortisol, aldosterone, thyroid hormones, ACTH and catecholamines within a free-ranging northern elephant seal (NES) population and its dependence upon gender, age, seasonality, time of day, reproductive state and fasting duration; 2) establish relationships between serum GC levels and levels found in fur, vibrissae and blubber; 3) perform ACTH and TSH challenges and characterize the activation of the hormone axes across multiple matrices; 4) determine the impact of baseline cortisol on thyroid hormones and function; 5) determine the impact of baseline aldosterone on electrolyte balance in elephant seals; 6) determine the natural life-history variation in sex hormones for both genders and impact of variation in baseline cortisol on reproductive hormones; and 7) examine the ecoimmunology of breeding and molting in elephant seals and determine the impact of variation on immune function. We leveraged the large existing research effort on NES to assess the biological significance of the variation in hormone levels including reproductive impacts in adults.

Methods

720 elephant seals were sampled across sexes, age classes (weaned pup, juvenile, adult) and haulouts. Matched blood, blubber, fur and vibrissae samples were obtained. Samples were obtained early and late in the haulouts to evaluate fasting, breeding and molting impacts on stress hormones. Blood samples were obtained from all other elephant seal projects to achieve a final database of 1232 blood samples. Cortisol measurements have been completed for the entire sample set and complete hormone profiles are complete for 720 samples.

ACTH pilot studies were performed to select dosages for studies. ACTH challenge experiments were performed in 17 molting juveniles and 18 breeding and molting adult males. ACTH trials were performed in 12 weaned pups to examine the time course of changes in blubber cortisol. A reference transcriptome for evaluating tissue level impacts of cortisol in muscle and blubber was developed. TSH trials were completed in 12 weaned pups. Cortisol levels at deployment and recovery were compared to foraging success in 332 adult females and natality rates in 153 adult females tagged and tracked as part of the TOPP efforts.

Results

Comparison of serum cortisol to time to sample collection in all study groups suggests that serum values represent baseline. Similarly, no diel pattern for cortisol was evident in any sample group. Patterns of change across life history stages have been determined for all hormones (e.g., Fig.1). Mean cortisol is lowest when seals return from foraging trips, but individuals show unusually high cortisol at all sampling periods suggesting high baseline stress. Cortisol and aldosterone levels are correlated in all study groups, suggesting the potential importance of aldosterone as a stress hormone in marine mammals. Aldosterone levels were not associated with electrolyte levels across all sample periods, but when analysis was restricted to animals that had just returned from sea, serum NA^+ increased with aldosterone (r^2 = 0.58) suggesting the potential for stress impacts on electrolyte balance.

Thyroid hormones show strong negative relationships to cortisol, particularly T3 in most groups, suggesting a strong suppression of the thyroid axis in response to stress. We found unusually high rT3 levels in elephant seals and a strong positive relationship between cortisol and rT3 levels. This effect is present in all age classes and life history stages examined, except in lactating females. These data suggest that the potential for metabolic suppression in response to

chronic stress is unusually strong in elephant seals. Parallel findings in the NMMF dolphin studies suggest this may be a feature of marine mammals.

Analysis of hair samples shows that cortisol levels in hair are low compared to terrestrial carnivores, but are measurable. Variation in hair cortisol was significantly related to serum cortisol levels, suggesting hair has utility for stress measurements. Vibrissae cortisol levels showed distinct patterns along the vibrissae length, and varied strongly between study groups. The vibrissae root cortisol was a useful proxy for serum cortisol. Blubber samples contained extremely high progesterone levels which require use of a cortisol analysis with no cross-reactivity to progesterone. Blubber cortisol measurements from 298 samples showed that blubber cortisol varied serum cortisol (r^2 = 0.41). Blubber cortisol was not a sensitive predictor of low level variation, but did a good job of detecting animals with high serum cortisol.

NES were very sensitive to ACTH, elevating cortisol to up to 10 times baseline. ACTH was as strong a secretagogue for aldosterone as for cortisol. Elevations in cortisol and aldosterone impacted glucose, lactate, NEFA, and BUN. These effects varied between study groups, suggesting modifications of tissue responses to cortisol with life-history stage. We are currently analyzing blubber cortisol levels in response to ACTH challenges. NES were strongly responsive to TSH suggesting a tractable system for studying thyroid physiology. TSH caused a strong and rapid elevation in both T4 and T3.

We found strong relationships between serum cortisol levels and long-term foraging success in females. Cortisol levels at implantation or return directly predicted natality. These data suggest elephant seals may use natural stress responses to nutritional status to make breeding decisions as mediated by the interaction of cortisol with the gonadal axis. We identified variation in several key sex hormones in female seals. Progesterone peaked at the end of the molt, the likely period of implantation. Progesterone concentrations at implantation were negatively associated with cortisol levels suggesting a proximate mechanism for cortisol suppression of natality.

We measured dramatic immune responses to breeding in both male and female elephant seals. In females, cortisol concentrations were negatively associated with IgE , a marker for parasite immune response. In breeding males there were strong differences in the form of immune responses with dominance rank. Cortisol appears to play a role in modulating immune response showing strong positive associations with innate immune markers and negative associations with some adaptive immune markers. These findings suggest modifications of the impact of chronic elevation of cortisol on immune function compared to terrestrial mammals.

Initial transcriptomics work in muscle and blubber has suggested coordinated gene responses to acute stress and potential impacts of acute cortisol release on oxidative stress. This initial work has transitioned into a follow up project looking at chronic stress and the potential for developing genomic biomarkers for blubber that differentiate between acute and chronic stress.

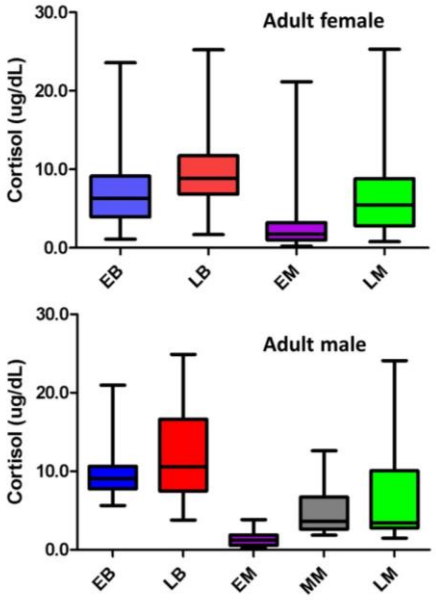

Figure 1. Max-min whisker plots of serum cortisol in adult female (n=800) and male (n=128) NES

Notes:

Quantifying Stress in Marine Mammals: measuring biologically active cortisol in cetaceans and pinnipeds

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Background

Stress hormones (glucocorticoids – either cortisol or corticosterone) are easily measured in blood and are an important measure of stress. However, most circulating glucocorticoids are at least temporarily bound by corticosteroid binding globulin (CBG), their key carrier protein in blood. While bound by CBG, glucocorticoids cannot leave the circulatory system to exert biological effects (Breuner et al. 2013). It is therefore important to be able to measure free (i.e. unbound) glucocorticoid concentrations. This requires knowing the binding affinity of CBG for glucocorticoid for each species of interest, and then measuring the amount of CBG present in a given individual plasma sample (maximum corticosteroid binding capacity, MCBC). We measured the binding characteristics of CBG in 17 species of marine mammals and compare the suitability of the major techniques for measuring MCBC.

Objectives

Our primary objectives were to: (1) measure the equilibrium dissociation constant (K_d) for 17 species of marine mammals; and (2) measure MCBC in blood samples from individual animals varying in sex, season of sample collection, life stage, and stress history.

Methods

Plasma or serum samples were obtained from 17 species of marine mammals collected by aquariums and marine mammal researchers. Equilibrium dissociation constants (K_d) of CBG for each species were determined using saturation binding and nonlinear regression. CBG binding capacity for individual plasma samples was estimated by incubating plasma with a fixed, saturating concentration of cortisol. For both K_d and binding capacity determinations, separating bound from free hormone was accomplished by one of three separation techniques: dextran-coated charcoal, a cell harvester with glass fiber filters, or microdialysis.

Results

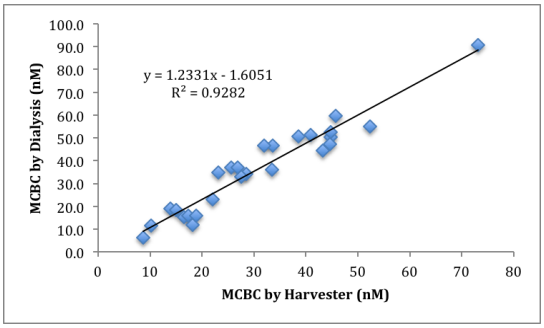
The K_d values for the 17 marine mammal species are reported in Table 1. These represent fairly high affinity K_d values relative to those reported for birds and terrestrial mammals (Desantis et al. 2013, Delehanty et al. 2015), but within normal ranges. Saturation binding curves were run using pooled plasma, and the number of individuals contributing to the pool range from 1 to more than 10. This means that estimates of binding capacity from saturation binding curves are only useful for a coarse comparison of binding capacity. However, even from the saturation binding curves, the binding capacity from the 17 species of marine mammals was notable for its huge variation, ranging from 15.9 nM to 599 nM.

We measured MCBC in individual samples collected by our collaborators, primarily bottlenose dolphins. All MCBC methods require separating CBG-bound from free hormone. We compared three such methods: dextran-coated charcoal (DCC), cell harvester with glass fiber filters, and microdialysis. Microdialysis is the gold standard because the separation occurs across the dialysis membrane at equilibrium. In contrast, both DCC and harvester methods separate bound from free hormone only after the solution has reached equilibrium. DCC adsorbs free hormone when incubated with the plasma sample, but over the course of the incubation, some CBG-bound hormone is released from the CBG and is adsorbed by the DCC. Therefore, a correction is required to calculate how much hormone was bound by CBG at the time the DCC was first added to the plasma solution. The harvester method separates bound from free hormone by capturing CBG on glass fiber filters. However, not all CBG is captured by the filters, and we used microdialysis to estimate the loss through the filter. Both DCC and the cell harvester produced MCBC estimates that were highly correlated with dialysis estimates ($r^2 \geq 0.9$; Figure 1), but both underestimated MCBC. DCC yielded values closer to the dialysis method.

Based on these results, we recommend the use of DCC to measure MCBC, preferably with a correction made by measuring a subsample with dialysis.

Notes:

A.



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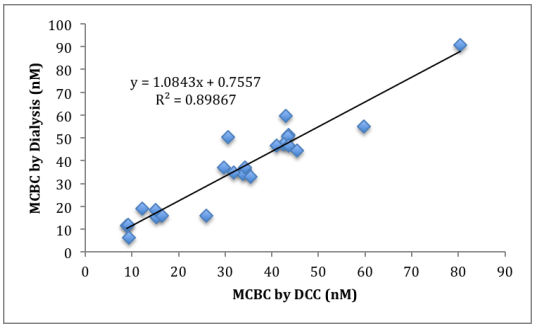

Figure 1: Relationship between estimates of maximum corticosteroid binding capacity (MCBC) arrived at by (A) cell harvester, and (B) dextran-coated charcoal (DCC) with dialysis. Each method was used to estimate the MCBC of a set of samples collected from 24 individual bottlenose dolphins (*Tursiops truncatus*).

Table 1: Binding characteristics of corticosteroid binding globulin of marine mammal species. Equilibrium dissociation constant (K_d) and maximum corticosteroid binding capacity (MCBC) for the pooled plasma used for the K_d determination are presented as means (± SE). Note that for bottlenose dolphins, there was very high variance in MCBC because two separate plasma pools were used and the pools varied considerably in CBG content.

Species name	Common name	K_d (nM) at 4°C	K_d (nM) at 37°C	MCBC (nM)
Enhydra lutris	Sea Otter	0.76 (±0.3) n=6	6.4 (±1.1) n=2	28.5 (±5.3) n=6
Mirounga angustirostris	Northern elephant seal	0.99 (±0.08) n=3	3.8 (±0.4) n=2	65.6 (±6.0) n=3
Zalophus californianus	California sea lion	0.34 (±0.02) n=2	1.6 (±0.3) n=2	64.6 (±4.1) n=2
Eumetopias jubatus	Stellar sea lion	0.57 (±0.1) n=6	3.9 (±0.4) n=2	127.5 (±10.4) n=6
Leptonychotes weddellii	Weddell seal	1.3 (±0.2) n=5	10.9 (±0.6) n=2	598.7 (±45.4) n=2
Lobodon carcinophagus	Crabeater seal	0.58 (±0.04) n=2	2.1 n=1	462.8 (±26.6) n=2
Arctocephalus pusillus doriferus	Australian fur seal	0.26 (±0.05) n=2	1.40 (±0.2) n=2	117.0 (±2.9) n=2
Arctocephalus gazella	Antarctic fur seal	0.26 (±0.04) n=2		116.8 (±5.0) n=2
Callorhinus ursinus	Northern fur seal	0.33 (±0.09) n=2		82.8 (±4.7) n=2
Halichoerus grypus	Grey seal	0.26 (±0.06) n=2	0.52 (±0.08) n=2	107.8 (±2.4) n=2
Pagophilus groenlandicus	Harp seal	0.18 (±0.05) n=2		42.9 (±0.9) n=2
Phoca vitulina	Harbour seal	0.24 (±0.03) n=7	1.34 (±0.03) n=2	156.5 (±7.3) n=4
Delphinapterus leucas	Beluga	0.17 (±0.02) n=6	0.92 (±0.09) n=2	15.9 (±0.7) n=6
Pseudorca crassidens	False killer whale	0.38 (±0.05) n=2		23.8 (±5.5) n=2
Phocoena phocoena	Harbour porpoise	0.26 (±0.1) n=4	1.00 (±0.0) n=2	27.9 (±3.5) n=4
Lagenorhynchus obliquidens	Pacific white-sided dolphin	0.52 (±0.25) n=4	1.83 n=1	27.2 (±4.5) n=4
Tursiops truncatus	Bottlenose dolphin	0.29 (±0.05) n=2	2.64 (±0.3) n=2	27.6 (±11.9) n=4

References:
Breuner CW, Delehanty B, Boonstra R. (2013) Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Funct Ecol 27: 24–36.
Delehanty B, Hossain S, Jen CC, Crawshaw GJ, Boonstra R. (2015) Measurement of free glucocorticoids: quantifying corticosteroid-binding globulin binding affinity and its variation within and among mammalian species. Conserv. Physiol. 3:1–13.
Desantis L, Delehanty B, Weir JT, Boonstra R (2013) Mediating free glucocorticoid levels in the blood of vertebrates: are corticosteroidbinding proteins always necessary? Funct. Ecol 27:107–119.

Determining baseline stress-related hormone values in large cetaceans

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Background

Whales secrete a lipid-rich cerumen into their external ear canals in typical mammalian fashion; however, in certain whale species this wax accumulates chronologically over an individual's lifetime to form a plug. Similar to other lipid rich tissues, cerumen is a lipophilic matrix and has the potential to act as sinks for both anthropogenic (contaminants) and biotic (hormones) lipophilic compounds. Based on their lipophilic nature, compounds such as POPs and stress-related hormones (e.g. corticosteroids) should also accumulate in whale earplugs.

Currently, there are no large cross-sectional datasets of baseline stress or contaminant biomarkers in free ranging marine mammal populations. Without such data, there is no context with which to interpret the biological significance or anthropogenic impact of individuals and populations. The research community, as part of its environmental stewardship, can utilize these markers to assess the acute and chronic impacts that its actions might have on marine mammals. This approach would permit better mitigation of potential impacts and ensure that anthropogenic activities do not come at a deleterious cost to marine mammal populations.

Objectives

During this study, specific objectives included 1) to determine stress-related hormone baseline values from reconstructed lifetime hormone and contaminant profiles in both historic (<1972) and contemporary archived whale earplugs (>1972), 2) determine the potential relationship between stress-related hormones and contaminants concentrations within an individual organism (earplug) and 3) compare and contrast stress-related hormones and contaminants levels between historical and contemporary earplug samples (among species).

Methods

Whale earplugs (N = 20) from four species; finback (*Balaenoptera physalus*; n = 12), Humpback (*Megaptera novaeangliae*; n = 4), blue (*Balaenoptera musculus*; n = 3) and Minke (*Balaenoptera acutorostrata*; n = 1; were collected from archived collections.

All earplugs used in this study had corresponding collection data including species, date and location of collection. All earplugs were stored at 4◦C until analyzed. Bisecting each earplug into equal halves using a ceramic knife allowed for lamina discrimination and aging. Repeated counts (±2% CV) of dark and light lamina, with each pair estimated as one year (GL), confirmed age estimation for each earplug. All weighed (mg) extracted laminae were placed into vials with PTFE caps and stored in amber vials under nitrogen at −30◦C.

Cortisol extractions were assayed using a competitive immunoassay kit (Enzo Life Sciences; 900-071) as well as using UHPLC/MS/MS-ESI with validation and parallelism as described by Hunt et al. (2014). Each extraction ran in duplicate on a 96-well microtiter plate (Beckman Coulter DTX 880 Multimode Detector).

Results

Twenty (N=20) baleen whale earplugs representing an unprecedented 147-year time series of reconstructed cortisol trends spans 1869 to 2016 from individual whales from four species (fin, humpback, blue and minke whales) aged 1.5 to 63 years resulted in 1109 individual lamina samples from 9 males and 11 females (Figures 1 and 2). Mean earplug mass was 11.3±8.8 g whereas the mean lamina mass from all 20 earplugs was 0.074±0.056 g. Fifteen (n=15) of the twenty earplugs from museum archives represented collection dates from 1909 to the 1972 (pre-MMPA) whereas five earplugs (n=5) represented recent acquisitions (>1972; post-MMPA).

Mean cortisol concentrations per lamina of earplug for each species and sex are as follows; fin (n =12), 5.8 ± 5.7 ng/g ($p > 0.05$ between sexes); humpback (n = 4), 6.1 ± 2.3 ng/g ($p < 0.05$; males, 2.7 ± 3.4 ng/g; females, 6.5 ± 1.7 ng/g); blue (n = 3), 5.1 ± 1.5 ng/g ($p > 0.05$ between sexes) and minke whales (n = 1), 6.2 ± 1.9 ng/g. Cortisol from 20 baleen whale earplugs

calculated as a percent difference from baseline values revealed positive trends for both sexes when plotted against age (Figure 2; $p < 0.0001$, both male and female).

Notes: *this can be figures, references, etc*

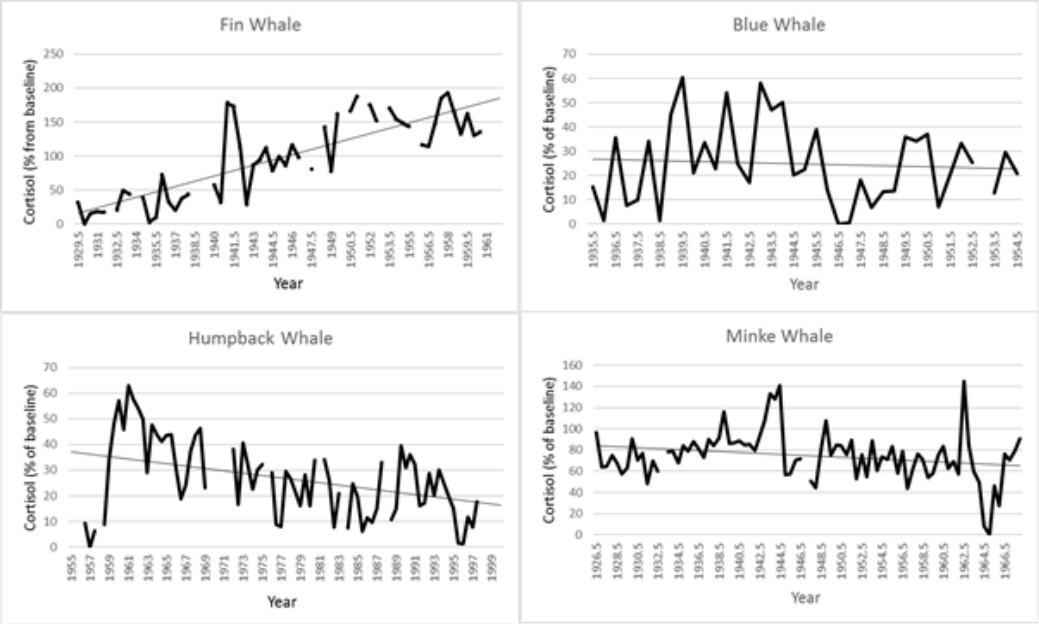

Figure 1. Representative reconstructed cortisol (percent difference from baseline values) profiles plotted against year for the four species of baleen whale earplugs used during this study

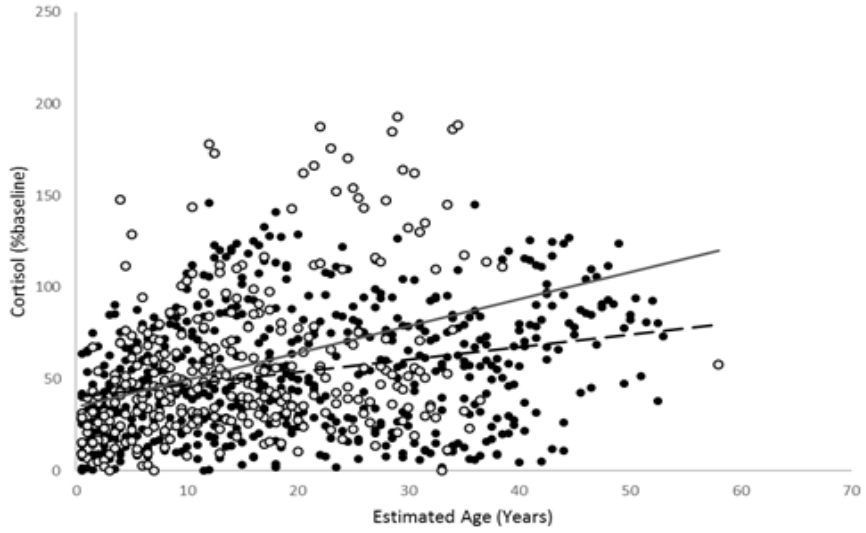

Figure 2. Cortisol extracted from individual lamina (N = 1109) from earplugs of males (light circles) and females (dark circles) plotted against estimated age (based on lamina counts) in four species of baleen whales. The solid regression line is associated with male cortisol whereas the dashed line is female cortisol

Comparative and Cumulative Energetic Costs of Odontocete Responses to Anthropogenic Disturbance

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Background

Cetaceans respond to anthropogenic activities in a variety of ways. However, the consequences of these responses are often difficult to quantify in biological currencies. Recent studies have empirically measured the energetic consequences of some responses that may have acute or chronic impacts. The current investigation involves separate but related studies. The first addresses, in a comparative framework, metabolic costs of sound production and vocal modification across different sound types and odontocete species. The second component addresses cumulative energetic costs of behavioral responses to disturbance. These analyses will provide quantitative information that can be incorporated into models such as the Population Consequence of Disturbance (PCoD).

Objectives

This investigation comprises five main objectives: (1) compare the metabolic costs of social sound and click production in bottlenose dolphins, (2) determine the mass of muscle groups used for sound production in bottlenose dolphins and other odontocetes, (3) develop a predictive bioenergetic model of the metabolic costs of sound production across odontocetes, (4) assess interspecific differences in the physiological performance and energetic demand of muscle groups used for sound production, (5) quantify the cumulative energetic costs of responses to human disturbance.

Methods

For objective 1, the metabolic cost (above resting metabolic rate, RMR) of producing social sounds (Holt et al. 2015) and producing clicks (Noren et al. in review) for 2 min were compared. The metabolic cost and acoustic parameters of social sounds are reported by Holt et al. (2015). The metabolic cost of click production was estimated from the difference in oxygen consumed during 2 min of submerged clicking and 2 min of submerged silence (control trials) conducted during the same month. For both sound types, the cumulative energy flux density levels (cEFD) of the sounds produced were adjusted to source level. For objective 2, mass of muscle groups used during sound production were determined in several odontocetes (harbor porpoise, bottlenose dolphin, killer whale, and two beaked whale species) via segmentation analysis of CT scans to determine muscle volume, which was converted to mass using muscle density. The metabolic cost of social sound production (objective 3) was predicted from the relationship between body mass and % of total body mass devoted to sound production muscles across odontocete species, the cost of dolphins producing social sounds for 2 min, and a scaling factor that accounts for the nonlinear relationship between body mass and RMR. For objective 4, myoglobin concentration, acid buffering capacity, and muscle fiber typing analyses were conducted to gain insight into the physiological performance and energetic demand of sound producing muscle groups. The energetic costs of modifying swim speeds and daily activity budgets were modeled to assess the cumulative energetic costs of responses to human disturbance (objective 5). Accordingly, the literature was surveyed to determine species-specific responses to disturbance as well as the energetic costs of these responses. The metabolic cost of modifying swim speeds was calculated for killer whales using published data on the effects of disturbance on swim speeds and the cost of swimming as a function of speed (results reported in Noren et al. 2017). The metabolic cost of modifying daily activity budgets with increasing hours of vessel presence was calculated for killer whales and bottlenose dolphins following derived methods of Christiansen et al. (2010) and Williams et al.

(2006). The energetic costs of different activity states from Williams et al. (2006) were used for killer whales. The energetic costs of different activity states for bottlenose dolphins were based on previously published bottlenose dolphin RMRs (Yazdi et al. 1999, Noren et al. 2013) and multipliers of RMR for the different activities from Williams et al. (2006).

Results

For the production of both social sounds and clicks, there were significant positive relationships between metabolic cost and vocal cEFD. Metabolic costs of clicking, both in terms of cost range and slope, appeared to be lower than those of whistling. The amount of muscle devoted to sound production across odontocete species was remarkably similar across species (mean = 2.0 %). The two beaked whales appeared to devote a smaller amount of their body mass to sound producing muscles compared to other species examined. Because percent body mass devoted to sound production muscle is consistent across species, only a correction factor to scale metabolic rate with body mass was needed to estimate the metabolic cost of producing social sounds across odontocetes. Consequently, the modest metabolic cost of sound production empirically measured in bottlenose dolphins is an even smaller cost, relative to RMR, of larger odontocetes, including killer whales. Due to the limited sample size of stranded specimens, only biochemical properties of sound production muscles from bottlenose dolphins and harbor porpoise are included in the analysis. Overall, vocal muscles had significantly lower myoglobin concentration [Mb] and acid buffering capacity (β) than locomotor muscles of the same species. Furthermore, muscle fiber type varied across sound production muscle groups and varied somewhat by species. Notably, there were significant differences in [Mb] and fiber type between the nasal musculature on the right (RNM) and left (LNM) in Tursiops, but not in Phocoena. Specifically, the LNM of bottlenose dolphins, where whistles are produced, has properties that equate to a higher aerobic capacity, which are likely related to the greater energetic demand of producing whistles compared to click production. Indeed, the lower aerobic capacity of the RNM, where clicks are produced, mirrors that function. The prevalent disturbance source, suite of behavioral responses, and cumulative energetic impact of human disturbance varies across odontocete species (Noren et al., 2017). Overall, the cumulative energetic cost of ephemeral behavioral responses (e.g., surface active behaviors, modifying sound production) and changes in swim speeds and activity budgets increases daily energy expenditure by ≤4% in most odontocetes. Across odontocetes, decreased energy acquisition as a result of reduced foraging undoubtedly has a larger impact on individuals than the increase in energy expenditure associated with behavioral responses.

Notes: *References*

Christiansen, F. et al. 2010. Effects of tourist boats on the behavior of Indo-Pacific bottlenose dolphins off the south coast of Zanzibar. Endangered Species Research 11, 91-99.

Holt, M.M. et al. 2015. Vocal performance affects metabolic rate in dolphins: implications for animals communicating in noisy environments. The Journal of Experimental Biology 218, 1647-1654.

Noren, D.P. et al. 2013. The metabolic cost of communicative sound production in bottlenose dolphins (Tursiops truncatus). Journal of Experimental Biology 216, 1624-1629.

Noren, D.P. et al.2017. Comparative and cumulative energetic costs of odontocete responses to anthropogenic disturbance. Proceedings of Meetings on Acoustics 27, 040011.

Noren, D. P. et al. in review. Echolocation is cheap for some mammals: dolphins conserve oxygen while producing high-intensity clicks.

Williams, R. et al. 2006. Estimating relative energetic costs of human disturbance to killer whales (Orcinus orca). Biological Conservation 133, 301-311.

Yazdi, P. et al. 1999. Energy expenditure of swimming bottlenose dolphins (*Tursiops truncatus*). *Marine Biology 134, 601-607*

Building a virtual model of a baleen whale: Phase 3 - The Cranial Vibroacoustic Antenna

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Background

There is worldwide interest in the potential effects of anthropogenic sound on baleen whales but relatively little is known about their hearing characteristics. Long wavelength, low-frequency sounds are likely to have significant interactions with the large bodies of whales. The large size of mysticetes prevents meaningful bioacoustic work on these animals in captivity. One way to study the vibroacoustic physiology of mysticetes is to construct a model that can be used to study the interactions between the whale's anatomy and incident sound waves.

Objectives

Construct audiograms and head-related transfer functions (directional hearing maps) for a fin whale (*Balaenoptera physalus*) and a minke whale (*Balaenoptera acutorostrata*).

Methods

Vibroacoustic computational models are, in essence, based on the physics of the interactions between masses and springs, using the principles of finite element (FE) analysis. These FE techniques can be applied to a broad spectrum of species and acoustic stimuli. When possible, our models are constructed of the entire organism. This allows us to investigate interactions within the functional context of the organism in its environment, to address sound propagation and transmission across tissue interfaces and between suites of structures. It also allows us to calculate the distribution of acoustic pressures, shear stresses, dissipated energy and heating effects, excessive strains or displacements due to resonance, and the potential to induce cavitation. More importantly, these methods provide a means to calculate reasonable approximations of hearing sensitivity by producing synthetic audiograms.

Industrial x-ray CT scanners make it feasible to scan mysticetes. Our team has pioneered a suite of techniques that combine the anatomic geometry from CT scans with measurements of tissue elasticity and our finite element analysis software. This combination produces a versatile computational environment for vibroacoustic simulations. Our vibroacoustic FE methods have been validated, meaning that the simulation results closely approximate experimental results gathered by bioacoustic or psychoacoustic methods. Our publications and reports reveal newly discovered mechanisms and pathways for sound reception in toothed whales and baleen whales (Cranford and Krysl, 2015; Cranford et al., 2010, 2015).

Results

Collectively, our research has produced the first CT scan of an entire baleen whale (Fig.1), the first audiogram for a baleen whale (Cranford and Krysl, 2015), and the first evidence for directional hearing in myticetes.

Apparently, the mysticete skull is part of the sound reception apparatus, the cranial vibroacoustic antenna (Fig. 2). The cetacean head works like an acoustic antenna, gathering inputs from many directions and processing them according to frequency, amplitude, and input trajectory (Cranford et al., 2010, 2015).

Studies of minke whale sound reception are nearing completion. Directional hearing results for the fin whale are complete and planned for the minke whale. Anatomic geometry of the TPC (Fig 3) and skull kinesiology are keys to understanding the functional morphology of mysticete hearing. Work on these components of functional morphology are underway.

A comprehensive understanding of directional hearing in mysticetes could dramatically reduce the number of "takes" the US Navy is responsible for under the Marine Mammal Protection Act of 1972. Under current practices, individual marine mammals are considered omni-directional receivers; that is, they receive sound equally from all directions. Even considering the limited evidence we now have,

this is almost certainly not the case for mysticetes across the majority of oceanic anthropogenic noise sources and spectra.

Notes:

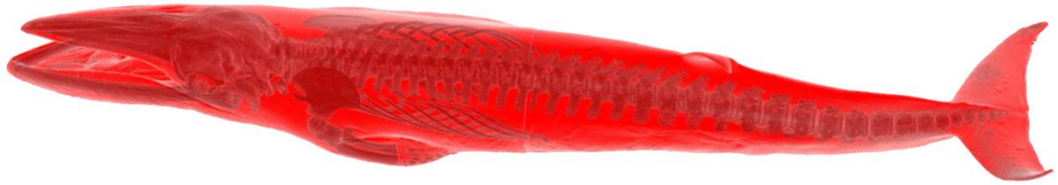

Figure 1 - Entire juvenile minke whale reconstructed from CT scan data.

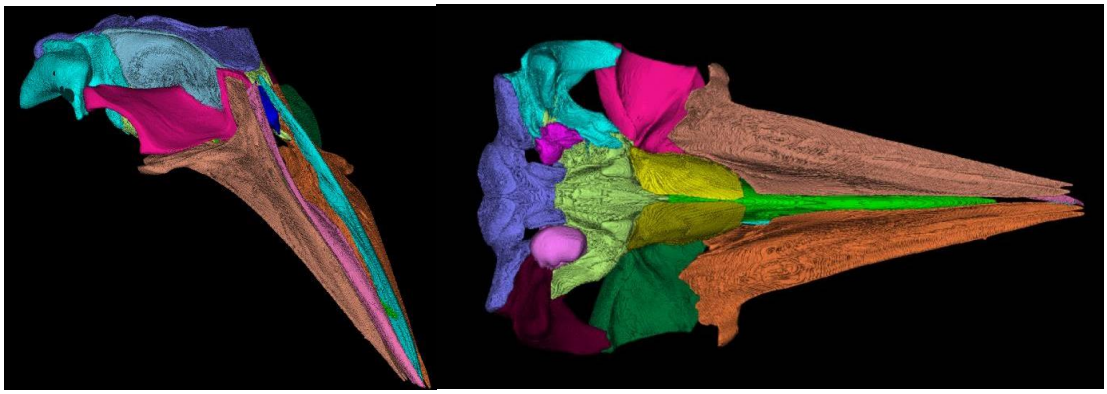

Figure 2 - The Cranial Vibroacoustic Antenna reconstructed from CT scans of a minke whale. The image on the left is a right dorsolateral view and the image on the right is a ventral view. Several bony components of the kinesic skull have been segmented and colored.

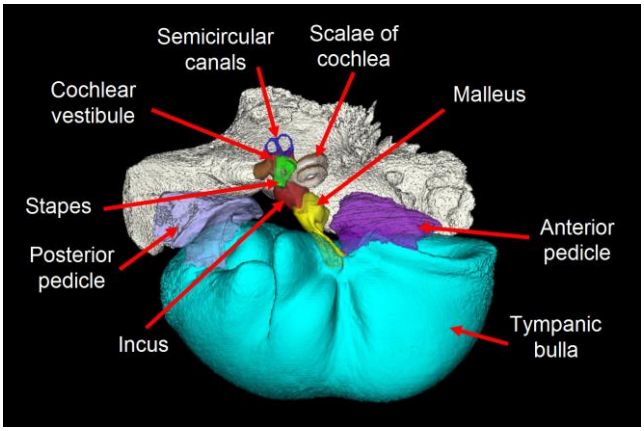

Figure 3 - Details of the fin whale TPC from CT data.

References

Cranford, T. W., and Krysl, P. (2015). "Fin Whale Sound Reception Mechanisms: Skull vibration enables low-frequency hearing," in PLoS ONE (Public Library of Science, San Francisco), p. e116222.

Cranford, T. W., Amundin, M., and Krysl, P. (2015). "Sound production and sound reception in delphinoids," in Dolphin Communication and Cognition: Past, Present, and Future, edited by D. L. Herzing, and C. M. Johnson (MIT Press, Cambridge), p. 328.

Cranford, T. W., Krysl, P., and Amundin, M. (2010). "A new acoustic portal into the odontocete ear and vibrational analysis of the tympanoperiotic complex," in PLoS ONE (Public Library of Science), p. e.0011927.

A Population Consequence of Acoustic Disturbance Model for Cuvier's beaked whale (Ziphius cavirostris) in Southern California

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Background

Beaked whales were the predominant species in several stranding events associated with the use of Mid-Frequency Active sonar (MFAS) (D'Amico *et al.* 2009). A significant population of Cuvier's beaked whales (*Ziphius cavirostris*, *Zc*) have been documented on the Southern California Offshore Range (SCORE) were MFAS operations routinely occur (Falcone *et al.* 2009).

DeRuiter *et al.* (2013), reported behavioral disturbance when *Zc* were exposed to a simulated sonar playback from a local source within 2000m. Such disturbance was also documented for Blainville's beaked whales (*Mesoplodon densirostris*, *Md)* at AUTEC around sonar operations and in response to directed playbacks (Tyack *et al.* 2010). However, judging the cumulative impact of repeated exposure and displacement is difficult. By monitoring *Md* vocal activity and sonar around multi-ship MFAS operations, a risk function for behavioral disturbance was derived (Moretti *et al*. 2014). In addition, passive acoustic data are being used to estimate the cumulative effect of disturbance in terms of loss of foraging dives over an entire year of data at AUTEC as an application of the population consequences of disturbance (PCoD) framework(New *et al.* 2013). This program will extend these methods to *Zc* at SCORE.

Objectives

The primary objective of this project is to extend the methods used to estimate abundance, behavioral risk function derivation and population consequences of disturbance for *Md* at AUTEC to a new species and location: *Zc* at SCORE.

Methods

The *Md* dive counting abundance estimate developed at AUTEC (Moretti *et al.* 2010) was adapted to SCORE and applied to a five-year period. Semi-automated methods were used to detect vocalizing groups. The data were randomly sampled and the rate of false positives and false negatives were calculated. Correction factors derived from the detection statistics were added to the estimating equations.

Sonar data were also extracted from multiple operations throughout a two year period using the same data archives. The receive levels were estimated and the behavioral risk function derivation method developed at AUTEC was applied. Since *Zc* group vocal periods (GVPs) are detected predominantly on the western side of the range, in this initial analysis, only the hydrophones on the western half of the range were considered. To complete the analysis, more advanced models will be examined in an effort to expand the dataset and the range of exposure levels.

The *Md* PCoD model developed at AUTEC is being applied to *Zc* at SCORE. Isolation of *Zc* echolocation clicks on DTags strongly suggests that foraging only occurs during deep dives (Johnson *et al.* 2004). Therefore, the number of these deep dives per day can be used as a proxy for energy gain (New *et al.* 2013). A mean daily dive rate is being derived from published data and from animals tagged directly within SOCAL. Recent data from depth recording satellite tags on *Zc* at SCORE are being applied (Schorr *et al.* 2014).

The energetic gain from each of these deep dives will be calculated from an estimate of daily energy requirements for adult females in different reproductive states (pregnant, lactating, not-pregnant). In order to estimate the weight of these animals, we will follow the approach developed by Hooker *et al.* (2002) and Bloch *et al.* (1996) for bottlenose whales using data from stranded animals and general length-weight relationships for cetaceans. The additional daily costs of pregnancy and lactation are calculated using the approach developed by Lockyer (2007) for a range of cetacean species.

The frequency of periods of disruption are estimated using passive acoustic data collected on the Southern California Antisubmarine Warfare Range (SOAR) throughout the year along with SCORE records of range activity These provide an estimate of potential exposure to disruption.

Photo-ID data from SCORE provide demographic metrics for the model, such as the proportion of mothers, calves, and juveniles in the population at a given time, and the amount of time calves remain

associated with their mothers. Initial estimates from the Bahamas suggest *Md* calves remain with their mothers and continue to nurse for at least 2 years, though it is unlikely that calves are entirely dependent on their mothers during this period. Preliminary data from SCORE suggest this period may be shorter for Zc here. The rate and proportion of independent feeding is unknown. The worst case assumption is that calves do not feed independently through this period.

Zc group data are being examined before and during MFAS operations. An estimate of the number of dives "lost" is calculated and a mean number of dives lost per operation estimated. The number of dives lost is converted to an estimate of energy lost. The ratio of dependent calves to adult females is estimated as a function of energy intake via foraging. The effect of multiple exposures based on the number of operations on the range is used in the model and the ratio of dependent calves to adult females estimated with and without sonar.

Results

Correction factors for the detection of *Zc* dive starts for both false positives (.827, CI +/- 0.004, 95%) and false negatives (1.35, CI +/- 0.004, 95%) were calculated from random data samples. These were applied to produce corrected abundance estimates on a monthly and yearly basis. The results suggest no decline in the *Zc* population over the five year period (Fig. 1).

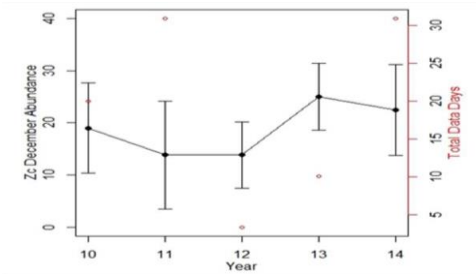

Figure 1. Yearly SCORE abundance for the month of December from 2010 to 2014.

Sonar and *Zc* data were combined from the western side of the range to produce a preliminary risk function for Zc. Hydrophone receive levels were used to estimate the exposure level. These are being calibrated and must be projected back to near surface values.

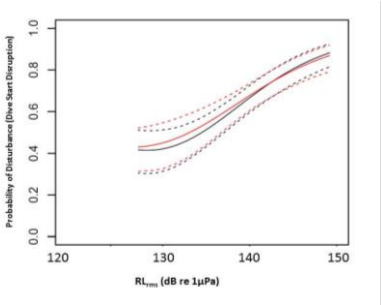

Figure 2. Preliminary result showing probability of GVP disturbance by sonar received level is shown as estimated by fitting a generalized additive model (GAM). The black curves show the result from the GAM fit to the full (black) and the red the reduced (zero exposure levels removed) dataset (solid lines) with a 95% confidence interval (dashed lines).

Two PCoD models have been completed. The first examines the changes in the ratio of dependent calves to adult females over a user defined number of years as a function of caloric intake with and without sonar present. The second looks at the ratio by considering a user defined number of individuals and estimating the inter-calf interval as a function of caloric intake with and without sonar disruption. Both have been completed and run on AUTEC data and are being adapted for *Zc* at SCORE. Operational and GVP data are being used to estimate the dive loss due to sonar. These data will then be used to inform the models and estimate the long-term effect of sonar disturbance.

PCoD lite: Using an interim PCoD protocol to assess the effects of disturbance associated with US Navy exercises on marine mammal populations

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Background

Policy makers and managers are increasingly concerned about the effects of disturbance on wildlife populations. However, forecasting population level consequences of changes in individual behavior is difficult. Recently, working groups established by the US National Academy of Sciences and the US Office of Naval Research developed conceptual models of the population consequences of disturbance (PCoD) for marine mammals (NRC 2005).

Although there is an extensive literature documenting the effect of human disturbance on wildlife behavior, the fitness consequences of behavioral changes have been demonstrated for only a few species. For most species there is little or no empirical evidence to quantify the relationship between behavioral or physiological change and fitness. To fill this knowledge gap, we have developed an interim version of the PCoD framework that uses expert elicitation (EE). The approach is 'interim', because the values provided by experts should be replaced with empirically-derived values as soon as they become available.

In order to issue an incidental harassment authorization to the US Navy under the Marine Mammal Protection Act, the Office of Protected Resources must ensure that "the specified activity …cannot be reasonably expected to, and is not reasonably likely to, adversely affect the species or stock through effects on annual rates of recruitment or survival".

Objectives

The interim PCoD framework was designed to assess the potential impact of disturbance associated with the construction and operation of offshore renewable energy developments on marine mammal populations in UK waters. For this project, the framework is being adapted so that it can be used to forecast the potential effects of disturbance associated with Navy exercises on Blainville's beaked whale (*Mesoplodon densirostris*) and sperm whales (*Physeter macrocephalus*) at the Atlantic Undersea Test and Evaluation Center (AUTEC), Bahamas, and at the Hawaii Range Complex (HRC), Hawaii.

In this interim report we describe the changes we are making to the population models that underpin the interim PCoD model, and document the expert elicitation process we have undertaken to parameterize the relationships that are used to forecast the potential effects of disturbance on the vital rates of Blainville's beaked whales and sperm whales and suggest some potentially useful output metrics from these models.

Methods

Expert elicitation seeks to accumulate opinions from experts about parameters for which there is currently little or no data. In this process each expert provides estimates of, and some indications of uncertainty for the parameters of interest. We used the 4-step interval approach developed by Speirs-Bridge et al. (2010), in which experts were invited to give their best estimates of a quantity, the lowest and highest plausible values, and an estimate of the confidence they attached to the interval they created.

We conducted expert elicitation exercises, first using an online questionnaire for beaked whales (no online version was done for sperm whales). A total of 106 leading experts in the field of marine mammal science were approached. Overall, 38 experts responded to the initial survey. The next step in the expert elicitation process was to invite all of the experts who participated in the survey to attend workshops, at which the results from the initial elicitation were presented and experts were asked if they would like to reconsider their opinions. This is the Delphi process, in which experts are asked to reconsider their opinions in the light of what other experts have said. This has been shown to substantially improve the reliability of the elicitation results (Burgman et al. 2011). At the sperm whale workshop, additional time was spent to calibrate expert responses and develop an EE approach expert were involved in. All

outputs were analyzed using approaches developed in Donovan et al. (2016).

Incorporating these results, we modified a version of the Interim PCoD approach to forecast the potential effects of all Navy training and testing activities conducted during the course of a year on Blainville's beaked whale populations on the range at the Atlantic Undersea Test and Evaluation Center (AUTEC) and on the Hawaii Range Complex (HRC). We used the same approach to forecast the potential effect of these activities on the sperm whale population on HRC. For AUTEC we used a timetable of training events and probabilities of behavioral response provided by D.Moretti. For HRC we used the outputs from the Navy Acoustics Effects Model that were used in the preparation of the 2013 Hawaii-Southern California Training and Testing EIS/OEIS.

Results

For beaked whales the predicted effects of all the training and testing activities conducted during the course of a year on the survival and fertility of beaked whales depended on what assumptions were made about the duration of a behavioral reaction, and the amount of time animals spend in the vicinity of Navy activities. If animals were assumed to resume normal behavior on the day after a training or testing event, some effect on survival or fertility was predicted in 13-19% of all simulations for AUTEC, but in less than 10% of simulations on HRC. However, these changes in demographic rates had only a small effect on population growth rate. If the effects of a behavioral response were assumed to continue for up to 2 days after the disturbance event, as has been observed at AUTEC, some effect on survival or fertility was predicted in 23-34% of simulations for beaked whales. The predicted effects of these changes on population growth rate were relatively small in the case of HRC, but they were predicted to reduce population growth rate by more than ΔRPBR in 8-25% of simulations for AUTEC.

For sperm whales, we restricted our analysis for sperm whales to the potential effects of testing and training activities at HRC (Claridge & Dunn (pers. comm) indicated that no adult female sperm whales have ever been observed in the Tongue of the Ocean). For HRC, no effect on inter-calf interval was predicted in any of the 10,000 simulations we conducted for a sperm whale population exposed to Navy training and test activities on the range without "residual disturbance" nor in any of the 10,000 simulation with "residual disturbance. The only predicted effect for sperm whales was a reduction in individual growth in a small number (3-5) of the 10,000 simulations with "residual disturbance".

It should be recognized that the results presented in this report are not intended in any way as an assessment of the potential effects of Navy training and testing activities on beaked whale or sperm whale populations. Nor is it intended to influence regulatory policy or control over these activities. Rather, we have attempted to show how the Interim PCoD approach can be used with information that is routinely collected on some Navy ranges, such as AUTEC, or that is provided by the Navy Acoustics Effects Model to forecast these potential effects.

Next, we compared some of these forecasts with outputs from a preliminary version of a PCoD model for Blainville's beaked whales based on bioenergetic principles. The outputs of the bioenergetics model are very sensitive to the values used for a number of parameters, most of which cannot be directly measured. However, the outputs from a version that is compatible with the different age structures that have been observed in the beaked whale population at AUTEC and in an undisturbed population at Abaco are within the range of predictions of the Interim PCoD model.

Notes:

Burgman, M. A., McBride, M., Ashton, R., Speirs-Bridge, A., Flander, L., Wintle, B., Fidler, F., et al. (2011). Expert status and performance. PloS one, 6(7), e22998. doi:10.1371/journal.pone.0022998.
National Research Council. (2005). Marine Mammal Populations and Ocean Noise : Determining when noise causes biologically significant effects. The National Academy Press. Washington D.C.
Speirs-Bridge, A., Fidler, F., McBride, M., Flander, L., Cumming, G. & Burgman, M. (2010). Reducing overconfidence in the interval judgments of experts. Risk Analysis, 30: 512–523.

The Health Black Box in PCoD: Understanding the Onset of Health Impacts Caused by Disturbance

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Background

We need to have a more detailed grasp on the health component of PCoD to understand how behavioral and physiological responses to repeated disturbances are integrated in the demographic investment decisions of individuals. We are currently primarily using blubber volume/mass as a demographically relevant proxy for health, but this measures has limitations and its relationship to calving/pupping success is noisy.

Both physiological and ecological studies point to the blubber as a tissue playing an active role in linking cetacean behavior to demography. More broadly, in mammalian studies, individuals placed under foraging constraints will also modulate their fat stores. In addition to the energetic constraint placed on individuals by decreased fat stores, adipose tissue also plays a signaling role influencing cellular biology in multiple tissues. This regulatory role affects activity budget and energy partitioning and therefore demographic contributions. Many of these signals can be detected by the expression of the genes and metabolites involved in their biological pathways. Identifying how these pathways interact under foraging restriction, and in stressful environments, would provide a better resolution for the PCoD health process. This would have two advantages. Firstly, gaining a mechanistic understand of how health changes under repeated disturbances will provide a better resolution to link perturbations to demographic contribution decisions. Secondly, this provides a practical solution for PCoD monitoring and assessment because we have means to sample the adipose tissue of free-ranging individuals.

Here we aim first to develop a conceptual understanding of how cellular biology may change in relation to foraging restriction using a system biology approach. To do so, we first use a graded calorie restriction (CR) manipulation in mice to determine how gene expression changes in tissues (hypothalamus, liver, and adipose) involved in key mechanisms linking foraging perturbations to reproductive investment decisions. We aim to understand the dynamics of changes following restriction, particularly if we could observe state-shifts in cellular networks which could be related to reproductive investment.

We then challenged the predictions emerging from the mouse experiment with plasma metabolomic observations emerging from a fasting manipulation in bottlenose dolphins to assess potential departure from the standard mammalian model in cetaceans and determine topological changes in the metabolomic network that could indicate shifts in 'health' state. Finally, we aim to challenge these results with longitudinal observations from live-captured free-ranging bottlenose dolphins.

Objectives

Given our understanding of the way calorie restriction influenced the hypothalamus transcriptomics of the mice, we needed to assess the way in which adipose signaling to hypothalamus changes under calorie restriction and whether this related to hypothalamic changes and reproductive investment. We also assessed whether the multi-tissue transcriptomic networks underwent a state shift in interactions as CR increased to gain insight in network dynamics as it relates to PCoD.

We aimed to develop software to provide a reproducible analytical pipeline to identify metabolites and estimate and analyze metabolomic networks. We used this software to estimate whether the plasma metabolomic network changed after fasting in bottlenose dolphin and particularly we assessed whether, following theoretical prediction, a dynamical network biomarker (DNB) emerged, which could be used as a pre-state shift signal.

Methods

The full CR experimental design is available in Lusseau et al. 2015. We focused here in estimating gene differential expression in the adipose, as its mass decreased drastically, to determine the signaling role of adipose in response to CR. We also estimated topological changes in gene interaction networks in hypothalamus, adipose and liver with increased CR. This was to assess whether these networks underwent a bifurcation as CR increased which could be related to decreased reproductive investment (measured as the concentration of Mups in the male mice).

We subsequently estimated the bottlenose dolphin plasma metabolomic network using pre-existing observations before and after fasting (paired sampling design). We determined topological changes using network analyses.

Results

The hunger pathway and the HPA played a key role in relating increased CR to decreased reproductive investment (Figure 1). In this, adipose played a key signaling role, with p53 signalling pathway being downregulated at the highest CR treatment level. p53 has been associated with the reallocation of energy investment between somatic maintenance, growth and reproduction and here it is associated with reduced Mups. The multi-tissue network analysis does show an overall non-linear response to CR with a bifurcation leading to a topological change at the highest CR treatment level associated with reduced Mups. The mouse model therefore confirms the prediction of cellular network state shifts linked to demographic decisions (somatic maintenance v. reproductive investment) which can be detected using a system approach.

First analyses of the bottlenose dolphin metabolomics shows a change in network topology, with amino acid metabolism metabolites playing a larger structural role pre-fasting, while lipid metabolism metabolites playing this role post-fasting (Figure 2). A subset of those formed what can be interpreted as a DNB, with greater within-DNB metabolite interactions and weaker interactions with other metabolites as well as greater between-individual variability in intensity for those metabolites than for the others (Chen et al. 2012). Taken together these analyses show that a netwrk approach to omics observations provide a mean to refine our view of the health process in PCoD.

Notes:

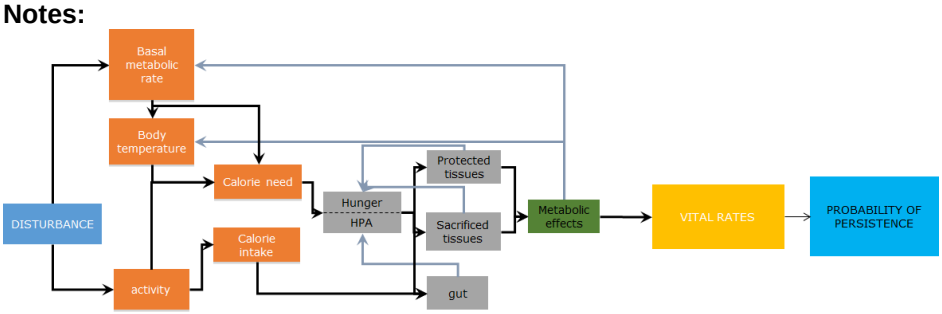

Figure 1. PCOD – health: the PCOD framework is expanded to account for the main interactions within the body that link response to disturbances to the individual vital rates.

Pre – fasting Post fasting

Figure 2. Changes in metabolomic network topology and relative importance of 'super' pathways. Links are the conditional mutual information (CMI) shared between two metabolites. Nodes are metabolites of which the size represents the eigenvector centrality of each metabolite; the larger, the more structurally important the node is to the topology of the metabolomic interaction network. Node color corresponds to super-pathways – white: unidentified; green: lipid metabolism; red: amino acid metabolism; blue/green: peptid metabolism; brown: nucleotide metabolism; blue: energy metabolism; orange: carbohydrate metabolism; pink: cofactor & vitamins.

Reference Chen et al (2012) Scientific Reports 2:342; Lusseau D. et al (2015) Scientific Reports 5:13198

A Power Analysis and Recommended Study Design to Directly Detect Population-level Consequences of Acoustic Disturbance

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Background

Increasing levels of anthropogenic sound in the World's oceans is recognized as a potential threat to marine mammal populations. Navy mid-frequency sonar has been clearly implicated in causing strandings and deaths of beaked whales. But a much more difficult question is whether these relatively isolated small-scale events and less acute, chronic exposure to sonar and other anthropogenic sounds are likely to have population-level consequences. Since 2006, photo-ID mark-resight data for Cuvier's beaked whales, *Ziphius cavirostris*, have been collected at the Navy's Southern California Offshore Range (SCORE).

Passive acoustic array data have been collected for a similar time frame. But the ability to make inferences from these data about population dynamics or the potential population-level consequences of anthropogenic sound have not been evaluated. In this study, we aimed to estimate various demographic parameters from the photo ID and acoustic data, including annual apparent survival, trends in use and the number of beaked whales using SCORE. Additionally, we conducted a statistical power analyses to determine the power of mark-recapture type monitoring to detect population trends or other demographic indicators of population impact. Based on this analysis, we sought to provide guidance for improving long-term monitoring schemes based on these survey methods to best monitor the health and status of beaked whale populations and potential human impacts thereon.

Objectives

1) Use existing monitoring data (photo ID and passive acoustic) to estimate population parameters for Cuvier's beaked whales at SCORE
2) Conduct power analyses individually for the fixed hydrophone array and mark-resight (photo ID) monitoring schemes, to compare effectiveness of each design in terms of their ability to detect beaked whale population trends and suggest revisions to sampling design to improve the effectiveness of each
3) Use photo-ID datasets to assess and improve the reliability of different measures of abundance from the acoustic hydrophone datasets, noting that the hydrophones record activity levels, not actual abundance
4) Evaluate sampling design schemes that will enable the hydrophone-array and photo-ID data to best complement each other; i.e., describe an optimal "mix" of these data types to maximize inference about beaked whale population dynamics
5) Contextualize data for the SCORE area within broader patterns of beaked whale population change throughout the California Current, in effort to improve inference about the spatial extent of potential Navy impacts in the Southern California Bight.

Methods

A robust estimation method for population size, survival rate, and population growth rate of *Ziphius*, given available data, is prerequisite to conducting a power analysis. We fit various mark-recapture open models (Cormack-Jolly-Seber, Jolly-Seber) to the photo ID monitoring data, using both frequentist (MARK) and Bayesian (JAGS) implementations. Inferences at this time are based on Bayesian estimation of a POPAN parameterization of the Jolly-Seber model, applied to right-side only photo ID data. Survival rate and entry rates to the population are estimated as constant through time, while resight probability is time-dependent and includes random effects for temporal and individual heterogeneity. Several estimated parameters from the POPAN model (e.g., annual resight probability and associated random effect variances, animal abundance) are then used as a basis for conducting power analysis. Parametric bootstrapping is used to simulate the field sampling process under different process models (e.g., different survival rates) and observation models (e.g., differing sampling time frames). Frequentist estimation

of mark-recapture models (e.g., Pradel) fit to the simulated data are used to understand conditions under which useful inferences about changes in population health or status can be expected.

Results

Photo ID data collected from 2007 – 2016 generated > 100 individual capture histories but only 74 based on right-side only photos. Resight rates were low, with 55 animals never resighted, 15 resighted once and 4 resighted two or more times. This translated into annual resight probabilities ranging from 0.04 to 0.12; such low rates challenge model convergence and estimation precision of model parameters. Our preliminary estimate of annual apparent survival is 0.90 (SE = 0.05). Estimates of annual population size decreased from around 223 (CV ≈ 0.45) in 2007 to 160 (CV = 0.65) in 2016, corresponding to an annual growth rate of $\lambda \approx 0.97$ (SD = 0.05), depending on the particular model. The population size estimates from the photo ID data will be compared to independent acoustic-based estimates of population and trend, noting that these are only partially comparable metrics because the latter provide estimates of abundance on SCORE at a particular time (and *use* of SCORE through time), whereas the photo ID data provide estimates of how many animals use the area during the year (which will be greater than that is on the range at any particular time) and how this changes annually. Power analysis is ongoing, but we are able to identify some auxiliary information types that would improve our ability to assess population health. These include additional movement tagging data, which would allow some distinction between true and apparent survival, and biopsy data to better understand population reproductive rates and changing age structure, which would enable better interpretation of current survival and trend estimates.

Notes:

Photogrammetry with an unmanned aerial system to assess body condition and growth of Blainville's beaked whales

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Background

Recent studies indicate that beaked whales move away from navy sonar sources, with disruption to their foraging behaviour. To assess and mitigate the impacts of this disruption, the Population Consequences of Disturbance (PCOD) framework has been developed to trace the effects of disturbance through life functions to population status. Notably, bioenergetics models suggest that beaked whales require relatively high quality habitat in order to meet their energy requirements, and that regular displacement from preferred feeding habitats could potentially impact survival and reproduction through compromised growth and body condition. However, key steps of this model can be hard to parameterize, particularly for cryptic species like beaked whales, for which functions such as feeding success are particularly hard to observe.

In this study, we used aerial photogrammetry to directly measure the body condition and size of Blainville's beaked whales (*Mesoplodon densirostris, Md*) using an Unmanned Aerial System (UAS). As such, we tested the utility of UAS-photogrammetry for filling key data gaps that have constrained direct assessment of whether nutritional stress from disturbance is a realistic mechanism for population-level responses.

Objectives

We aimed to test the utility of using UAS for photogrammetry of *Md* during a field study off SW Abaco Island, northern Bahamas. Aerial images collected in June 2016 were matched to photo-identifications to provide key morphometric measurements for whales with known histories. These included lengths for whales of different ages, to test the ability to measure growth, and width profiles for adult females, to test the ability to detect differences in body condition and pregnancy.

Methods

We used a remote hexacopter (APH-22; see Durban 2015, Journal of Unmanned Vehicle Systems 3:131-135) to obtain vertical images of *Md*. This is a small (1.9kg, 82cm wingspan), vertical take-off and landing UAS that enabled safe launch and retrieval from a 6.8m rigid-hulled inflatable boat (RHIB) to hover over whale groups. Images were collected using a micro 4/3 camera (Olympus E-PM2, 16MP RAW files) with a 25mm f1.8 lens, mounted on the hexacopter to be downward-pointing. When directly above the whales, the camera was remotely triggered to record high-resolution still photographs (Olympus RAW format) at one second intervals to maximize the chance of obtaining "flat" images for unbiased photogrammetry measurements. The RHIB repositioned during flights to maintain a consistent contact distance, typically between 200 and 400m, to the whales.

To facilitate matches to individual beaked whales with known histories, we also collected lateral photo-identifications from the RHIB. Since 1997 BMMRO has compiled a photo-identification catalog of 167 different individual *Md* in this sheltered, coastal study site. Aerial images and measurements were assigned to specific individuals based on the pattern of healed wounds from the bites of cookie-cutter sharks (*Isistius sp.)* that are readily distinctive from the air and matchable to BMMRO's catalog. Additionally, *Md* are readily assigned to age/sex classes from photographs of dentition and scarring enabling us to link measurements to whales of specific age classes.

Results

A total of 30 flights were successfully flown over *Md*, averaging 10 minutes in duration (maximum = 16 minutes), providing repeated photographic opportunities during seven different encounters. The average altitude of flights was 33m (108ft), with a maximum of 47m (154ft), and the average distance covered during each flight was 593m (max = 993m). A total of 10 different whales were represented in 3644 images, including three adult males, three adult females, two sub-adults and two dependent calves. Concurrent boat-based photo-identifications allowed nine of these whales to be matched with known histories, and the other whale was confirmed as adult male from photographs of its erupted teeth.

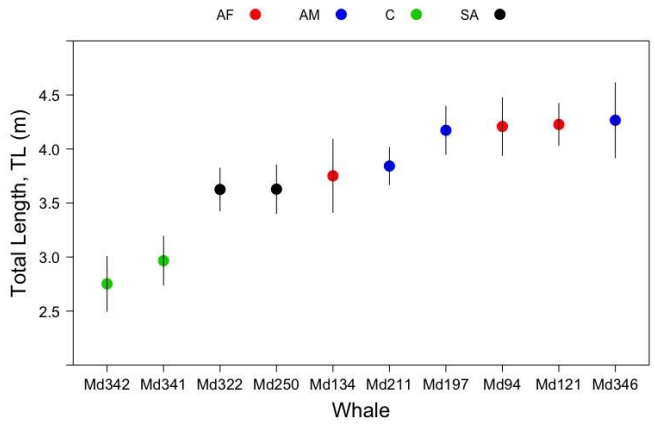
Measurements in image pixels were scaled to real size using data on altitude and lens focal length. Bias in the altitude estimates from the hexacopter's 15-channel GPS receiver was evaluated using measurements from 35 images of the known-size RHIB taken on 8 different days; average error was

<1% (<4cm compared to 6.8m boat). Realized precision was assessed from variability in the total length (TL) measurements of the same whale: all ten whales were represented by >20 measurement-grade images (mean = 34, maximum = 53 images). The average TL estimates ranged from 2.75m to 4.27m, with a mean coefficient of variation (cv=SD/mean) of 6.6%. There was a clear trend in estimated TL from calves (~1.5yrs of age) to adults, with sub-adults measuring as intermediate in length (Figure 1). There was no significant difference between the maximum length of adult males and females, but there was individual variability in the adult size of both sexes (Figure 1), providing insights into the social implications. Namely, the largest adult male (Md346) was repeatedly seen with reproductive females and their calves during the study while the smallest male (Md211) was associated with an immature female, despite both males being estimated to be of a similar age.

Width profiles were measured in pixels at 5% increments along the TL and were represented relative to the pixel measurement of TL in the same image. Significant differences in width profiles were identified, notably between adult females (Figure 2). Only one female (Md121) was encountered without a calf, and she was suspected to be pregnant based on measurements of increased width at mid body (Figure 2). Subsequent follow-up photo-identification studies documented her with a newborn calf in September 2016, demonstrating our ability to document pregnancies based on shape profiles from vertical images. There were also significant differences in the profiles of the two females with dependent calves. The thinner of the two (Md94) was the mother of the largest calf (Md 341, Figure 1), likely reflecting the increased energetic cost of prolonged lactation.

This study successfully demonstrated the utility of UAS-photogrammetry for directly measuring body condition, size and identifying pregnancy status of beaked whales. The ability to detect pregnancy will allow us to monitor the covariates underlying reproductive success. Photogrammetry measures of body condition and growth can provide an integrated assessment of feeding success and food availability, to inform considerations of nutritional status as part of the PCOD framework.

Notes:



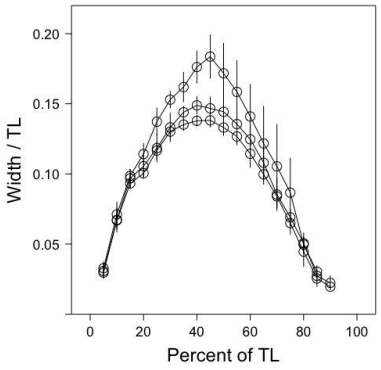


Figure 1 (left): Estimates of total length (TL) for ten individual Blainville's beaked whales: three adult males (AM), three adult females (AF), two sub-adults (SA) and two dependent calves (C).
Figure 2 (right): Width profiles for the three adult females at 5% increments along the total length (TL) of the whale. All estimates are presented as means +/- sd.

Developing a bioenergetic model for baleen whales to assess population consequences of disturbance – Phase 1

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Background

The need for quantifying and managing cumulative impacts of non-lethal human activities (e.g. shipping, naval activities, fisheries, renewable energy, oil and gas exploration and tourism) on marine mammals is becoming increasingly important as their exposure to anthropogenic activities are rapidly intensifying. Human disturbance has been shown to have population level consequences in some targeted populations, however the underlying mechanism of how these effects arise is unknown. In order to predict population consequences of disturbance (PCoD) on marine mammals, we need to understand how behavioral changes can influence individual vital rates (e.g. reproduction and survival), and ultimately population dynamics.

Objectives

The aim of this project is to develop a mechanistic framework to assess PCoD on baleen whales (**Fig. 1**) by accomplishing the following objectives:

Aim 1 Link animal behavior to bioenergetics
Aim 2 Link bioenergetics to body condition
Aim 3 Link body condition to vital rates
Aim 4 Link vital rates to population dynamics

In order to capture a range of life history strategies in baleen whales in our model, we will take a multi-species approach, using humpback whales (*Megaptera novaeangliae*), right whales (*Eubalaena sp.*) and minke whales (*Balaenoptera acutorostrata*) as study species (two families, three genera).

This presentation will focus mainly on **Aim 3**, which is to "*determine the relationship between body condition and vital rates*", with a emphasis on reproduction. Results will be presented for Southern right whales.

Methods

Unmanned Aerial Vehicles (UAV) were used to measure the body size of Southern right whales at the Head of Bight, South Australia, an important breeding ground, between the 24th of June and the 25th of September 2016. The UAVs were flown at altitudes of 20-50m above a surfacing whale to obtain aerial photographs of their body. The size of the whales in the photographs was scaled from the known altitude of the UAV, which was measured using a laser range finder attached to the UAV. Photogrammetry methods were then used to measure the size of the whales (length and width) from the photographs. The body size measurements were used to calculate the body volume of the whales, which was used as an index of body condition. By taking repeated measurements of the same whales throughout the breeding season (Southern right whales can be individually identified from the air based on the callosity pattern on their heads), intra-seasonal variation in the body condition of individual whales could be assessed.

To determine the relationship between body condition and reproduction in Southern right whales, the rate of growth in volume of individual calves were compared to the rate of loss in body condition (i.e. volume) of their mothers. The effect of maternal size (i.e. length) and body condition on the rate of loss in maternal condition (the amount of energy transfer to the calf) was also investigated.

Results

During the three months field season, we successfully carried out 878 UAV flights, and obtained a total of 2,897 body condition measurements. We measured 238 whales, including 89 lactating females, 89 calves and 60 unaccompanied adults. We managed to obtain repeated body condition measurements from individual females with calves over periods of up to 86 days.

We found a significant negative relationship ($F_{1,59}$=14.22, P<0.001, R^2=0.20) between the rate of change (growth) in body volume of calves and the rate of change in body volume of their mothers (**Fig. 2**). For every m^3 of body reserves invested by the mother, the calf would gain 0.13m^3 (SE=0.035), or 130 liters, in body volume.

The rate of loss (energy transfer) in maternal body volume was positively correlated to the absolute size (i.e. length) of the mother and her relative body condition (FBC).

Relevance for ONR

By understanding how female body condition affects the growth of their calves, we are able to predict the effect of behaviorally mediated changes in body condition, caused by anthropogenic disturbance, on reproduction.

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Figures

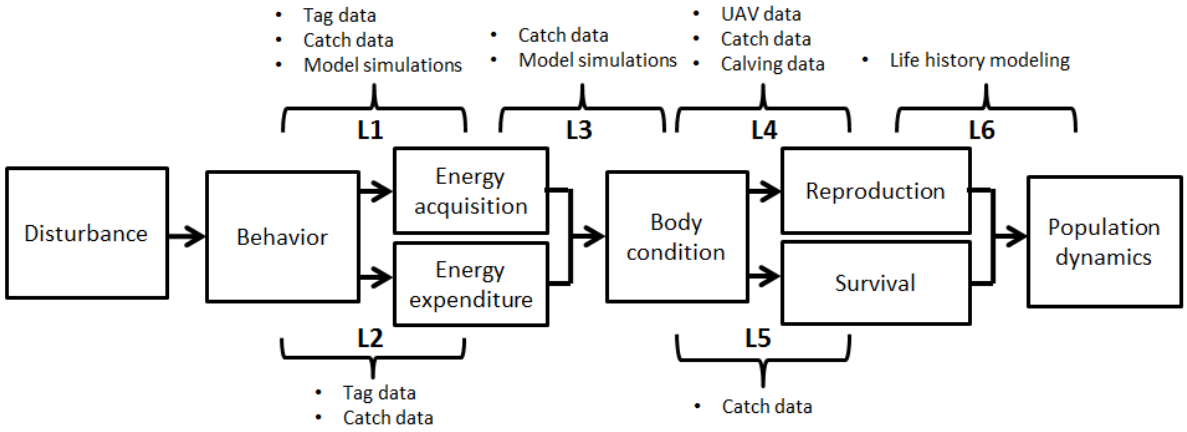

Fig. 1. *The proposed mechanistic model to assess population consequences of disturbance (PCoD) on Southern right whales. The solid lines show the links (L) in our mechanistic model, and the text above/below each link specifies the empirical data that will be used to inform the link.*

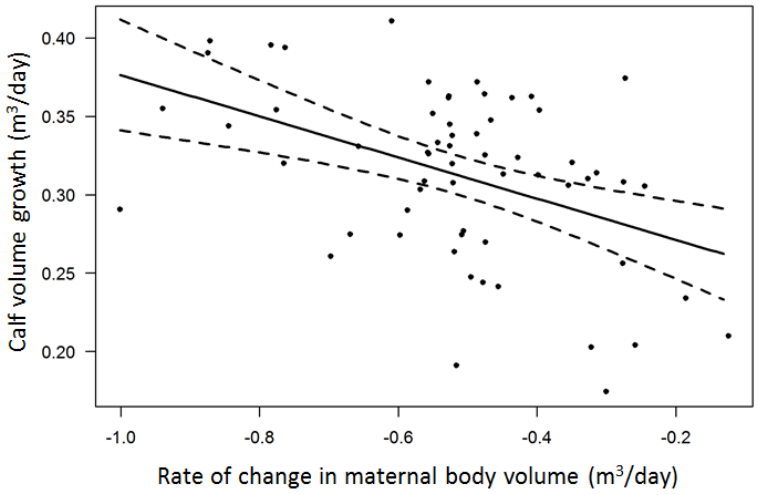

Fig. 2. *Relationship between calf growth rate in body volume and maternal rate of change in body condition (i.e. volume). n=63 whales.*

Linking deep-water prey fields with odontocete population structure and behavior

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Background

Beaked whales are known to feed in the Tongue of the Ocean (TOTO), Bahamas. These species, appear to be particularly sensitive to mid-frequency sonar based on both observations and experimental investigation and a number of mass strandings have occurred coincident with naval exercises. Recent experimental and observational work on beaked whales has demonstrated clear behavioral responses, primarily avoidance of sound sources, in these species, but has also shown that they continue to inhabit areas with regular sonar disturbance. Field studies in TOTO have shown consistent, spatially heterogeneous habitat use within the Tongue of the Ocean. Prey is a likely driver of these predictable patterns. However, while previous efforts in this area have assessed the integrated biomass of prey, this work was limited only to depths shallower than the active foraging depths of beaked whales. Our efforts coupling deep and shallow water sampling of squid on the Navy's SCORE range off San Clemente Island in southern California have shown that integrated prey biomass using conventional approaches focusing only on the upper water column may be misleading in terms of the quantity and accessibility of beaked whale resources. We sought to leverage new technology funded by DoD to describe deep-water prey fields and link these descriptions with existing extensive data on biology, behavior, and population demographics.

Objectives

We sought to obtain direct measurements of the prey environment for beaked whales to provide a critical, and previously unavailable, mechanistic link for understanding beaked whale ecology in the Tongue of the Ocean, Bahamas. These data will provide key contextual environmental data for a relatively well-studied research site and species of interest and the ability to integrate such data into estimates as well as quantitative predictions for the potential consequences of disturbance from human activities, including Navy operations.

Methods

Between July 2 and 14, 2015, active acoustic sampling integrated into a deepwater AUV was combined with ship-based acoustic sampling to provide measures of potential beaked whale prey throughout the water column, ground-truthed with net tows. Beaked whale habitat use was quantified using a combination of visual observations from the ship using 15 X 80 Fijinon binoculars and passive acoustic sensors, including archival recorders affixed to the AUV, a listening array towed from the ship, and using the AUTEC range hydrophone array. The sampling design was informed by historical patterns of habitat use from extensive long-term studies in the same area (Claridge et al, Moretti et al).

Results

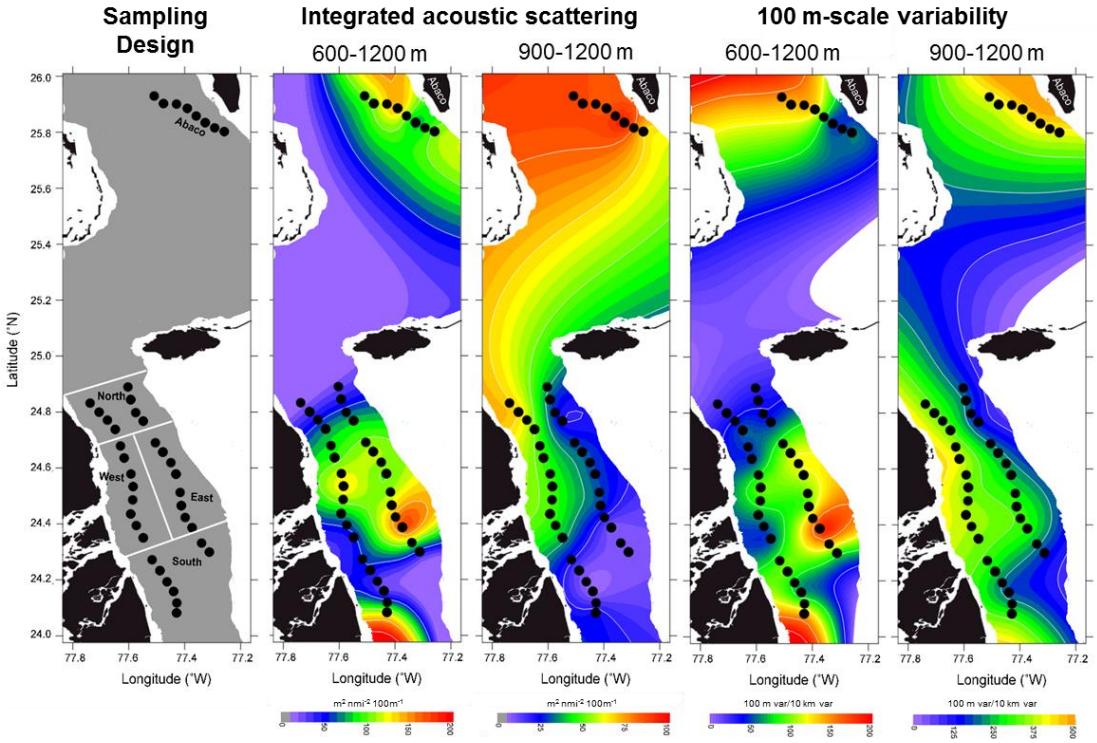
Our sampling was designed to be able to test hypotheses about food availability in various sections of the AUTEC range, the potential costs of animals leaving the range during Naval activities, and to assess differences from the Abaco area known to have a population of larger animals. Sampling utilized a blocked design to examine prey as a function of historical habitat use by beaked whales. Sampling included four, 30 km total length v-shaped transects in each of 5 zones, a low use on range habitat (East), a high on range habitat (West), two areas where displacement occurs immediately adjacent to the range (North and South), and an area with different beaked whale demographics but undescribed differences in prey (Abaco).

We were able to complete all 20 of the planned sampling Vs, resulting in 40 statistical units divided evenly amongst 5 treatment blocks. We found significant differences between the two major regions we sampled, Abaco and AUTEC, within the Tongue of the Ocean (TOTO). First, these habitats are different in their physical setting. While Abaco showed a generally consistent profile of salinity in the upper ~400 meters which slowly decreased at deeper depths the TOTO sites had a lens of high salinity water centered around 150 m. Due to the high evaporation rates and solar heating during summer months in the Bahamas, density instabilities can be generated. Abaco behaves as expected for an open-ocean system and these instabilities are freely re-equilibrated by large-scale oceanographic circulations involving vertical and lateral flow. However, TOTO, bounded on all sides by land or shallow waters acts as a semi-isolated system. Throughout the food chain, biotic features were significantly deeper at Abaco than in the TOTO sites, beginning with the subsurface chlorophyll max, including midwater scattering layers, and the peak depth of larger animals consistent with squid. In addition, animals ranging from krill and bristlemouth fish larvae to deepwater acoustic targets identified as squid were significantly larger at Abaco. Migrating animals at Abaco moved over a greater vertical range each night and scattering layers and had a higher overall biomass. We observed indications of stronger coupling between productive surface waters and

meso- and bathy-pelagic ecosystems in Abaco than TOTO. Differences between Abaco and TOTO suggest that Abaco may support a more energy-efficient food web.

Within the AUTEC range, significant differences were observed in both mesopelagic and bathypelagic resources. However, total biomass and estimated squid size did not vary within the TOTO regions. To examine the potential drivers of habitat use in beaked whales, we included a variety of environmental and prey metrics as independent variables in a discriminant function analysis with sampling zone as the grouping variable. All of the variables in the first function, together explaining 78.5% of the variance, included, described prey within the depth range that beaked whales have been observed foraging. The most important were the density of 'squid' targets and the patchiness of 'squid' targets at 100-m scales. Abaco was most similar to samples from the highly used West zone, suggesting prey features in both regions drive the consistent patterns in beaked whale foraging observed throughout the region. Integration of these results into ongoing efforts to understand and model the resident populations in these two regions is ongoing.

Notes: Collaborators include Mark Moline (University of Delaware), Brandon Southall (SEA), Diane Claridge (BMMRO), and David Moretti (NUWC)



The midpoint of each sampling unit surround is shown in the left-most panel with white lines delineating the pre-planned sampling zones. Prey metrics measured from the echosounder-equipped AUV are mapped in the remaining panels using a minimum curvature interpolations.

The diet composition of beaked whales and melon-headed whales from the North Pacific

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Background

Knowledge of the diet of a species is crucial for understanding it's behavior and ecology, and also has relevance to assessing the impact of potential changes in behavior or spatial use that may be associated with anthropogenic activities. Assessing diet for many species of cetaceans is difficult, given that most foraging occurs far below the surface and that stomach contents of stranded animals are rarely available. Very little information on food habits of most species of beaked whales or of melon-headed whales (*Peponocephala electra*) is available from any region of the world.

Objectives

The goals of this project are to describe the diet composition of beaked whales and melon-headed whales in the North Pacific. Prey remains obtained from the stomach contents of 16 Cuvier's beaked whales stranded or bycaught from across the North Pacific and from ten stranded melon-headed whales in Hawaii were examined. Objectives include prey identification for each prey item to the species level, determining size and mass estimates of individual prey items when possible, and the analysis of prey contribution to whale species diet by both abundance and mass. This allows for a detailed description of the diet composition of beaked whales and melon-headed whales and provides insight into the foraging behavior and ecology of these whales in the North Pacific.

Methods

Stranded or bycaught beaked whales and melon-headed whales were identified to species based on body length, coloration, and/or head shape. Stomach contents were collected during necropsy and initially frozen and later thawed. Stomach contents were curated at the Marine Mammal Laboratory (MML) at the Alaska Fisheries Science Center, Hawai'i Pacific University, the Natural History Museum of Los Angeles County, the Santa Barbara Museum of Natural History, or at the Smithsonian Institution. Following thawing, invertebrate remains were then preserved in 70% ethanol for longer term storage or immediately processed for sorting. Stomach contents were rinsed through a progression of sieves with decreasing mesh sizes of 1.4 mm, 0.94 mm and 0.50 mm. After sorting, cephalopod beaks and fish bones were preserved in 70% ethanol. Fish otoliths were stored dry in gelatin capsules. All remains were identified to the lowest possible taxon using the private reference collection of W.A. Walker and the fish bone, otolith and cephalopod beak reference collections housed at the MML. A voucher series of select beaks and otoliths representing each prey taxon were removed from the individual stomach samples and incorporated into the MML reference collections.

The total number of each species of cephalopod was estimated as the number of lower beaks present. The total number of each fish species was estimated based on the greater number of left and right otoliths. In some instances the number of fish prey was estimated based on the greater number of left or right paired cranial bones. Crustacean abundance was estimated using the number of individual carapace remains in each stomach or the greater number of anterior or posterior carapace portions in the case of partial remains. All prey measurements were obtained to the nearest 0.1 mm using an optical micrometer or Vernier calipers in the case of larger remains. Dorsal mantle length and total weights were estimated by measuring lower beak rostral length for the cephalopod decapods and lower beak hood length for the cephalopod Vampyromorpha and Octopoda. Fish otoliths and diagnostic bones were measured for fish prey and carapace remains for shrimp. Appropriate regression equations were then applied to estimate prey size and weight.

Results

The melon-headed whale stomachs contained a total of 728 food items ranging from 1-205 items per stomach. Six contained only cephalopods and four contained both cephalopods and fishes. Relative frequency by number indicated that fishes comprise 23.8% of the diet by number. Cephalopods were found in 100% of the stomachs examined and contribute 76.2% of the diet by number. However, when estimating prey contribution by mass, 86.0% is comprised of cephalopods and only 14.0% of fishes. Fish remains represented 9 families and 25 species. Myctophid lanternfishes were the most abundant making up 17.6% of the total prey by number but only 6.6% by mass. A total of 555 lower beaks were identified representing 15 families and at least 25 species of cephalopods. The highest contribution of cephalopod prey by weight was represented by the Enoploteuthidae family (28.0%). Enoploteuthid squid were present in eight of the ten stomachs examined and represented 34.8% of the prey contribution by number. The Cranchiidae family was represented in three of the ten stomachs examined. Cranchiidae accounted for 12.0% of the prey contribution by number and 8.0% by mass. The Cycloteuthidae family represented 13.5% of the prey contribution by mass and 6.9% by number and was represented in half of the stomachs examined. A single diamond squid, *Thysanoteuthis rhombus*, was found in one of

the melon-headed whale stomachs and accounted for 5.9% of the total prey mass. With the exception of the Enoplotheuthidae and Onychoteuthidae families, no other cephalopod families were represented in more than half of the stomachs examined.

The 16 Cuvier's beaked whale stomachs contained a total of 11,441 food items ranging from 1-7,997 items per stomach (median = 61). All stomachs examined contained cephalopod remains. Fishes were present in three of the 16 stomachs (18.9%) and crustacean remains were present in five (31.3%). The one individual with 7,997 prey items, an adult male from California, had remains of cephalopods (20 species from 10 families) and fishes in the stomach. Overall, the diet composition of cephalopods, fish and crustaceans varied when considering the contribution by prey number as compared to prey mass. Cephalopods represented 98.0% of the diet by number and 87.7% by mass. Fishes represented only 1.1% of the diet of the whales by number but 12.1% by mass. Despite being present in 31.3% of the stomachs, crustaceans only represented 1.5% of the diet by number and 0.3% by mass.

Fish remains found among the Cuvier's beaked whale stomachs represented five different species, with each species representing a different family. Four of the five fish species identified were represented by only an individual specimen in one of the stomachs. The giant grenadier (*Albatrossia pectoralis*), from the family Macrouridae, was present in high abundance in one specimen which accounted for virtually all of the fish prey contribution by mass (12.1%) and by number (1.1%). Crustacean remains were present in five (31.3%) of the stomachs, but only represented a small contribution by both prey number (1.5%) and mass (0.3%). Two families of crustacean were present (Oplophoridae and Pasiphaeidae), representing at least three different species with a greater contribution of Pasiphaeidae by number (0.9%) and mass (0.2%). Cephalopods were found in all of the stomachs and represented the highest contribution to the diet both by number (98.0%) and mass (87.7%). A total of 11,136 lower beaks were identified that represented 16 families and at least 37 different species. The families Cranchiidae, Gonatidae and Octopoteuthidae were present in over half of the stomachs and were substantial in their contributions to the whales' diet both by number and mass. Nine of the species of the family Gonatidae had the greatest contribution by mass (40.4%) and also contributed significantly by number (26.4%). The highest contribution to the diet by number was represented by the family Cranchiidae (27.2%), with *Taonius borealis* comprising 21.3% by number. Cranchiidae contributed less to the diet when considered by prey mass (10.7%) with *T. borealis* also contributing most to the diet by mass (8.7%). The family Octopoteuthidae, represented by 3,086 specimens of a single species, *Octopoteuthis deletron*, contributed 27.0% by number and 20.2% by mass. Although only present in three stomachs, the family Vampyroteuthidae, represented by the vampire squid, *Vampyroteuthis infernalis,* contributed 14.2% by number and 12.4% by mass. The other 12 families of cephalopods represented in the stomachs contributed less than 5% to the diet by number or mass

Notes:

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Day 3: Wednesday March 22, 2017

2017 ONR

Marine Mammal & Biology

Program Review

Comparing the foraging efficiency of beaked whales on and off naval ranges

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Background

Beaked whales respond to naval sonar by displacement away from the sonar and by disruption of foraging behavior. Less is known about longer-term responses of beaked whales in areas where sonar is used, which is necessary assess the potential population impacts on protected marine mammals.

The purpose of this project is to evaluate whether beaked whales show chronic changes in foraging behavior in a disturbance site even when the disturbance is not present. We study this by comparing the detailed energetics of foraging behavior of beaked whales in the disturbance site to a comparison site where disturbance is rare. Foraging energetics was selected because observed responses affect foraging and successful PCAD models have been based on energetics. We ultimately aim to test whether observed changes in foraging may be linked to biologically significant reductions in body condition or population health.

This project represents collaboration between the University of St Andrews, Bahamas Marine Mammal Research Organisation, Woods Hole Oceanographic Institution and the Naval Undersea Warfare Center.

Objectives

The objective of this project is to improve the understanding of chronic disturbance responses of Blainville's beaked whales (*Mesoplodon densirostris*) to sonar by comparing foraging efficiency and performance of animals at a site where naval sonar exercises are frequent to sites where naval sonar exposure is uncommon.

This project complements ongoing behavioral response studies which assess acute responses of cetaceans to exposure to Navy sonar. The combined results of these studies will provide greater power for assessing the importance of both acute and chronic responses of cetaceans exposed to sonar.

Methods

This study is designed to compare foraging behavior of Blainville's beaked whales at the Atlantic Undersea Test and Evaluation Center (AUTEC) range in the Bahamas, where sonar activities are frequent, to foraging at displacement sites near AUTEC and at a comparison site off south Abaco Island (SA, 170 km away), where whales are not regularly exposed to sonar. A 7-m rigid-hulled inflatable boat (RHIB) was employed as the principle survey and tagging vessel. At AUTEC, a 13-m sailboat was also used as the focal follow and tag recovery vessel.

Whale Tag - Data on foraging performance and efficiency are collected via the deployment of DTAGs, which record acoustic and fine-scale 3-dimensional movement data. The DTAG is attached to whales with 4 suction cups, using a 7-m hand-held carbon fiber pole. The tag contains a VHF transmitter used to track the tagged whale during deployment and to retrieve the tag after release. DTAGs record stereo sound at the whale as well as depth, 3-axis accelerometer and magnetometer information. DTAG audio was sampled at 192 kHz and other sensors at 50 Hz, allowing for a fine scale reconstruction of whale behavior. The acceleration data can be used to estimate relative fluking effort during foraging, and the acoustic data record echolocation-based search, and attempts to capture prey are marked by rapid accelerations of clicks called buzzes (Johnson et al. 2004).

High-resolution images were collected of each whale and the natural variation in dorsal fin shape and body scarring patterns were used to identify individual animals and compare to existing photo-id catalogs of individuals at each field site.

Results

Three Blainville's beaked whale tagging cruises have been conducted: two at SA in 2015 and 2016 and one cruise at AUTEC in 2016. These efforts resulted in 30 workable vessel days, during which 2599 km were covered over a period of 226.5 hours.

In SA, groups of Blainville's beaked whales, ranging in size from 1 to 8 (mean 3.6), were observed on 15 occasions. Detailed observations were made totaling 63 hours, during which 2281 identification images of 24 individuals were taken, 21 of which were previously observed and photographically catalogued by BMMRO. At AUTEC, 4 sightings of Blainville's beaked whale were made and groups contained either one or two individuals (mean 1.8). The AUTEC whales were observed for 6.9 hours and 634 photo-ID images were collected of 5 different individuals, 3 of which had been previously catalogued by BMMRO. Blainville's beaked whale sightings in SA were typically made near the 1000 m isobaths, whereas at AUTEC sightings occurred beyond the 1000 m isobath, near the center of the Tongue of the Ocean.

Over the 2015 and 2016 field efforts, eleven Blainville's beaked whales were successfully DTAG'ed. Nine deployments were made in SA (3

adult males, 3 adult females, 2 sub-adult males and 1 sub-adult female) and 2 at AUTEC (both sub-adults, one male and one of unknown sex). Of the 11 whales, one AUTEC and 8 SA animals were individuals with known life histories, adding to the depth and value of the data collected. Body condition data were also collected for 3 of the tagged whales two weeks later during a photogrammetry study in SA. At SA a total of 104.6 hours of DTAG data have been collected, with deployments lasting a maximum of 18.5 hours. At AUTEC 20 hours of data were collected in 2016, which when pooled with 6 DTAG datasets collected at AUTEC between 2006 and 2008 yields a total of 118.5 hours of DTAG data from AUTEC.

Between Site Variation – Two high quality comparable datasets now exist for the SA and AUTEC field sites; foraging data from 9 different individuals tagged in SA in 2015 and 2016 is being compared to similar data collected from 8 different animals between 2006 and 2016 at AUTEC. Preliminary data from the two locations are summarized in Table 1. The combination of parameters presented suggest slightly lower foraging rates at AUTEC, but it is not yet clear whether these differences are significant. To best represent the data and establish whether statistically significant differences in the recorded foraging parameters exist between the study sites, detailed comparative analyses will be undertaken (e.g. Johnson et al. 2008).

Variation in Foraging Between Age Class, Sex and Reproductive Status – Preliminary analysis suggests some variation in the foraging behavior of different sexes, age classes, and reproductive states. Subtle differences in foraging between age classes and study sites if stable over years may influence the reproductive success of different sub-populations. Where anthropogenic disturbance causes chronic changes in foraging, these subtle differences may impact population dynamics. Detailed analysis of the foraging parameters summarized here is required and will be incorporated into models to estimate population-level effects as part of this study.

This study has successfully collected a wealth of typically difficult to acquire data using a cost effective, small team approach.
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Notes: Table 1. Foraging dive and prey capture attempt summaries for the 17 Blainville's beaked whales tagged in the Bahamas between 2006 and 2016. The data presented are mean values for each foraging parameter, with standard deviations in parentheses.

Study Site	N	Dive Duration (min)	Max. Dive Depth (m)	Click Duration (min)	# of Buzzes	Buzz/Min	Buzz Depth	Inter Dive Interval (min)
South Abaco	9 animals 47 dives	49.5 (5.2)	1011.6 (195.5)	29.8 (5.8)	60 (24.7)	2.0 (0.7)	900.7 (123.7)	135.3 (40.5)
AUTEC	8 animals 39 dives	52.9 (7.8)	990.0 (192.8)	31.1 (6.3)	54.5 (11.3)	1.8 (0.5)	892.7 (119.2)	155.4 (63.3)

References

Johnson, M., Madsen, P. T., Zimmer, W. M. X., de Soto, N. A. & Tyack, P. L. (2004). Beaked whales echolocate on prey. Proceedings of the Royal Society, Biological Sciences, 271 Suppl., S383–6.

Johnson, M., Hickmott, L.S., Soto, N.A. and Madsen, P.T. (2008). Echolocation behaviour adapted to prey in foraging Blainville's beaked whale (*Mesoplodon densirostris*). Proceedings of the Royal Society of London B: Biological Sciences, 275, 133-139.

Foraging behavior of beaked and other deep diving odontocetes in the Kona coast of Hawaii Island

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Background

The goal of our research is to understand the foraging behavior of deep diving odontocetes including beaked, sperm and pilot whales and Risso's dolphin, so that acoustic encounters with Navy assets can be minimized. Understanding of the characteristics and dynamics of the prey field is critical in understanding the foraging behavior and life cycle of odontocetes. To understand foraging behavior of the prey, the oceanographic conditions affecting the presence of the prey and how the whales interact with the prey field all needs to be better under understood.

Objectives

1 Obtain an understanding of the biomass environment in which deep diving odonto- cetes are foraging on.
2 Determine the pattern of foraging for deep diving odontocetes on the Kona coast line.
3 Demonstrate the utility of the DIDSON high-resolution sonar in obtaining biomass images at depth commensurate with typical foraging depth of deep diving odontocetes.

Methods

We used three main tools to determine the distribution and abundance of the prey field,
understand the relation-ship between deep diving whales and the prey field, and understand how these whales interact with the prey field.

The first tool consisted of EARs, three were deployed for about a year was to in the Kona study area. The EARs operated at 80 kHz and deploted at depth up to 1 km.

The second tool consisted of EK-60 scientific echosounders operating at 38 and 70 kHz. Volume backscatter surveys were performed along and across slope along the steep island bottom. The acoustic volume scattering along the survey tracks will be related to density estimate obtained with the presence of foraging deep diving dolphins and with results of profiler discussed in the next paragraph.

The third tool consisted of a specially fabri- cated profiler to investigate the composition, density and the characteristics of the micro- nekton in the deep layers that deep diving odontocetes forage on. The DIDSON high resolution imaging sonar and a low-light video camera system are used with the profiler.

In order to perform the necessary field work we have collaborated with two different organiza- tions which allowed us to maximize the effectiveness of our field work, to obtain the use ancillary data, to expand our study area and stretch our financial resources. This method has allow us to obtain a more thorough and comprehensive understanding of foraging behavior of our targeted species. We have collaborated with NOAA (Pacific Island Fishery Science Center) cruises in the Northwest Hawaiian Island National Monument (Papahanaumokuakea Marine National Monument, and off the Kona coast of the island of Hawaii. We have collaborated with Schmidt Oceanographic Institute to conduct a cruise off the Kona coast of the island of Hawaii. We have also performed our own research off the Kona coast on two field trips using a relatively small fishing vessel (38 ft) that was modify to support the EK-60 echosounder transducers and had a fast pinch grabber winches to handle the DIDSON profiler and to deploy EARs

Results

In the waters of Kona pilot whales are most prevalent in locations where the isobaths was between 1000 and 2500 km and between 250 and 2000 m for Blainville's beaked whales. The key oceanographic parameters characterizing the foraging regions were bathymetry, temperature at depth, and a high density of midwater micronekton layer (the deep scattering layer) which probably serve as prey for the prey of the whales. The micronekton of the deep scatter layer off the Kona coast of Hawaii Island and potentially around other main Hawaiian Islands is critical in sustaining the deep diving odontocete populations.

Hotspots of biomass determined from the EK-60 were found around the different islands and atolls in the NWHI. Deep diving odontocetes were often spotted foraging around these hotspots, but not always. Where odontocetes were detected, a hotspot of biomass was almost always detected. There were also hotspots at which no odontocetes were detected visually or acoustically.

Two broad scattering layers were found: a deep layer from 370 to 670 m and a shallow layer from 0 to 270 m. The highest densities of both deep and shallow scattering organisms were associated with deep slopes of banks and atolls. Beaked and short-finned pilot whale sightings occurred in locations of high scattering density associated with slopes of atolls and banks.

EAR data suggests that temporal variation in foraging activity was species-specific beaked whales foraged more at night in the north, and more during the

daytime off Kalua-Kona. No daytime/nighttime preference was found in the southern end of the sampling range. Sperm whales foraged mainly at night in the north, but no daytime/nighttime preference was observed off Kona and in the south. A generalized linear model (GLM) was then applied to assess whether location and chlorophyll concentration affected the foraging activity of each species. Chlorophyll concentration and location influenced the foraging activity of both these species of deep diving odontocetes.

EAR data revealed that foraging was the highest in the north and the lowest in the south. Interestingly, the foraging activity decreased at all locations from the beginning of the study up to the 12^{th} month of the study. After the 12^{th} month, the foraging activity started to increase again. It seems that some odontocete species do not stay associated with the island of Hawaii all year round, causing the overall foraging activity to decrease and to increase again when they come back to the island.

Estimate of micronekton densities ranging from 1 to 6 animals/m^3 and individuals as long as 3 m were detected. These densities were orders of magnitude higher than those estimated from trawls and average sizes of animals were much larger as well. A mixed model was used to characterize density and length of animals as a function of depth, location, time of day, and month. Location and month varied with both the density and length of animals, while depth only varied with the density. The DIDSON proved to be a good tool for open ocean / deep sea estimation of density and size of marine animals.

Higher average monthly and yearly satellite surface chlorophyll-a levels were consistent with the spatial variability of acoustic abundance. These results were further validated by the spatial orientation of the "hotspots" found strictly around emergent land masses and deep banks. The amount of backscatter and organism composition also varied temporally, but the offshore site seemed to have less variability than locations closer to shore.

DIDSON casts showed prey densities and size were higher when whales were present. However, whales were also absent at high density locations (similar for hotspot). Moreover, whale's foraging seems not to be influenced by the overall size of the potential prey except for sperm whales. When sperm whales are foraging, there were always a higher number of potential prey bigger than 20 cm. A total of 7068 animals were counted and sized. We estimated densities ranging from 1 to 7 animals/m^3 and individuals as long as 3 m were detected. These densities were orders of magnitude higher than those estimated from trawls and average sizes of animals were much larger as well. A mixed model was used to characterize density and length of animals as a function of depth, location, time of day, and month. Density and length of animals varied with location and month, with density also a function of depth of micronekton, especially larger ones.

DIDSON densities and EK-60 densities are not linearly correlated. More work is being done to establish the relationship between DIDSON and EK-60 measurements.

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Notes:

Finalizing the Dtag: implementation and testing of design improvements for reliability and availability

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Background

In this project we implemented design changes to improve reliability and manufacturability of the Dtag, a sound recording multi-sensor tag. Additionally, we established a lease pool to provide the community with access to tags at a moderate cost per project while also allowing for monitoring of tag field performance. Feedback from performance in the field will be being used to continuously monitor and improve the tag design.

Objectives

Dtags have proven to be a valuable tool for the study of marine mammal acoustics and fine scale motion. The success of the Dtag has resulted in an increased demand for the instrument from researchers both within the Navy and the marine mammal community. However, the previous Dtag (DTAG3 V1.0) design had cost, robustness, and reliability issues that made it unsuitable for the scaled-up manufacturing necessary to meet demand. To address this, we **A)** improved reliability and manufacturability of the tag, **B)** evaluated the new design by fabricating a small number of proto-type tags for testing, and **C)** implemented these design changes to create a pool of field-ready and cost-effective Dtags available to marine mammal researchers. A fourth goal, **D)** the design and fabrication of a robust stand-alone archival sensing unit for an ARTS compatible tag system, was added during the execution of the project.

Methods

The objective of the DTAG3 V1.0 design was to expand the capabilities of the Dtag-2 (longer duration, wider bandwidth, support for more sensors) while decreasing the size of the instrument to make it suitable for smaller delphinids. The new design was also needed because Dtag-2 components had become obsolete. To meet the challenge of reducing size while increasing capabilities, we explored several different packaging methods. Unfortunately, some of these new compact fabrication methods decreased the robustness of the system leading to tag failures in the field. This project involves four tasks to be performed over the course of two years. They are: **1)** implementation of design changes to address tag failure modes; **2)** improved design for manufacture; **3)** a small test build (4 units) of the new Dtags, which will be tested rigorously; and **4)** the fabrication of a quantity of the revised tags for use by the marine mammal science community via a lease pool. The development of this pool will alleviate the current Dtag availability bottleneck while providing a mechanism to incorporate experience from the field about long-term reliability of the tags.

Results

Fabrication and implementation of the Tag Pool: To date we have fabricated three DTAG3x-Prototype tags, four DTAG3-Core units, and six DTAG3x V1.0 tags, Figure 1. These tags are avalible for the field work using the month-to-month leasing model outlined in the proposal. Feedback from the field deployments will be used to revise the future designs, and funds from the lease will be used to fabricate additional tags for the pool. We are currently planning on building eight more DTAG3x V1.0 tags by the end of the year and will have 15 tags avalible to support field efforts in 2018. The goal of the lease program is to become self-sustaining. To this end demand for tags and feedback from from the community will be used to refine the business model to achieve the duel goal of providing affortable tags to the community while achieving sustainability.

DTAG3 Core Units: Along with the design, testing, and fabrication of the robust and

manufacturable DTAG (the DTAG3x) we worked successfully with Dr. Patrick Miller and colleagues at the University of St. Andrews to design a separate tag system that will be compatible with the Aerial Rocket Tag System (ARTS). While the design of the DTAG3x V1.0 has been focused on the integration of tag components to optimize packaging efficiency and reduce the overall tag size, the design of the DTAG3-Core was focused on the creation of a robust stand-alone archival sensing unit that could be deployed in the marine environment. This work was motivated by the need to develop a DTAG3 compatible system for the ARTS pneumatic launching system for tag deployment in certain field situations. The DTAG3-Cores are designed around the DTAGx V1.0 electronics, and leverage many DTAG3x V1.0 design elements including the connector, release and housing. As with the DTAG3x V1.0, a low viscosity epoxy casting resin together with vacuum investment was used for electronics encapsulation. Dr. Miller and his team designed an alternative housing system for the Core units. The housing system included the floatation, VHF transmitter, suction cups, and the interface with the ARTS system.

Field Testing: Field trials have been conducted with both Core units and DTAG3x-Prototype tags (Figure 1) built using the design concepts at two field sites: off the coast of Norway tagging sperm whales with the DTAG3x-Prototype tags and around Jan Mayen with the Core units focused on bottlenose whales. Additional field work was conducted off the coast of southern California as part of the SOCAL-BRS project. During the SOCAL work the DTAG3x-Prototype tags were used to tag Risso's dolphins, fin whales, and blue whales. The tags successfully collected data during all trials, and no tags were lost during the experimental work. However, the researchers reported occasional connectivity issues with the USB connector and the VHF antenna core was broken on tags during both experiments. These issues with early prototypes have been addressed in the final tag design and will be implemented with the remaining pool tags that are currently being fabricated. A database containing details of problems identified in the field will be used to continually improve the design of the DTAG and monitor resulting performance.

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Figures

Figure 1 A picture of one of the DTAG3x-Prototype that was successfully fielded this past summer.

Improving Large Cetacean Implantable Satellite Tag Designs to Maximize Tag Robustness and Minimize Health Effects to Individual Animals

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Background

Satellite tracking of large cetaceans is often conducted with intramuscular tags, raising concerns regarding their potential to cause long-term health effects to tagged individuals due to shearing forces at the blubber-muscle interface (Moore et al. 2013). Also, tag design flaws seen in recent tagging follow-up studies with humpback whales (*Megaptera novaeangliae*) (Robbins et al. 2016) may explain their relatively short and variable duration and strengthen the need to develop new, more robust tags.

Objectives

The specific objectives of this project are as follows:

1) Design, build, and test robust blubber and/or muscle penetrating tags, which will (a) resolve structural limitations of existing designs and (b) minimize tissue trauma while extending retention time;
2) Evaluate structural integrity of designs created in Objective (1) during laboratory experiments and in cetacean carcasses;
3) Examine structural tissue damage in the blubber, sub-dermal sheath and muscle caused by penetrating dummy implantable tags in cetacean carcasses, including manipulation to simulate live motion;
4) Assess performance of new tag designs in populations of large cetaceans where extensive follow-up studies can be performed (e.g. Gulf of Maine humpback whales)

Methods

Experiments to evaluate structural tissue damage near the muscle-blubber interface were conducted on cadavers of common dolphins (*Delphinus delphis*) in a laboratory setting. The extent of the mobility of the blubber over the muscle during the locomotory cycle was measured by inserting 17 gauge needles (1.5 mm diameter) on the midline anterior to the dorsal fin and dorso-laterally in two rows at sites equivalent to potential tag implant sites from anterior to posterior of the dorsal fin (Fig. 1). The needles were inserted in diads or triads, having one penetrating the blubber only and the other one/two through the blubber and at different depths (2 and 4 cm) into the muscle. The angle of penetration was measured for each needle using a protractor (Fig. 1), with the animal in the relaxed, dorsal flexed and ventral flexed positions. The change in angle for each needle was calculated between dorsal and ventral flexion. In addition, scaled dummy tags were inserted in dolphin cadavers and the animal was cyclically flexed dorsally and ventrally in a custom made oscillator.

In light of the design flaws observed during a follow-up study with humpback whales, modifications to an existing tag design (Gales et al. 2009) were introduced to improve tag robustness. These included elimination of an articulated anchoring system, integration of the anchor-transmitter interface, and redesign of the posterior end of the satellite transmitter, resulting in a new design (Mold 303B, Fig. 2). Laboratory experiments were conducted to further evaluate additional changes in this design, including the performance of the tag tip and of the retention devices in the anchoring system. These resulted in an alternate tag design (Mold 303E, Fig. 2).

The performance of these new tags were assessed through penetration and impact tests of miniature or full-size dummy tags in a controlled setting and through the deployment of actual tags in free-ranging humpback whales in the Gulf of Maine.

Results

Mobility of the blubber over muscle in the common dolphin experiments showed marked positional variation. Mean shearing ranged from 0.1 to 3.5 cm, with the greatest adjacent and caudal to the dorsal fin. In addition, needles at the more caudal sites were observed to bend at the blubber muscle interface with flexion. With the oscillator, there was variable gross trauma evident in the muscle around the tag tips. A minority of retention flaps in the scaled dummy tags were disconnected at the hinge in the caudal stations, which is also suggestive of greater shearing at those locations. In addition, detachment of these flaps suggested that similar features in full size tags may break when placed underneath the muscle-blubber interface, leaving tag parts into the whale's body after the tag is shed. This motivated some of the changes in design of the retention devices performed in this study.

If shearing documented in common dolphins is comparable in large whales, divots and regional swellings observed with intramuscular tags are likely the result of tissue loss and repair, respectively. Placing tags para-sagittally anterior to the dorsal fin

would cause the least trauma, but the potential pain caused by these tags remains a concern.

Changes to correct previously observed design flaws were implemented in tag molds 303B and 303E (including the removal of the articulated anchor and the integration of the anchor/transmitter interface). These resulted in significant greater tag transmission durations and reduced tissue responses related to tag implantation (Robbins et al., 2016). Yet, breakage was occasionally documented at the welding joint designed to replace the previous mechanical anchor/transmitter interface. Manufacturing the tag housing as a single unit using a process known as Direct Metal Laser Sintering (DMLS) has shown to be a more robust approach in a laboratory setting. Deployments of actual tags built with this method are expected to occur in the summer of 2017.

Laboratory experiments demonstrated the three-bladed tip design implemented in Mold 303E was superior to the double tip of Mold 303B as it facilitates tag penetration and reduces likelihood of poor tag implantation. In addition, the three sets of retention petals of Mold 303E are less likely to break in comparison with the actively deployed flap design of Mold 303B.

Preliminary results from deployments of molds 303B and 303E in free ranging whales suggest superior transmission duration of the latter. However, small sample sizes preclude a more robust statistical comparison between the two designs. Deployment of additional tags in the summer of 2017 will provide additional data to assess the performance of these tags.

Bibliographic References

Moore, M., et al. 2013. Rope trauma, sedation, disentanglement, and monitoring-tag associated lesions in a terminally entangled North Atlantic right whale (*Eubalaena glacialis*). Mar. Mamm. Sci. 29:E98-E113.
Gales, N. et al. 2009. Satellite tracking of southbound East Australian humpback whales (*Megaptera novaeangliae*); challenging the feast or famine model for migrating humpback whale. Document SC/61/SH17 presented to the International Whaling Commission Scientific Committee, 12pp.
Robbins, J. et al. 2016. Evaluating Potential Effects of Satellite Tagging in Large Whales: A Case Study with Gulf of Maine humpback whales. Report to the National Fish and Wildlife Foundation, Grant #23318. 100pp.

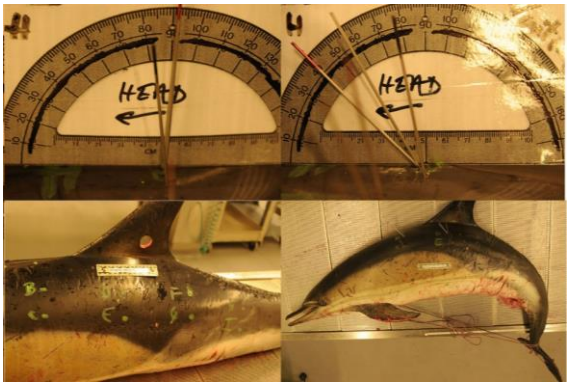

Fig. 1 – Experiments to evaluate tissue displacement (shearing) near the muscle-blubber interface using 17 gauge needles and a protractor (relaxed position on the left, ventral flexion on the right)

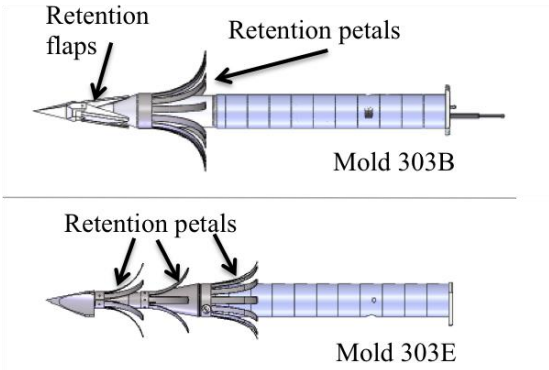

Fig. 2 – Transdermal implantable tag designs Mold 303B and 303E (drawings by Wildlife Computers, Redmond, WA)

Acquisition of oceanographic measurements from baleen whales: Field deployments of tags developed under grant ONR (N00014-13-1-0854)

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Background

It is critical for the understanding of ongoing climatic perturbations in the Arctic that frequent and reliable data on oceanographic regimes are collected. Diving ocean predators can be fitted with instruments that record the animal's location, diving depth, and concurrent oceanographic parameters creating a "real-time autonomous sampling platform" allowing for data collection from remote or ice covered waters where sampling by conventional ship-based techniques or profiling floats is expensive or impossible. Sampling of oceanographic data, including salinity (conductivity), temperature and depth, by use of instrumented marine mammals is an attractive technique that has been used widely with seals and to a lesser extent with narwhals and belugas. The oceanographic data collected by marine mammals can be incorporated into existing oceanographic monitoring of ocean trends and variability or they can be used as proxies for prey availability and habitat preferences, but also for real-time operational purpose where for instance the effects of salinity on sound transmission is important.

Objectives

The objectives of this project are:

•To develop CTD satellite transmitters for baleen whales that can collect and transmit data on location, depth, temperature and salinity at specific depths.
•To develop and test the deployment techniques for two tag designs on bowhead whales, blue whales and/or humpback whales in Greenland and possibly Iceland.
•To evaluate the reliability and quality of CTD data collected in arctic ice covered waters by slow swimming bowhead whales and from the open ocean by fast swimming blue and humpback whales.

Methods

The only CTD tag that have shown accuracy useful in oceanography is the CTD-Satellite Relay Data Logger (CTD-SRDL) developed by Sea Mammal Research Unit, Univeristy of St. Andrews (e.g. Boehme et al. 2009). It uses an inductive cell from Valeport Ltd., which has the advantage of being highly durable, and with little risk of corrosion, the disadvantage is the relatively large size of the sensor and that it is affected by an offset by the object the tag is placed on. This means that the results need to be calibrated, which is very challenging on a large whale. As an alternative approach we have been cooperating with Wildlife Computers (Seattle) to develop an electrode-based conductivity sensor with low power consumption, small size and high resolution (0.001). This has never been done before and has taken three years to develop. In spring 2017 five of these tags will be deployed together with five CTD-SRDL on bowhead whales in Greenland. One whale will carry both tags for comparison of data accuracy and number of CTD casts received. A calibrated SeaBird CTD probe will be lowered at the site of the deployments for subsequent correction for conductivity offset. The attachment and longevity on the whales will also be evaluated. Depending on the outcome of the first field campaign, the Wildlife Computer CTD tag may be modified to ensure that further deployments later in 2017 will give the most accurate data.

The bowhead whales west of Greenland (the so-called Baffin Bay-Davis Strait stock) are primarily attracted to waters of polar origins in Canada but make annual visits to areas in West Greenland affected by warm Atlantic water. Both the strength of the south going polar current along the western part of Baffin Bay and the warm north going side branch of the Gulf current on the eastern part are of importance for the understanding of the coupling between global warming and changes in the circulatory patterns

in the North Atlantic. Using bowhead whales as a relatively inexpensive way of collecting data on temperature and salinity would augment existing sampling regimes and would allow for a more consistent sampling during winter. The bowhead whale is the first target species in this project as they can be approached by boat to a distance where tags can be deployed with a pole system with high precision. Deployment of tags on humpback whales and blue whales will also be attempted to demonstrate the feasibility of the technique on a range of baleen whales in different habitats.

Results

The CTD tag for large cetaceans based on conductivity electrodes has now been developed. It has the resolution and accuracy needed for measuring long-term trends in ocean temperature and salinity as well as real-time input to forecasts and ocean monitoring. The salinity data should also be stable regardless of target species.

Notes:

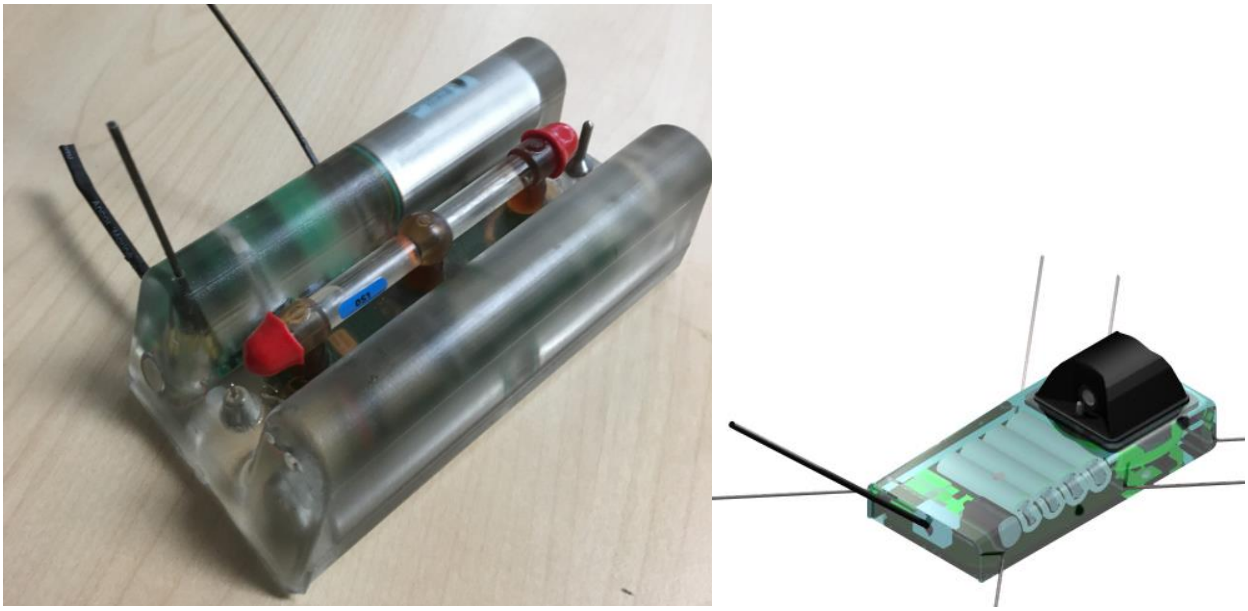

Fig. 1. To the left is the newly developed CTD tag by Wildlife Computers. It has two fast response thermistors in each end of the electrode conductivity sensor in the middle of the tag. The Argos and GPS antennas are seen on the left side of the tag. To the right is a drawing of the redesigned CTD-SRDL tag from Sea Mammal Rearch Unit, University of St. Andrews. The black dome is the Valeport inductive conductivity cell.

References

Boehme, L., Lovell, P., Biuw, M., Roquet, F., Nicholson, J., Thorpe, S.E., Meredith, M.P. and Fedak, M. 2009. Technical Note: Animal-borne CTD-Satellite Relay Data Loggers for real-time oceanographic data collection. Ocean Sci., 5: 685–695.

TrackPlot Enhancements: Support for Multiple Animal Tracks and Gyros

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Background

TrackPlot software has been used by the marine mammal research community to carry out detailed analysis of the kinematics of foraging marine mammals. Prior to this work the software contained no support for gyroscopes which have recently become available in low cost tags. In addition to giving more accurate angular velocity information, gyroscopes provide a means of decomposing acceleration measurements far more accurately than can be accomplished using the widely applied ODBA method. This in turn should enable better estimates of energy expenditure during various phases of foraging.

Objectives

The goal of this project has been to create software to support the visualization and kinematic analysis of marine animal movements derived from archival tag data. Tags are supported that have sensors for pressure, acceleration, magnetics and optionally, rotations. TrackPlot software creates pseudotracks and these may be georeferenced given a set of fixes. With the use of gyros true dynamic accelerations may be computed. This may make it possible to supplant the Overall Dynamic Body Accelerations ODBA metric, which despite its name, measures rotations as much as accelerations. Specific objectives were:

1) Support for the analysis of gyroscope data in combination with accelerometer and magnetometer data.
2) Support for the visualization and kinematic analysis of foraging groups
3) Support for longer tag deployments,
4) Enhanced support for the presentation of results

Method for gyro-related work

In order to determine the extent to which low cost MEMS gyros can be used to calculate true dynamic accelerations, bench tests, using an industrial lathe and field tests, with tagged Steller sea lions, were carried out. Four trained Steller sea lions were equipped with biologging tags swam alongside a boat cruising at steady speeds in the range of 4 to 10 kph. At each speed, and for each dive, Gyro-Informed Dynamic Acceleration (GIDA) was computed using a method incorporating gyroscope data with accelerometer data. This new metric is based on the average gain in speed per flipper stroke divided by mean stroke periodicity. This is called APBA—Averaged Propulsive Body Acceleration.

Method for software development

Software development is carried out using C++ and OpenGL. QT is used for the windows and menus.

Results

A method for combining gyroscope and accelerometer data has been implemented and evaluated for the purpose of estimating dynamic body accelerations.

Bench tests suggest that dynamic body acceleration can be calculated to an accuracy of approximately 0.4 m/s^2. This is adequate to resolve accelerations due to propulsion for a marine mammal of a few hundred kg. When applied to data from tagged Steller sea lions accelerations due to flipper strokes can be clearly identified (Fig. 1). Brief spikes of forward acceleration are interspersed with 2-3 sec of deceleration due to drag. Other results suggest that the widely used ODBA metric greatly overestimates dynamic body accelerations.

However the accuracy of MEMS devices precludes accurate estimation of true dynamic accelerations of larger whales, where accelerations of more than a fraction of a m/s^2 are unlikely.

The TrackPlot package has been modified to provide support for the display of tracks from multiple tagged animals shown simultaneously. Fig. 2. Shows tracks from three animals. Other software enhancements include support for longer tracks, better georeferencing, and support for high resolution images.

Notes:

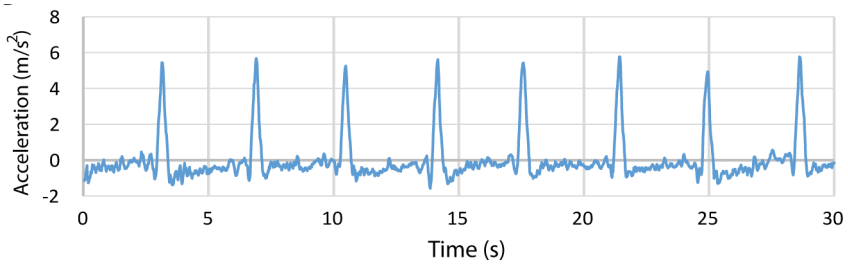

Figure 1. Dynamic forward body accelerations obtained from a tag attached to a horizontally swimming Steller sea lion. The animal was swimming at approximately 6 kph. The results show that forward accelerations approximate results from a theory-based drag calculation.

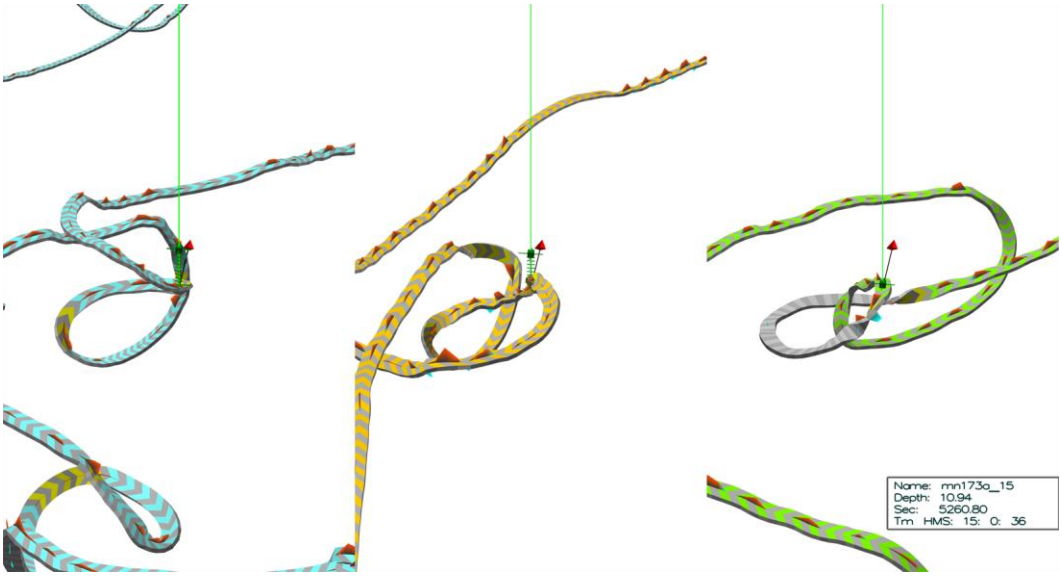

Figure 2. TrackPlot screen shot showing three tracks displayed together. Tracks from three tagged Humpback whales synchronized by time shown during a bubble netting event. Two of the animals were tagged with Acousondes and one was tagged with a CATS tag. All three animals surface within a few seconds. This feature supports the interpretation of group behaviors.

An Investigation of Fin and Blue Whales in the NE Pacific Ocean using Data from Cascadia Initiative Ocean Bottom Seismometers

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Background

Ocean bottom seismometers (OBSs) are increasingly often being deployed in sizeable networks (Fig. 1) for intervals of about a year to monitor earthquakes and image earth structure. Many of these deployments are in regions that are also of interest for baleen whale studies. Because OBSs record the low-frequency calls of fin and blue whales, the seismic data sets provide a unique opportunity to investigate the distribution and behavior of these two species. The long-term goal of this project is to use data from OBSs deployed in the Northeast Pacific Ocean to develop, evaluate and compare techniques to estimate fin and blue whale densities. The expectation is that when fully developed, these techniques can be routinely applied to OBS data sets of interest.

Objectives

This project has the following objectives:

1. Implement established techniques to automatically detect fin and blue whale calls in OBS data from the Cascadia Initiative.
2. Develop an automated method to track blue whales using data from the Cascadia Initiative OBS data set to complement a technique previously developed to track fin whales.
3. Develop a technique to estimate the location and density of fin whales from a single OBS using multipath arrivals and compare this to a method that is based on total received energy with a longer-term goal of comparing both methods to a third technique that uses particle motion. In shallow water where the fin whale multipath arrivals do not overlap, neither the multipath nor particle motion techniques are applicable to estimate ranges, so we plan to explore the use of received call amplitudes.
4. Implement and compare techniques to estimate blue whale densities based on received call amplitudes and total received energy and verify them based on call tracks.

Methods

To detect fin whale calls, we use a matched filter that is tuned to the rate of frequency downsweep of the 20 Hz fin whale call. For blue whales, we use a spectrogram cross-correlation method with the detector tuned to fundamental frequency of the Northeast Pacific blue whale call. Both detectors were verified using data sets of manually identified calls.

The Cascadia Initiative network has an instrument spacing of 70 km in deep water. To track blue whales using a time-difference of arrival method requires using arrival times for stations where the detections have very low amplitudes. Our method starts with a data set of call detections for which the detection threshold has been set low. Each higher-amplitude detection is then used as a master call to apply a multiple-animal time-of-arrival method which finds all the detections that may be associated with the master call. Finally, a Bayesian inversion approach is used to create probability density functions for the whale locations.

The spacing of OBSs in the Cascadia Initiative experiment is too large to track fin whales. Our multipath ranging technique uses both the relative timing and amplitude of whale arrivals to estimate the range to the animal. A fundamental challenge is that it is unknown *a priori* whether the first observed arrival is the direct path or a multipath. We consider three hypotheses in which the first arrival is the direct path, the first multipath, and the second multipath, respectively, and select the hypothesis that provides the best fit to the relative timing and amplitude data. Ranges obtained from a given instrument can then be used to estimate call density using point transect distance sampling.

We are also estimating whale density from total received energy in the specific frequency band of the calls. The central idea behind the method is to use whale calling rate and source level, combined with acoustic propagation modeling, to estimate the amount of acoustic energy that would be received at a given sensor from a whale. Then different densities of whales in an area are simulated, along with distributions of whale vocalization parameters (call rate, source level), in a Monte Carlo model to estimate the total energy received as a function of whale population density. This function is then inverted to achieve a function that maps received level at a sensor to whale population density in the vicinity of the sensor for a given time period.

Results

The blue whale tracking method is consistently able to track call sequences but is less reliable with individual calls. Because the large OBS spacing requires the inclusion of low-amplitude detections, many spurious detections are inevitably included in the analysis and these lead to a significant number of spurious locations. However, the application of a simple sequence filter to identify groups of calls with similar times and locations eliminates nearly all bad locations and resolves tracks. We are in the process of compiling a track data set for the full 4-year duration of the Cascadia Initiative experiment. We are also looking at the patterns of strong detections on individual instruments. These reveal large temporal variations in the spatial distribution of whales in the experiment footprint.

The multipath ranging technique has been evaluated at two contrasting locations, the Endeavour segment of the Juan de Fuca Ridge, with rough basaltic basement, and a flat site in the middle of the Juan de Fuca Plate, with several hundred meters of sediment. Synthetic amplitudes obtained from modeling ray divergence do not fit the data well and we implement a supervised bootstrap method to obtain an amplitude model from the data. At the mid-plate site, reflections from the base of the sediment layer can bias range estimates to smaller values and we implement a method to correct for this effect. At both sites, we infer that we are able to successfully range to all non-overlapping calls within ~4 km of the OBS, and after correcting for the proportion of overlapping calls, we demonstrate that the ranges can be used for density estimation.

One technical challenge for the received energy technique is the conversion of seismometer ground velocity measurements into acoustic pressure levels, as this process requires assumptions about the incidence angle. We have measured received levels in the frequency band of fin whales (15-25 Hz) and estimated whale densities for each two-week period throughout the 2011-2012 deployment, mapping density throughout the network for a year. Blue whale estimation is more challenging because their call frequency range overlaps that of the much more numerous fin whales.

Because density estimation techniques are dependent on the cue rate, we have investigated the characteristics of fin whale calls in the Northeast Pacific over 10 years. We find a significant temporal evolution of call frequency and inter-pulse intervals and some subtle spatial variations. These results have implications both for using calling patterns to identify fin whale populations and for the interpretation of inter-annual variations in call density. In order to constrain one of the parameters for amplitude modeling, we have also investigated the depth of fin whale calling based on the interference between the direct path and downward sea surface reflection. We infer that most calls in this region are made at ~50 m depth.

Notes:

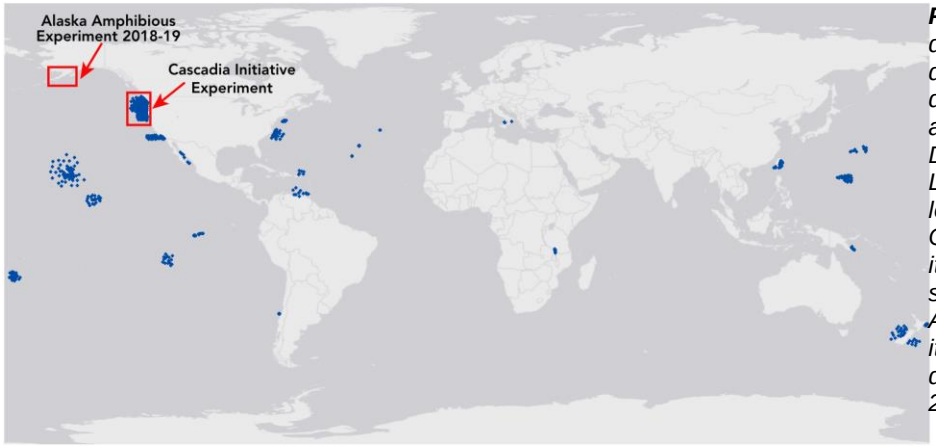

Fig. 1. *Location of 853 OBS deployments of >100 days duration (765 are for >250 days) for which data is available through the IRIS Data Management Center. Labeled boxes show the location of the 2011-15 Cascadia Initiative Community Experiment used in this study and the footprint of the Alaska Amphibious Community Experiment which will deploy about 80 OBSs in 2018-19.*

Cetacean community ecology in the waters of Sri Lanka and the Bay of Bengal

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Background

The Indian Ocean contains arguably the highest diversity of cetaceans in the world's oceans, yet research in this region is extremely limited. The strong environmental variability imposed on the northern Indian Ocean by the seasonal monsoons likely causes a wide variety of niches in both space and time that support the observed diversity of cetaceans. In addition to shelf, slope, and oceanic habitats, there are regions dominated by the input of fresh water (e.g., Bay of Bengal), by evaporation and low river runoff (e.g., Arabian Sea), as well as coastal currents, eddy activity, and large-scale oceanic currents. Moreover, the Arabian Sea and Bay of Bengal have well-developed oxygen minimum zones (mesopelagic regions with O_2 concentrations <0.5 ml l^{-1}) that likely have a significant influence on the behavior and distribution of cetacean prey.

Our long-term goal is to understand the physical and biological oceanographic processes that influence the distribution and occurrence of tropical and subtropical cetaceans. The northern Indian Ocean is particularly well suited for investigating these processes because of the large spatial and temporal variability in environmental conditions imposed by the monsoons. However, very little is known of the distribution, abundance, or behavior of cetaceans in the oceanic waters of the Bay of Bengal.

Objectives

We hypothesize that the cetacean community of the oceanic Bay of Bengal and the waters of Sri Lanka varies with seasonal changes in water masses and circulation associated with the monsoons. During the summer and the following autumnal inter-monsoon period, the cetacean community of Sri Lanka will be dominated by oceanic species endemic to the Arabian Sea, whereas during the winter and vernal inter-monsoon period, the cetacean community should be dominated by neritic species and species endemic to the Bay of Bengal. In the oceanic Bay of Bengal, we hypothesize that cetacean community composition will exhibit significant seasonal variability associated with strong monsoonal forcing of the upper ocean, and that the spatial distribution of cetaceans will be influenced by the depth of the oxygen minimum layer (which, in turn, influences the availability of prey in the upper ocean). To address these hypotheses, we sought to take advantage of an extraordinary opportunity to participate in the Air-Sea Interactions in the Northern Indian Ocean (ASIRI) project sponsored by the ONR Physical Oceanography program and the Naval Research Laboratory (NRL) to characterize (1) the cetacean community in the waters around Sri Lanka and in the oceanic Bay of Bengal during the fall and spring inter-monsoon periods, (2) the relationship between cetacean spatial distribution and mesoscale oceanographic features, and (3) the relationship between cetacean community composition and variability in seasonal oceanographic conditions associated with the periods immediately following the southwest and northeast monsoons.

Methods

Our goal was to conduct cetacean sighting surveys aboard the R/V *Roger Revelle* during cruises to the central Bay of Bengal planned for intermonsoon periods of fall 2013, spring 2014, fall 2014, and spring 2015. We participated in the fall 2013 pilot cruise during which collocated physical oceanographic and cetacean observations were collected in the oceanic waters of the central Bay of Bengal. An international survey team consisting of American, Mexican, Indian, and Sri Lankan scientists used 25x150 "big eye" binoculars, 7x50 hand-held binoculars, and the naked eye to observe cetaceans during the "passing mode" survey. Unfortunately, the ASIRI program changed focus after 2013 from the intermonsoon periods to the monsoons, precluding cetacean sighting surveys because of persistently poor sighting conditions during the monsoons (i.e., high winds, rain). We were encouraged to pursue our research objectives through partnerships with Sri Lankan and Indian researchers, which have taken some time to cultivate.

Results

A total of 1,669 km of trackline were surveyed in Beaufort 5 or less sea conditions during the fall 2013 cruise, and 52 sightings of 12 different species were recorded. Simultaneous physical and biological oceanographic observations were collected from profiling instruments (uCTD and CTD-fluorometer) and echosounders (Sarma et al. 2016, Wijesekera et al. 2016). Pre-cruise and nightly training exercises were conducted to train the Sri Lankan and Indian marine mammal observers (Tandon et al. 2016).

With the change in the ASIRI science focus from the intermonsoon to the monsoon periods, we did not participate in any more ASIRI cruises. We instead concentrated on relationship building with our Indian colleagues at the National Centre for Biological

Research (NCBS) in Bangalore, India and the National Institute of Ocean Technology (NIOT) in Chennai, India so that we could continue to pursue the project research objectives. Activities undertaken to foster a close collaborative relationship with regional scientists include the following:

(1) Stafford and Baumgartner gave presentations on marine mammal research at NCBS and the Sri Lankan National Aquatic Resources Research and Development Agency (fall 2013).
(2) Baumgartner hosted Ms. Divya Panicker as a guest investigator at WHOI and at sea (spring 2014, spring 2015).
(3) At the invitation of Dr. Kartik Shanker (Indian Institute of Science), Stafford and Baumgartner wrote an article on marine mammal research in the journal Current Conservation (Stafford and Baumgartner 2014).
(4) Stafford and Baumgartner offered a 3-day short course on bioacoustics at NCBS for over 20 students and postdocs in the NCBS Program in Wildlife Biology and Conservation (December 2014).
(5) Baumgartner and Stafford presented their marine mammal passive acoustic work to the NIOT Ocean Acoustics program in Chennai, and met with the program lead, Dr. G. Latha, to discuss collaborative opportunities (December 2014).
(6) Together with Dr. G. Latha (NIOT), we were invited to contribute a chapter on real-time passive acoustic monitoring in a book to be published by Springer entitled "Observing The Oceans In Real Time – Instruments, Measurements And Experience," eds. R. Venkatesan, A. Tandon, M.A. Atmanand. We completed the chapter in 2016.

During our interactions with the Indian research and regulatory communities, we have learned that there are significant challenges for U.S. researchers conducting research in India. A more tractable strategy for pursuing our study questions involves having our Indian colleagues take the lead in the research. Toward that end, we have recently taken on an Indian scientist, Ms. Divya Panicker, as a PhD student in the Oceanography Program at the University of Washington, and will work to complete our project objectives through her thesis research. We are also currently planning a symposium for Indian marine mammal research to be held at NCBS during late 2017.

Notes:

Stafford, K.M. and M.F. Baumgartner. 2014. Marine mammal conservation and the role of research. Current Conservation 8:18-25.

Sarma, V.V.S.S., G.D. Rao, R. Viswanadham, C.K. Sherin, J. Salisbury, M.M. Omand, A. Mahadevan, V.S.N. Murty, E.L. Shroyer, M.F Baumgartner, and K.M. Stafford. 2016. Effects of freshwater stratification on nutrients, dissolved oxygen, and phytoplankton in the Bay of Bengal. Oceanography 29:222-231.

Tandon, A., E.A. D'Asaro, K.M. Stafford, D. Sengupta, M. Ravichandran, M.F. Baumgartner, R. Venkatesan, and T. Paluszkiewicz. 2016. Technological advancements in observing the upper ocean in the Bay of Bengal: Education and capacity building. Oceanography 29:242-253.

Wijesekera, H., E. Shroyer, A. Tandon, M. Ravichandran, D. Sengupta, S. Jinadasa, H. Fernando, N. Agrawal, K. Arulananthan, G. Bhat, M. Baumgartner, J. Buckley, L. Centurioni, P. Conry, J. Farrar, A. Gordon, V. Hormann, E. Jarosz, T. Jensen, S. Johnston, M. Lankhorst, C. Lee, L. Leo, I. Lozovatsky, A. Lucas, J. Mackinnon, A. Mahadevan, J. Nash, M. Omand, H. Pham, R. Pinkel, L. Rainville, S. Ramachandran, D. Rudnick, S. Sarkar, U. Send, R. Sharma, H. Simmons, K. Stafford, L. St. Laurent, K. Venayagamoorthy, R. Venkatesan, W. Teague, D. Wang, A. Waterhouse, R. Weller, and C. Whalen. 2016. ASIRI: An Ocean-Atmosphere Initiative for Bay of Bengal. Bulletin of the American Meteorological Society 97:1859-1884. doi:10.1175/BAMS-D-14-00197.1.

Baumgartner, M.F., K.M. Stafford, G. Latha. Near real-time underwater passive acoustic monitoring of natural and anthropogenic sounds. In Observing the oceans in real time – instruments, measurements and experience. eds. R. Venkatesan, A. Tandon, M.A. Atmanand. submitted.

Development of automated whistle and click classifiers for odontocete species in the western Atlantic and Temperate Pacific Oceans and the waters surrounding the Hawaiian Islands

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Background

Marine mammals are a diverse group and most species are acoustically active. As a result, passive acoustic methods have been widely adopted as a tool for monitoring and studying these animals. A fundamental need when analyzing passive acoustic data is to identify species from sounds in recordings. This can be difficult because many sounds produced by marine mammals exhibit high variability within-species and have significant overlap in time-frequency characteristics between species.

Statistical classification algorithms have been used to identify sounds produced by marine mammals, with varying degrees of success (e.g. Roch et al. 2011, Gillespie et al. 2013). Such classifiers generally include a small number species that might be encountered in an area and are based on only one sound-type. For example, classifiers for odontocete species are usually developed for either echolocation clicks or whistles. However, not every species produces whistles, and echolocation clicks are not produced in every behavioral state. Training classifiers using information from both types of sounds should make it possible to identify a larger number of species and schools and the combined information could lead to greater classification success.

Objectives

The primary objective of this effort was to develop automated acoustic classifiers for odontocete species in the northwest Atlantic, the temperate northeast Pacific, and the waters surrounding the Hawaiian Islands. These classifiers identify acoustic encounters (schools) to species based on information from whistles, echolocation clicks (hereafter 'clicks') and other characteristics ('ancillary variables', such as whistle and click rates, relative abundances of whistles and clicks and geographic location). The classifiers will be implemented into existing passive acoustic data processing software: the Real-time Odontocete Call Classification Algorithm (ROCCA) module in PAMGuard (Gillespie et al. 2008, Oswald et al. 2013), and Ishmael (Mellinger 2001).

The secondary objective of this work was to evaluate the need for regionally specific classifiers by examining geographic variation in the characteristics of whistles and clicks produced by species that are found in at least two of the three regions included in this effort.

Methods

Existing passive acoustic data were used to train and test the classifiers. Recordings were used only if they had visual confirmation of species identity, were from a single species school, and no other odontocete species were sighted within 3 nmi of the recording location. Whistles and clicks were automatically detected using PAMGuards whistle and moan detector and click detector, respectively. Whistle and click detections were passed to the ROCCA module, which automatically measured 50 variables from whistles, 7 variables from clicks, and calculated ancillary variables based on the output of the whistle and click detectors.

Seven variables measured from clicks, seven ancillary variables, and nineteen variables measured from whistles were for species that occurred in at least two regions of the three geographic regions. To determine whether significant geographic variation exists within species, variables were compared using Kruskal-Wallis tests and multiple comparison Dunn's tests with Benjamini-Hochberg adjustment (Benjamani and Hochberg 1995).

The classifiers for each region were developed using a three-stage approach modeled after Rankin et al. (2016). In the first stage, a representative dataset of whistles and clicks was identified for each species using a density clustering algorithm. In stage two, the representative datasets were used to train one random forest classifier for clicks and another for whistles. The training datasets had equal sample sizes across species for each region. The final stage was a random forest classifier that classified acoustic encounters based on the ancillary variables and the output of the whistle and click classifiers.

Classifiers were tested using all of the whistles and clicks that were not included in the representative dataset used to develop (i.e. train) the classifier.

Results

The results of both the whistle and click geographic comparisons support the hypothesis that classifiers should be trained using data collected in the geographic region where they will be used. For both clicks and whistles, most variables were significantly different when compared between regions. Due to this high level of variability between regions, a classifier trained with sounds recorded in one region would likely perform poorly if used to analyze sounds recorded in another region. In addition, combining data from different regions to create "global"

classifiers would result in higher variability in training datasets, making it more difficult to separate species into distinct classes.

The northwest Atlantic classifier included over 59,000 whistles and 261,000 clicks from 122 encounters and 6 species. When information from whistles, clicks and ancillary variables were used to classify encounters, 80.0% were classified correctly. This is significantly greater (Fisher's exact test, $p<0.0001$) than the 17% that would be expected by chance alone for 6 species. Correct classification scores for the encounter classifier ranged from 61.1% (*Stenella frontalis*) to 100% (*Grampus griseus*).

The Hawaii classifier training and testing datasets included over 116,000 whistles and 585,000 clicks from 255 encounters and 12 species. This classifier correctly classified 80.2% of encounters. This is significantly greater (Fisher's exact test, $p < 0.0001$) than the 8% correct classification that would be expected by chance alone for 12 species. Correct classification scores for the encounter classifier ranged from 51.1% (*Globicephala macrorhynchus*) to 100.0% (*Grampus griseus, Physeter macrocephalus*).

The temperate Pacific classifier training and testing datasets included over 500,000 whistles and 2 million clicks from 324 encounters and 10 species. This classifier correctly clasified 83.8% of encounters, which is significantly greater (Fisher's exact test, $p < 0.0001$) than the 10% correct classification that would be expected by chance alone for 10 species. Correct classification scores for the encounter classifier ranged from 61.4% (*Delphinus delphis*) to 100.0% (*Ziphius cavirostris*).

Notes:

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Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Series B Stat. Methodol.* 57: 289–300.

Gillespie, D., M. Caillat, and J. Gordon. 2013. Automated detection and classification of odontocete whistles. *J. Acoust. Soc. Am.* 134:2427-2437.

Gillespie, D., J. Gordon, R. Mchugh, D. Mclaren, D. Mellinger, P. Redmond, A. Thode, P. Trinder, and D. Xiao. 2008. PAMGUARD: Semi-automated, open source software for real-time acoustic detection and localization of cetaceans. *Proc. Inst. Acoust.* 30, no. 5.

Mellinger, D.K. 2001. Ishmael 1.0 User's Guide. NOAA Technical Memorandum OAR PMEL-120. Available from NOAA/PMEL/OERD, 2115 SE OSU Drive, Newport, OR, 97365-5258, http://www.pmel.noaa.gov/pubs/PDF/mell2434/mell2434.pdf.

Oswald, J.N., S. Rankin, J. Barlow, M. Oswald and M.O. Lammers. 2013. Real-time Call Classification Algorithm (ROCCA): software for species identification of delphinid whistles. In: Detection, Classification and Localization of Marine Mammals using Passive Acoustics, 2003-2013: 10 years of International Research, DIRAC NGO, Paris, France, pp. 245-266.

Rankin, S., F. Archer, J.L. Keating, J.N. Oswald, M. Oswald, A. Curtis, J. Barlow. 2016. Acoustic classification of dolphins in the California Current using whistles, echolocation clicks, and burst pulses. *Mar. Mamm. Sci.*

Roch M.A., H. Klinck, S. Baumann-Pickering, D.K. Mellinger, S. Qui, M.S. Soldevilla and J.A. Hildebrand. 2011. Classification of echolocation clicks from odontocetes in the Southern California Bight. *J. Acoust. Soc. Am.* 129: 467–475.

Does depth matter? Examining factors that could influence the acoustic identification of odontocete species on bottom-moored recorders

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Background

Substantial advancements have been made recently in the identification of odontocete species based on the properties of their whistles and clicks. However, the suitability of species classifiers trained using data from the sea surface to analyze recordings obtained at depth is currently unknown. The properties of whistles and clicks may be altered by propagation effects such as transmission loss and constructive and destructive interference and it is unclear how this may influence classification results when animals are at different depths and distances relative to recorders. If classifiers perform differently on data recorded at depth than at the surface, it may be necessary to re-train them to ensure accurate results. In this project, we examine how recorded species-specific signaling cues are affected by recording depth by using both surface-deployed and bottom-moored vertical arrays of hydrophones and autonomous recorders to obtain recordings at different depths in the water column from a variety of free-ranging odontocete species. We use these data to address the following questions: Does the depth at which dolphin whistles are recorded affect the signal properties observed? If so, does the performance of species classifiers developed using whistles recorded at the surface change when applied to data from bottom-moored recorders? Do reported species-specific click characteristics remain consistent across recording depths? If they do, are the cues consistent across behaviors, such as diving, surface milling and travel? The results of this work will lead to a greater understanding of the strengths and weaknesses of the acoustic species identification tools being employed for marine mammal monitoring and mitigation by the U.S. Navy.

Objectives

The primary objectives of this study are to 1) obtain field data from free-ranging odontocetes using both a surface-deployed and a bottom-moored vertical array off Lanai and Kona, Hawaii and San Diego, California, 2) collect surface array acoustic data from a trained Navy dolphin producing whistles and clicks at a known depth and known distances from the array, 3) Analyze the properties of whistles collected on both arrays and test classification results using the Real-time Odontocete Call Classification Algorithm (ROCCA) and 4) Analyze the properties of clicks collected using both arrays to determine whether the spectral properties of clicks remain consistent across recording depths.

Methods

Two types of vertical hydrophone arrays were used to obtain data for this project. One array was surface-deployed from a vessel and was composed of two vertical sub-arrays: a localization array with four cabled broadband hydrophones spaced 10 m apart, and a line array made up of five microMARS recorders spaced 50 m apart. The four hydrophones in the localization array were sampled simultaneously on a high-resolution portable recorder.

The second array was a bottom-moored vertical array composed of four second-generation Ecological Acoustic Recorders (EARs) spaced 90 m apart. The array also included a RJE International ARS-100 pinger, which provided a series of 4-7 kHz synchronization pulses every 30 minutes. These pulses were recorded on all four EARs and were used to time-align recordings during analysis in order to localize signaling animals and determine their depth and distance from the array. During the San Diego fieldwork, two SoundTrap 300HF recorders were also added to the bottom-moored array just above the first and third EARs to allow preliminary comparisons of signals recorded with two different types of recorders.

Data from both arrays were used to compare the characteristics of whistles recorded at different depths. Data obtained on the surface-deployed array from species that are included in ROCCA's tropical Pacific whistle classifier were used to test the null hypothesis that no significant differences exist in classifier performance on the same whistles recorded at different depths. Whistles with unknown species identity recorded on the bottom-moored array were similarly analyzed using ROCCA in order to determine whether recording depth significantly influenced species classification results (i.e. are the same whistles recorded at different depths classified as different species?).

The analysis of clicks involved a two-fold approach. The first was to examine the spectral characteristics of clicks recorded at different depths by calculating the average spectrum of the clicks obtained for the encounter. This information was used to determine whether patterns of spectral peaks and other click properties that some researchers use to make

species identifications occur independently of recording depth and are therefore truly distinctive features indicative of species specificity. The second approach was to compare the average click spectral properties of the different species recorded to determine which species are distinguishable and consistent at all recorded depths and which are variable. For those that are variable, the click structures were examined in detail to determine the source of the variability

Results

For most of the Kona EAR array and microMARS encounters, a high percentage of whistles were detected on all recorders. In contrast, for over half of the Lanai EAR encounters, 50 percent or more of the whistles appeared on only one EAR. These results show that for at least some encounters, a different set of whistles is available for classification analysis at different recording depths. These different sets of whistles may lead to different classification results at different depths.

Examination of spectrograms revealed that some whistles attenuated so that portions of their time-frequency contours were missing at some depths. These missing portions affected variables measured from these whistles and led to individual whistles being classified as different species when recorded at different depths. Most whistles were classified as the same species at all depths. However, more than 40% of whistles were classified as two or more different species when recorded at different depths. This could have a significant impact on studies focused on examining individual whistle contours.

The click analyses revealed that both depth-dependent variations within species and also homology in click spectral structure among species exist that could confound click-based classifications. Spotted dolphin clicks exhibited considerable variation in spectral structure across depths, while rough-toothed dolphin clicks were less variable. False killer whale and pilot whale clicks resembled one another to different degrees at different depths, most notably below 100 m, suggesting that they could be confused under different recording scenarios.

In addition to depth-dependent variations in click spectral structure, the instrumentation used to record clicks also had an effect on the observed click properties. The averaged spectra of clicks recorded using two microMARS (50 m and 200 m depth), two SoundTraps (50 m and 200 m depth) and the deepest hydrophone on the sub-array (35 m depth) varied substantially. The spectra had considerably greater structure (more peaks and notches) in the microMARS recordings than in either the SoundTrap or the sub-array hydrophone recordings. This latter finding is significant because the relationship between recording instrumentation and click spectral properties has yet to be described in the open literature.

Notes:

Unsupervised learning (clustering) of odontocete echolocation clicks

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Background

The primary long-term goal is to develop methods for clustering of marine mammal echolocation clicks by species without any training data. This will permit us to learn about species assemblages where little or no prior knowledge exists about these species' signal repertoire. Being able to monitor individual species can be effective for understanding how certain species use a region and habitat or how naval operations may affect their behavior. Understanding the movements and seasonality of potential species within an understudied region can enable targeted research such as sending observers at the right time to the right place.

Objectives

In the first phase of this project, we are working with data from the Southern California Bight where many of the species can be acoustically identified, enabling the development of clustering algorithms whose performance can be evaluated. The option year of this project will look at areas where our understanding is less developed such as data collected from the Gulf of Mexico or the Atlantic.

Methods

This work has focused on developing methods to distinguish between groups of marine mammals based on call characteristics. The focus has been on characterizing groups of echolocation clicks associated with toothed whale encounters by using distributional models although we have also worked with whistles and burst pulses on a more restricted population separation task.

The Kullback-Leibler (KL) metric is an information theory based asymmetric measure of dissimilarity between a pair of distributions. Values of zero indicate that the distributions are identical, larger values indicate the number of bits of divergence of one distribution from the other, and can be made symmetric by averaging the KL distortion in both directions. We use the symmetric form to measure the similarity between groups of features extracted from cetacean calls.

We demonstrated the efficacy of the metric by testing whether or not genetically and morphologically distinct pilot whale populations (Shiho and Naisa types) could be distinguished. Whistle and burst pulse features were extracted and distributional models were estimated. The KL divergence between the two populations was compared to intra-type KL divergences taken from randomized splits of each population.

Agglomerative clustering was accomplished by using the average-linkage algorithm resulting in dendrograms where each leaf is representative of an acoustic encounter. The dendrograms were partitioned using methods proposed by Langfelder *et. al.* which trim the dendrogram to core groups and then reinserts the removed leaves in either a top-down or bottom-up manner.

As many species in the Southern California Bight can be identified from descriptions in the literature, each encounter was compared to labels generated by human analysts. The adjusted Rand statistic was used to evaluate the how the human and machine generated clusters compared to one another.

Results

The KL divergence between groups of Shio- or Naisa- only types only accounted for 11 to 15% of the inter-type divergence between Shiho and Naisa pilot whales, suggesting that the KL divergence metric is capable of separating genetically and morphologically distinct populations (Van Cise *et al.*, 2017).

Species that have very flexible echolocation behavior and that cannot be identified reliably acoustically by analysts are problematic and we are reducing their contribution by splitting

analysis into whistling and non-whistling groups. The adjusted Rand index for all species in the Southern California Bight data set used in this study varies dependent on the clustering technique used from 0.0511 to 0.28 when whistling species are included and 0.38 to 0.73 when they are excluded. Values closer to 1 indicate better concurrence between the analyst and machine-generated partitions. The latter values would be considered good for clustering problems.

Publications:

Van Cise, A.M., Roch, M.A., Baird, R.W., Aran Mooney, T., and Barlow, J. (2017). “Acoustic differentiation of Shiho- and Naisa-type short-finned pilot whales in the Pacific Ocean,” J Acoust. Soc Am 141(2): 737-748.

Beaked Whale Group Deep Dive Behavior from Passive Acoustic Monitoring

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Background

While a significant body of knowledge regarding individual beaked whale behavior at depth has been established in the last decade, little is known about how beaked whales interact as a group while at depth. This lack of information makes it difficult to assess the effects of anthropogenic noise on individual behavior, long-term foraging success, and population health. This project uses Passive Acoustic Monitoring at the Atlantic Undersea Test and Evaluation Center (AUTEC) to track individual Blainville's beaked whales (*Mesoplodon densirostris)* during group foraging dives. These tracks will help establish baseline data for future behavioral response studies by providing insight into group foraging strategy at depth, including: prey capture attempts, spatial relationships among conspecifics, independent or cooperative prey hunting, and foraging strategy.

Objectives

1. Create a new Detection, Classification and Localization (DCL) method that can use passive acoustic data from the hydrophone array at AUTEC to track individual clicking beaked whales within group deep dives.
2. Create a model relating acoustic footprint statistics (e.g., click detection counts, number of hydrophones detected, etc.) to group size. Parameterize this model using surface visual observations.
3. Create a statistical model of beaked whale group deep dive behavior using the results of (1) and (2).

Methods

We enhanced a novel method of DCL developed under the ONR Advanced DCL program to provide the ability to DCL and track beaked whale clicks from multiple individuals within a diving group (Baggenstoss, 2013; Baggenstoss, 2015). A statistical model of beaked whale group deep dive behavior is in progress.

Results

During 2015, a MATLAB (MathWorks, Inc) Graphic User Interface program, whaleGUI, was created to enable user friendly DCL analysis of recorded hydrophone audio files. The DCL methodology was validated against 2007 Behavioral Response Study DTAG data for a group of 3 whales (one whale with DTAG). Archived data from 2005-2016 was analyzed using this tool. Diving *Md* groups were identified using click counts from the Marine Mammal Monitoring on Navy Ranges (M3R) click detection archives. Group size and compositions were determined by a field team. The start and end times of vocal groups with high click counts centered on either 7-hydrophone 1-nmi baseline array were noted. Audio wav files were extracted for these vocal groups and processed using whaleGUI.

Of the 56 group dives evaluated thus far, twenty groups (43 individuals) were sufficiently resolved with nearly continuous individual dive tracks to be included in evaluation of group dive dynamics. Whales were observed in groups of 1, 2, or 3 animals. Preliminary data exploration suggests beaked whales are capable of coordinating activity while at depth. There seems to be (at least) two types of behaviors, one in which animals travel together in parallel in a given direction (Figure 1, left), and another in which animals separate while at depth and then return to a common meeting point before their ascent (Figure 1, right). The primary foraging depth appears to be centered at approximately 900 m with a secondary layer at 1300 m (Figure 2). While individuals and foraging groups of 2 or 3 animals were observed at the shallower depth (n=32), only animals in a group of 3 were observed at the deeper foraging depth (n=11).

A model describing the size of *Mesoplodon densirostris* beaked whale groups as a function of the acoustical footprint of a group was obtained using a generalized linear model (GLM). This GLM model was then used to predict group sizes for groups detected on the AUTEC range. This led to the calculation of daily density estimates of beaked whale abundance using a modification of Moretti et al. (2010) dive counting method. The resulting procedure will allow for routine on-the-fly density estimation at AUTEC.

References

Baggenstoss, Paul M. (2013). "Processing advances for localization of beaked whales using time difference of arrival." *The Journal of the Acoustical Society of America,* 133.6: 4065-4076.

Baggenstoss, Paul M. (2015) "A multi-hypothesis tracker for clicking whales." The Journal of the Acoustical Society of America 137.5: 2552-2562.

Moretti, D.; Marques, T.; Thomas, L.; DiMarzio, N.; Dilley, A.; Morrissey, R.; McCarthy, E.; Ward, J. & Jarvis, S. (2010). A dive counting density estimation method for Blainville's beaked whale (Mesoplodon densirostris) using a bottom-mounted hydrophone field as applied to a Mid-Frequency Active (MFA) sonar operation. Applied Acoustics 71: 1036-1042

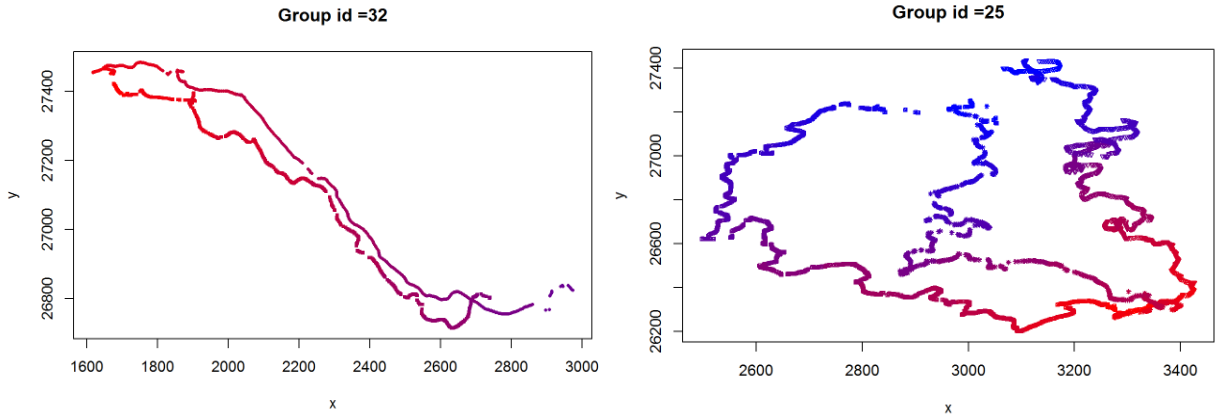

Figure 1. Example tracks of two groups localized during the vocalizing stage of their deep dives (depth not depicted, i.e. projected onto the sea surface). Left: 2 individuals performing “parallel” tracks. Right: 3 individuals that begin together, separate at depth, and return again to nearby positions before becoming silent.

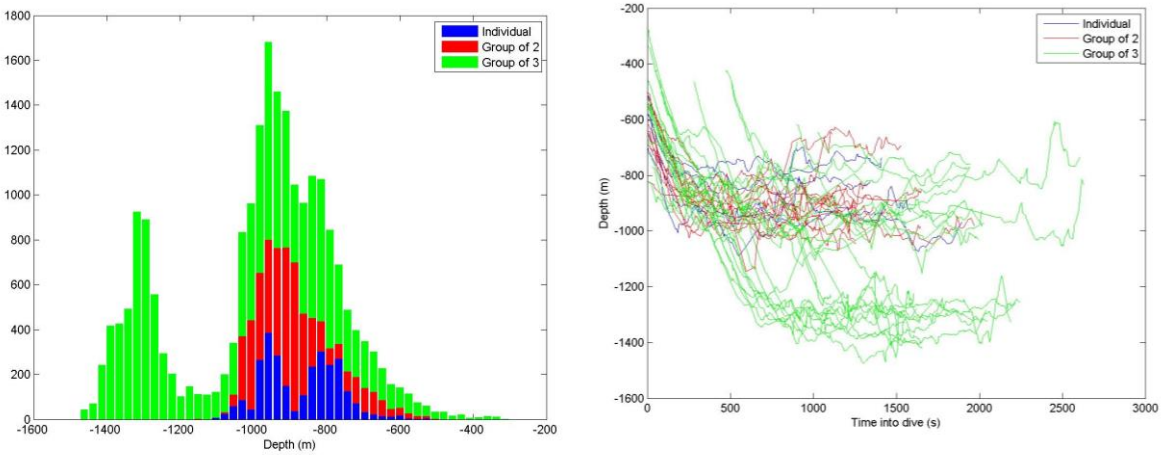

Figure 2. Depth distribution of track point observations by group size (top) and cumulative plot of depth track for all high quality group dives(bottom), n=20.

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Day 3: Wednesday March 22, 2017

POSTER SESSION

2017 ONR

Marine Mammal & Biology

Program Review

Endocrine markers for understanding stress response and reproduction in male humpback whales

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Background & Objectives

Nine populations of humpback whales have been removed from the ESA in a time of great environmental flux. As ecosystems shift in equilibrium, species shift habitat ranges, and as the North Pacific continues to experience anomalous warming, humpback whales may or may not face unexpected challenges. To prevent being caught unaware, long-term monitoring efforts are essential for tracking changes from baseline physiologic parameters. Hormonal biomarkers are useful indicators of the reproductive and metabolic state in mammals. The purpose of this study was to validate analytical methods for endocrine biomarkers to define reproductive and stress physiology in male humpback whales. The developed techniques allow for analysis of newly collected samples, as well as archived samples, and facilitate the merging of historical sample data with that of the new samples. Combined with behavioral and resight data, endocrine biomarkers provide a powerful tool in assessment of physiology and life history states.

Methods

Enzyme immunoassays (EIA) for both testosterone and corticosterone in blubber were validated for use in male humpback whales. Humpback samples from free-ranging animals (2004-2006; n = 317, 300 adult males, 7 male calves) were analyzed. Both EIAs were used to analyze concentration differences between blubber and skin in the same animal.

Results

Both testosterone and corticosterone assays proved to be valid for use for male humpback whale blubber. For the testosterone assay, serial dilutions (neat to 1:16) of the pool exhibited displacement parallel to that of the standard curve and proved accurate ($y=3.4 = 0.90x$, $r^2=0.99$). For the corticosterone EIA, serial dilutions (neat to 1:8) of the pool exhibited displacement parallel to that of the standard curve and proved accurate ($y=7.62 = 0.95x$, $r^2=0.99$).

Testosterone concentrations (ng/g) were significantly different between males sampled in Alaska and Hawaii ($p=< 2.2e-16$) and peaked in the winter breeding season. Corticosterone concentrations were not significantly different between Alaska and Hawaii (p-value = 0.7985) and were relatively consistent across the year. Blubber and skin from the same individuals (n=38) were also compared. Testosterone concentration was significantly higher (p-value=<0.05) in blubber samples than skin, whereas for corticosterone the relationship was varied. Relationships between group type and haplotype were also analyzed with no consistent trends found.

Notes:

Collaborators: Chris Gabriele, Marine Mammal Consortium, Kamuela, Hawai'i
Lab Manager: Kendall Mashburn, University of Alaska Fairbank, Juneau, AK

Activity budgets in long-finned pilot whales: individual, social and environmental context of behavioral responses to naval sonar

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Background

Time allocation to different activities and habitats enables individuals to modulate their level of exposure to different types of resources and risks, and can reveal important trade-offs between different fitness-enhancing activities (e.g., feeding vs. social behavior). Therefore, time budgets can be used to indicate biologically significant outcomes of behavior changes[1].

Objectives

We aimed to quantify individual-level time trade-offs in long-finned pilot whales under varying baseline conditions (time of day, water depth, social context) and individual contexts (body size, association with a calf). We then investigated how these changed during exposures to navy sonar and playback of killer whale sounds (potential predator/competitor).

Methods

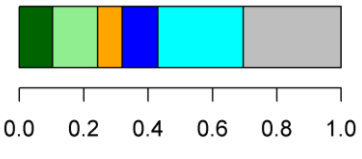
We quantified baseline and exposure time budgets for pilot whales using data from 19 tag records with surface and visual observations of the social context of tagged whales collected in the Vestfjorden basin off Lofoten in northern Norway 2008-2014 as part of the 3S research project[2]. The sonar exposures included 1-2 and 6-7 kHz sonar plus no-sonar control approaches. Data for each inter-breath interval (dive depth, duration, pitch variation, presence of echolocation clicks, group size, horizontal speed) were first classified into near-surface behaviors and dive types. The resulting behavior states were aggregated to time budgets, and the effect of navy sonar and other explanatory variables were tested in binomial regression models for time spent foraging.

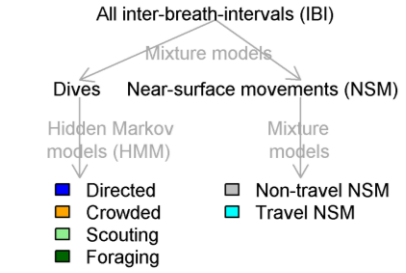
Results

We identified two near-surface behavior states (travelling vs. non-travelling) and four dive types: deep foraging dives ('Foraging'), shallower dives with echolocation indicating foraging or exploratory behavior ('Scouting'), non-vocal dives with large group sizes ('Crowded'), and short directional dives ('Directed'). On average, individuals spent most of their time (69%) resting and transiting near surface, 21% in shallow dives (depth <40m), and only 10% of their time in deep foraging dives, of which 65% reached the sea bottom. Individuals accompanied by calves or with a large body size spent more time foraging. Individuals spent less time foraging and more time transiting when in shallow water depths and when forming larger non-vocal aggregations of individuals in late afternoons. Simultaneous tagging showed that up to 50% of the activity budget was synchronized between conspecifics. Individuals reduced foraging time by 83% (29-96%) during the first sonar exposures. In exposures following the first sonar approach, there was a relative increase in time spent foraging. Such an order effect might indicate habituation or a change in response tactic. Our results highlight that individuals adjust their time budgets to both natural and anthropogenic factors, and that quantifying time budgets in ecologically relevant contexts can help us understand the level of time trade-offs individuals make in response to naval sonar.

Notes:

Figures: *(left) Individual-average time budget during baseline; (right) behavior state classification steps*





References: [1] Houston AI et al 2012. The cost of disturbance: A waste of time and energy? Oikos 121:597–604. [2] Miller PJO et al. 2011. The 3S experiments: Studying the behavioural effects of naval sonar on killer whales, sperm whales, and long-finned pilot whales in Norwegian waters. Scottish Ocean. Inst. Tech. Rep. SOI-2011-0.

Behavioral Response Evaluations Employing Robust Baselines and Actual Navy Training (BREVE)

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Background
A strong priority for the US Navy marine mammal program is to understand whether and under what circumstances marine mammals show a behavioral response to sonar. One research approach is to conduct controlled exposure experiments, where the reaction of free-swimming wild animals is observed to randomized exposures from experimental sound sources. The experimental subjects are typically fitted with animal-borne tags prior to the experiment. These experiments can be characterized as being high fidelity, high cost, short-term, (relatively) low sample size studies; appropriate analyses to maximize extraction of knowledge from the precious data is key, and this was the motivation for a three-year ONR-sponsored project MOCHA.

An alternative is an observational approach, where metrics of animal behavior are monitored in the context of real-world variation in sonar activity. Although this lacks the randomization associated with a pure experimental protocol, observational studies offer the potential for greatly increased sample size and also, importantly, allow observation of animals under real-word operational conditions.

There are broadly two types of observational study, those that focus on population-level responses (e.g., Moretti et al. 2014) and those that focus on individual-level responses. We focus here on the latter using tracks of individual whales derived from passive acoustic monitoring (PAM). We describe a new 3-year ONR-funded project, BREVE.

Objectives
We will focus on the Pacific Missile Range Facility (PMRF), Kauai, Hawaii, where large quantities of baseline and during-training data are available and automated acoustic processing methods are currently available for automatically detecting and localizing multiple baleen whale species (see Helble et al. 2015 and Martin et al. 2015). Species being considered include fin, sei, Bryde's, humpback and minke whales.

The research has two components. First, we will establish robust baseline behaviors for multiple baleen whale species, leveraging current, and to be refined, automated processing capabilities to process/re-process existing large datasets from PMRF. Outputs include individual animal acoustic tracks for some species. Second, we will extend and adapt methods for quantifying behavioral responses to mid-frequency active sonar (MFAS) doses that were developed in an earlier project (MOCHA) to the current proposed effort, and use the results from periods of Navy training, including mid-frequency active sonar operation, to compare against baseline behavioral data.

Methods
There are four main tasks: 1) Review current acoustic data and processing methods; 2) Selection of metrics to quantify whale movements and acoustic calls; 3) Analysis of data to generate metrics; and 4) Development and application of statistical analysis techniques.

The 'data' consists of recorded PAM data for dozens of range hydrophones at PMRF, PMRF range products during US Navy training, and various contextual data (e.g. weather, ocean conditions, proximity of other baleen whales that are localized and tracked, amount of surface ship activity and context of ship distances, and headings, to whales).

Metrics are key to the effort. While automated detection, classification and localization processes have been previously developed and applied to PMRF acoustic data we strive here to track individual whales and quantify the tracks with multiple metrics. By applying this process to seasonal baseline data we will establish robust baselines for comparing with results during US Navy training. The metrics represent: contextual information, large scale effects (e.g., mean estimated acoustic density of a species in an area of thousands of km^2), and finer-scale effects representing the individual ship-animal encounters during training. We will review metrics previously employed in localizing and tracking baleen whales at PMRF as well as metrics utilized in behavioral response studies (BRS).

Tracking individual whales will be carried out using relatively simple methods in the first instance with

potentially more complex methods, such as Kalman filtering (Elbert 1984) and Multi Hypothesis Tracking (Baggenstoss 2015), being developed later in the project. While PMRF range products provide GPS ship positions during training events, PAM methods are also utilized to localize the ships when MFAS transmissions occur. Maximal use of automated techniques will be stressed to allow efficient processing of large quantities of data during the effort.

Statistical methods will be developed that are appropriate for characterizing baseline behaviors, detecting behavioral responses and relating responses to covariates such as the dose of sound using the various metrics. Three types of methods are envisaged, all based on approaches developed during the MOCHA (Harris et al. 2016) and LATTE (Marques and Thomas 2015) projects. First are those based on large-scale metrics such as acoustic population density; such analyses may be similar to those of Moretti et al. (2014). Second are those based on multivariate individual-based behavior change metrics such as Mahalanobis distance (e.g., DeRuiter 2013). Third are Markovian models of animal behavioral state, using kinematic and other individual-based input metrics.

Results

For initial development work we have extracted tracks for minke whales and humpback whales from a period spanning a training exercise on PMRF in February 2014, as well as baseline track data from two weeks prior to the exercise. We have fitted splines to these tracks and extracted a suite of kinematic metrics from the tracks as well as aligning these with relevant contextual variables (environmental, ship-related and sonar-related). Initial analysis efforts will involve fitting univariate regression models to each metric as well as multivariate change-point detection methods. The aim being to establish which metrics are most robust and useful for establishing behavioral responses.

Notes: Baggenstoss, P.M. (2015). A multi-hypothesis tracker for clicking whales. J. Acoust. Soc. Am. 137(5), 2552-2562.
Elbert, T.E. (1984). Estimation and Control of Systems. Van Nostrand Reinhold, New York, 1984. 399-427
Harris, C.M., L. Thomas, D. Sadykova, S.L. DeRuiter, P.L. Tyack, B.L. Southall, A.J. Read and P.J.O. Miller. 2016. The challenges of analyzing behavioural response study data: An overview of the MOCHA (Multi-study OCean acoustics Human effects Analysis) project. Pages 399-407 in Popper, A.N. and A. Hawkins. (Eds), The Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology 875. ISBN 978-1-4939-2981-8.
Helble, T.A., G.R. Ierley, F.L. D'Spain, and S.W. Martin. 2015. Automated acoustic localization and call association for vocalizing humpback whales on the Navy's Pacific Missile Range Facility. J. Acoust. Soc. Am.137(1), 11-21.
Marques, T.A. and L. Thomas. 2015. A description of LATTE outputs. CREEM Technical Report 2015-2, University of St Andrews. http://hdl.handle.net/10023/7720
Martin, S.W., C.R. Martin, B.M. Matsuyama, and E.E. Henderson. (2015) Minke whales (Balaenoptera actorostrata) resond to Navy training. Jour. Acoust. Soc. Am. 137(5), 2533-2541.
Moretti, D., L. Thomas, T. Marques, J. Harwood, A. Dilley, B. Neales, J. Shaffer, E. McCarthy, L. New, S. Jarvis and R. Morrissey. 2014. A risk function for behavioral disruption of Blainville's beaked whales (Mesoplodon densirostris) from mid-frequency active sonar. PLoS ONE 9(1): e85064

Movements and diving behavior of beaked whales in Monterey Bay, CA: A comparative study site in the California Current Ecosystem

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Background

When assessing the nature and degree of anthropogenic impacts on a population, a key question is "compared to what?" This is difficult to evaluate when data does not exist for the population of interest prior to the introduction of the impact. This is the case with Cuvier's beaked whales that live in and near the Southern California Anti-Submarine Warfare Range (SOAR) and are frequently exposed to mid-frequency active (MFA) sonar systems during routine military training exercises. While we continue to expand our understanding of the behavior and demographics of this population, we lack comparative information from a sympatric, but undisturbed population, an essential component for assessing impacts from MFA sonar.

Objectives

Investigate Monterey Bay, CA as an ecologically-similar study site for Cuvier's beaked whales where MFA sonar is not used. If appropriate, compare the demographic rates and behavior of Monterey Bay and Southern California Bight whales.

Methods

Conduct two small-vessel survey efforts from Monterey Bay, CA for a total of 16 days, including photo-identification and satellite tag deployments. Evaluate effort-corrected sighting rates (controlling for habitat suitability and survey conditions) of Cuvier's beaked whales to determine the likelihood of adequate data collection there in the future. One effort would occur in October 2016, during the historical period of the lowest average wind speeds annually. The second field effort will occur flexibly in response to favorable weather forecast before September 2017.

Results

The first survey effort was 30 September to 7 October 2016. In 7 days, we covered 495.1 nmi during 50.1 on-effort hours. Seventy-one percent of effort hours occurred in "poor" or "fair" sighting conditions (accounting for the combined effects of wind, swell, and visibility), 20% occurred in "good" conditions and only 9% in "excellent" conditions (necessary for successful beaked whale encounters). We documented 50 sightings of 9 cetacean species, but no beaked whales were sighted. Inclement weather prevented concentration of effort in the deeper waters of the canyon or off the shelf break, preferred habitat for beaked whales. We connected with local researchers and whale watch operators and discussed the importance of their contributions of beaked whale data to our organization. As a result, we obtained historical beaked whale sighting locations and photographs, which will be added to our catalog.

While these interim results are not conclusive with regard to the suitability of Monterey for beaked whale research, it is worth noting that Isla Guadalupe, nearly as far south of SOAR as Monterey is north, may provide another comparative study site for Southern California Cuvier's beaked whales. Collaborators in Mexico, led by Gustavo Cardenas, completed the first dedicated survey for beaked whales at Isla Guadalupe in October 2016. Identification photos from that survey were combined with earlier opportunistic photos from Guadalupe and compared against 185 individuals from Southern California. Six internal Guadalupe matches were found, but there were no photographic matches between the two regions. While several tagged whales have moved between Guadalupe and Southern California in the past, exchange appears to be low. There is currently no documented exchange of individuals between Monterey and Southern California, which is preferable. However, given the apparently high encounter and resighting rates at Guadalupe, expanding beaked whale effort there, including tagging, may yield a sizable sample of comparative demographic and behavioral data from whales with minimal exposure to MFA sonar, particularly if Monterey is ultimately deemed unproductive.

Notes:

Acoustic niche partitioning of three high-frequency-vocalizing odontocetes in the Bering Sea

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Background

As the Arctic seas rapidly change with increased ocean temperatures and decreased sea ice extent, traditional Arctic marine mammal distributions will be altered, and non-traditionally Arctic species may shift poleward. Seasonal Arctic species typically include bowhead, humpback, right, gray, fin, and blue whales; odontocetes, specifically killer, sperm, and beluga whales; and several pinnipeds species. Until recently, recording constraints of power and storage limited sampling rates, thus preventing the detection of high-frequency-vocalizing species (< 22 kHz) in Arctic waters. The Bering Sea falls inside the US exclusive economic zone (EEZ) and is a critical fisheries hotspot for national food security. It borders the Russian EEZ, and has been occupied by Chinese vessels as recently as 2015. Detailed knowledge about species inhabiting the region contributes to the local environmental characterization and prediction capabilities for gaining every possible tactical advantage in Naval operations that support Food, Energy, and Water (FEW) goals.

Objectives

(1) To document the distribution of odontocetes along the Arctic corridor from the Bering Sea to the Chukchi Sea and develop predictive models of species occurrence.
(2) To identify any shifts in odontocete range or distribution along the Arctic corridor over time.
(3) To relate observed shifts to key environmental parameters.

Methods

The technology in Passive Acoustic Listeners (PALs) records at sampling rates up to 100 kHz and provides memory space and allotment for annual deployments (Nystuen, 1998). Using one of the first long-term data sets to record relatively high frequencies in the Bering Sea from PALs, clicks from killer whales (Oo), sperm whales (Pm), and Pacific white-sided dolphins (Lo) have been manually detected at station M2 in the southern Bering Sea from 2008-2013. Time series of the acoustic presence and relative acoustic activity of these three species were constructed and compared with ice cover and ice thickness.

Results

Current analyses and results target the first objective. Oo, Pm, and Lo acoustically coincide with ice cover at different lags, suggesting acoustical niche partitioning. Furthermore, not all species call similarly in the presence of varying ice thickness. Not-yet-verified clicks from Cuvier's or BW40 beaked whales, Risso's and Northern right whale dolphins, and belugas may have been detected through either manual or automated (PAMlab 8.0) analyses and are under further investigation. Linear regression comparing acoustic activity to ice metrics is thus far statistically significant for sperm whales in 2010 insomuch that their acoustic activity was positively associated with higher percent ice cover (but not with thicker ice).

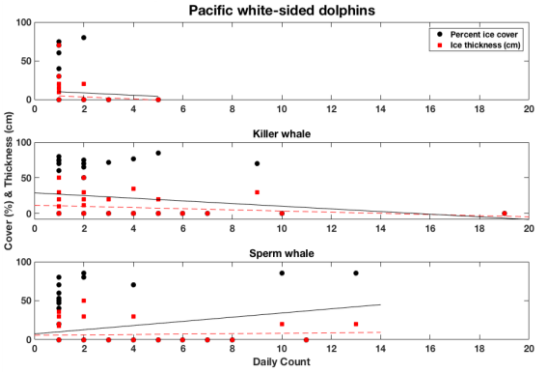

Figure 1. Daily 2009-2012 acoustical activity "counts" of Lo (top), Oo (middle), and Pm (bottom) compared to concurrent ice cover percent (in black circles and solid lines) and thickness in cm (in red squares and dashed lines).

Notes: Nystuen, J. A. (**1998**). "Temporal sampling requirements for autonomous rain gauges," J. Atmos. Oceanic Tech. **15**, 1254-1261.

Integrating remote sensing methods to measure social delphinid baseline behavior and responses to Navy sonar

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Background

Oceanic delphinids (*e.g.*, common dolphins, bottlenose dolphins) are generally not endangered or threatened and have typically not been observed in mass-stranding events associated with sound. Potential responses are often inferred from laboratory observations, with very different exposure contexts from free-ranging animals, or include anecdotal observations of responses that lack calibrated exposure measurements or detailed aspects of behavior. These provide some insight into the nature and variance of potential response, but each has limitations. There have been behavioral response measurements with tagged Risso's dolphins in the southern California Behavioral Response Study (SOCAL-BRS), but no such data exist for other common delphinids that are less amenable to tagging. Our goal is to experimentally study potential behavioral responses of free-ranging delphinids in controlled conditions using a novel integration of remote sensing technologies.

Objectives

I. Develop integrated, cross-disciplinary methods to simultaneously track group movement and behavior using integrated remote sensing methods.
II. Apply group-sampling methods using these integrated technologies to better characterize the typical (undisturbed) range of behavioral parameters for these species.
III. Obtain direct measurements of group behavioral changes, if any, resulting from experimentally controlled simulated Navy MFAS for three delphinid species that occur in large numbers in Navy range areas.

Methods

Building on our recent and ongoing research, we will develop and utilize a novel integration of (1) shore- and vessel-based visual sampling; (2) unmanned aerial systems (UAS) for aerial photogrammetry; (3) and remote-deployed passive acoustic sensors to document aspects of baseline behavior and potential behavioral responses in three delphinid species (common, bottlenose, and Risso's dolphins). We will evaluate potential responses to simulated mid-frequency active sonar (MFAS) using controlled exposure experiments (CEEs). The resulting data will be necessarily and categorically different from previous response studies involving tagged individuals. Beyond the fact that getting tags to remain attached on some of these species has proven infeasible, these social species typically occur in groups and group members likely interact in their response to external stimuli, making the group likely the more relevant unit of analysis, as they are in this study.

Notes: Nystuen, J. A. (**1998**). "Temporal sampling requirements for autonomous rain gauges," J. Atmos. Oceanic Tech. **15**, 1254-1261.

Multi-scale observations to more fully quantify behavioral responses in a beaked whale

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Background

The off-range beaked whale studies (ORBS) project aims to use controlled exposure experiments (CEEs) on *Hyperoodon ampullatus* near Jan Mayen, Norway, to investigate the influence of source-whale distance on response thresholds and severity, and to provide data on beaked whale behavioral responses to naval sonar far away from U.S. naval ranges. CEEs conducted in 2013-2015 indicated strong avoidance during two CEEs, at ~5 km [1] and at 0.8 km, and no avoidance at 0.3 km during a CEE with a low source level.

Objective

To conduct multi-scale sonar CEEs on northern bottlenose whales, to develop a capability to carry out a study of how source-whale distance affects behavioral responses.

Methods

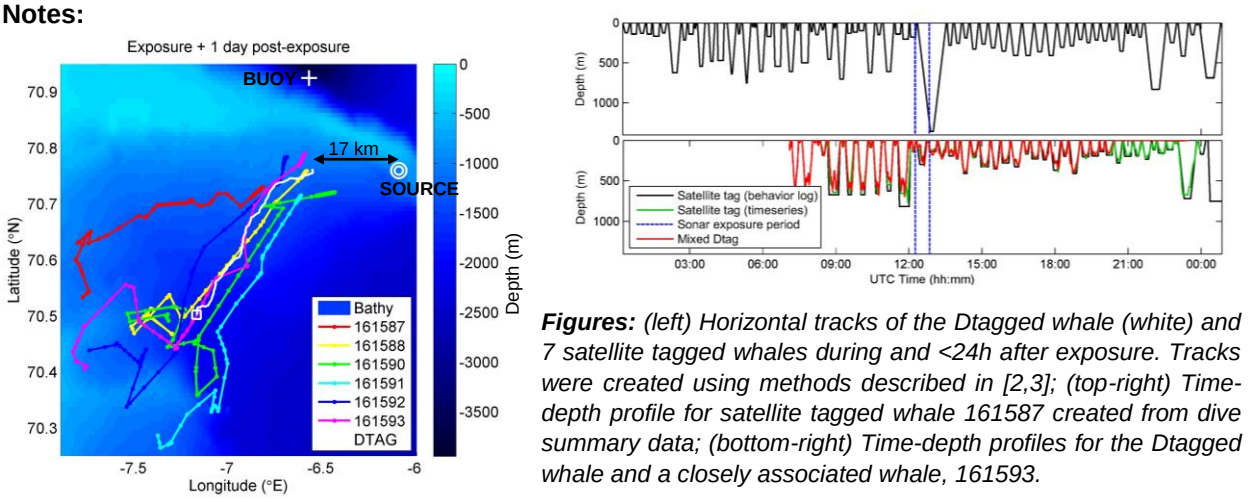
Whale responses were recorded using multi-scale observation tools: short-term suction-cup attached Dtags, medium-term SPLASH10-292B satellite tags, bottom-moored acoustic recording buoys, and visual observations from a crow's nest. Sonar sounds in the 3-4 kHz band were transmitted using the NUWC source at 214 dB re 1µPa-m for 20 min after a 15-min ramp-up.

Results

We successfully conducted one multi-scale CEE to *Hyperoodon* with a distant sonar source (figures, below). Six satellite tags (13-27 km from source) and one mixed-Dtag (17 km from source) were deployed prior to exposure, and all whales moved away with direct horizontal paths while diving to intermediate depths. An extended period without click detections was recorded by the acoustic buoy North of the tagged whales.

The use of satellite tags and acoustic buoys alongside Dtags greatly increased the value of the sonar experiment, by recording consistent responses of multiple animals at varying distances from the source during a single sonar exposure.

Notes:



Figures: *(left) Horizontal tracks of the Dtagged whale (white) and 7 satellite tagged whales during and <24h after exposure. Tracks were created using methods described in [2,3]; (top-right) Time-depth profile for satellite tagged whale 161587 created from dive summary data; (bottom-right) Time-depth profiles for the Dtagged whale and a closely associated whale, 161593.*

References:
[1] Miller PJO, Kvadsheim PH et al. (2015a). Northern bottlenose whales are highly sensitive to noise disturbance. Royal Society Open Science 2: 140484. [2] Johnson DS, London JM, Lea MA, Durbin JW (2008). Continuous-time correlated random walk model animal telemetry data. Ecology 89, 1208-1215. [3] Wensveen PJ, Thomas L, Miller PJO. (2015). A path reconstruction method integrating dead reckoning and position fixes applied to humpback whales. Movement Ecology 3, 31.

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Day 4: Thursday March 23, 2017

2017 ONR
Marine Mammal & Biology
Program Review

Large Scale Density estimation of blue and fin whales (LSD)

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Background

Effective management and mitigation of marine mammals in response to potentially negative interactions with human activity requires knowledge of how many animals are present in an area during a specific time period. Many marine mammal species are relatively hard to sight, making standard visual methods of density estimation difficult and expensive to implement; however density estimation from passive acoustic monitoring data can be an attractive, cost-effective alternative. A particularly efficient passive acoustic monitoring design is a "sparse array", where sensors are distributed evenly over a large area of interest – however a consequence of this design is that each vocalization cannot be heard at multiple sensor locations, restricting the choice of density estimation methods. Sparse array methods have been developed and demonstrated (e.g. Küsel et al., 2011). While these studies represent an important step forward in making density estimation methods more generally applicable at reasonable cost, they are only applicable to small local ocean areas, or they require unrealistic assumptions about animal distribution around the sensors, or both. This effort utilizes sparse array data from the Comprehensive Nuclear Test Ban Treaty Organization International Monitoring System (CTBTO IMS) and Ocean Bottom Seismometers (OBSs) to develop and implement a new method for estimating blue and fin whale density that is effective over large spatial scales and is designed to cope with spatial variation in animal density. The method developed for the targeted low frequency vocalizations of blue and fin whales will be directly applicable to other species and frequency ranges using sparse arrays of fixed or remotely deployed PAM systems. Outputs will be of direct relevance to Navy risk assessment models.

Objectives

This effort develops and implements a methodology for estimating blue and fin whale density from passive acoustic data recorded on sparse hydrophone arrays in the Equatorial Pacific Ocean at Wake Island. The method is then applied to the same species in the Indian Ocean at the Diego Garcia CTBTO location.

1. Develop and implement methods for estimating detection probability of vocalizations based on bearing and source level data from sparse array elements.
2. Validate using OBS data, where additional independent information on detectability is available.
3. Use all available and relevant data to develop multipliers for converting calls-per-unit-area to blue and fin whale density – i.e., estimates of average call rate.
4. Estimate the regional density and spatial distribution of blue and fin whales in the Equatorial Pacific Ocean, using CTBTO data from Wake Island.
Estimate regional density and spatial distribution of blue and fin whales in the Indian Ocean, using CTBTO data from Diego Garcia.

Methods

Low frequency (1-120 Hz), continuous data recorded by the CTBTO IMS for over a decade at Diego Garcia (2002-present: Indian Ocean), and Wake Island (2007-present: Equatorial Pacific Ocean) have been acquired from the Air Force Tactical Applications Center/ US National Data Center. The CTBTO IMS instrument configuration allows for call bearing and, in some cases where the vocalizing animal is close, localization from which source level can be estimated. These data, coupled with sound propagation models in the study area, are being used to estimate the distribution and density of calling whales in the monitored area.

A detector characterization analysis gives the probability of detecting a call as a function of signal-to-noise ratio (SNR). Call "abundance" at the location of each call is then estimated with a Horvitz-Thompson-like estimator, where each detected call is scaled by its associated probability of detection to account for undetected calls also produced at that location (e.g., Borchers et al., 2002). The resulting estimates are smoothed in space with a spatial model to give an estimated density surface. Taken together, this represents a novel approach to density estimation that has wide applicability.

OBS data have also been used to inform method development. An array of 24 OBSs was deployed off the coast of Portugal for 12 months. Both range and bearing to fin whale calls can be estimated using the OBSs (Harris et al., 2013). Therefore, using this array, density results obtained using bearing data can be

directly compared with density results obtained using standard distance sampling.

Results

Results from this ongoing effort reflect 1) a pilot study and method simulation from three months of data estimating fin whale density at Wake Island (Fig 1), 2) long time series patterns and trends in fin whale distribution, source levels, and estimated animal density at Wake Island, and 3) highlighted differences in observed fin whale behavior between Pacific and Indian Ocean populations (Fig 2).

Notes:

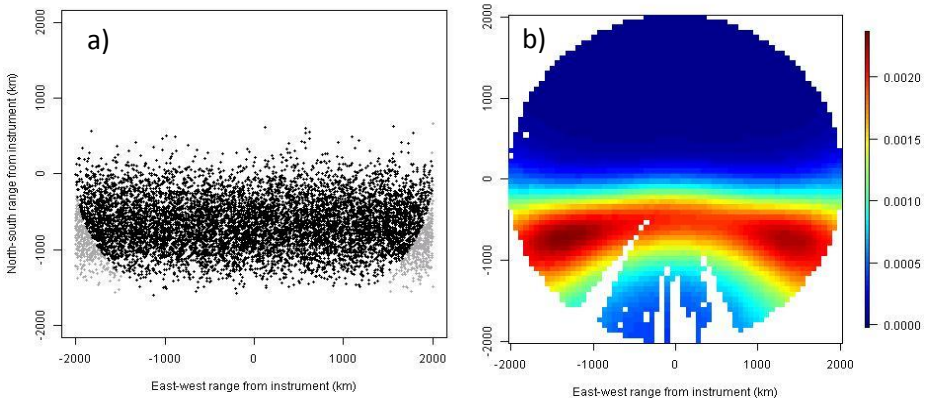

Figure 1 (a) Simulated whale calls inside (black dots) and outside (gray dots) a 2000 km maximum detection range (b) spatial map of call density (signals/km^2) predicted using the bearing data associated with Fig.1a.

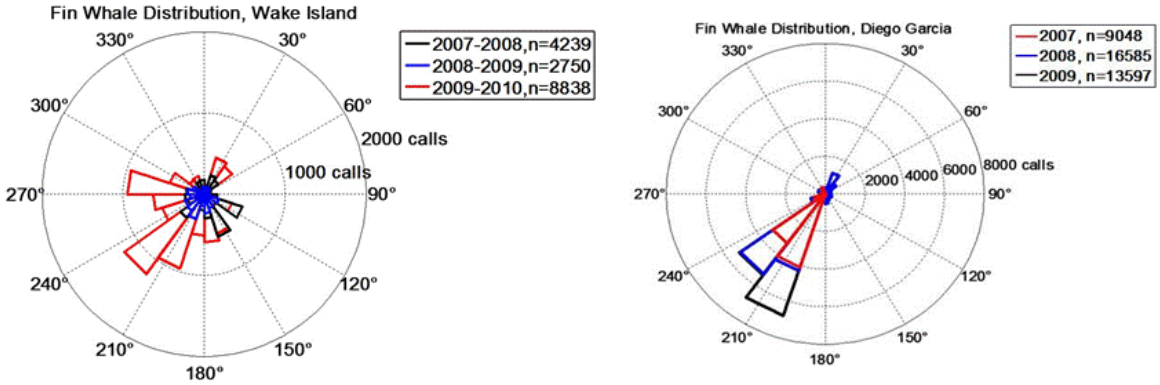

Figure 2. Fin whale distribution is not uniform at either CTBTO IMS location. Whale distribution at Diego Garcia (right) is annually consistent, whereas it is widely variable at Wake Island (left).

References:

Borchers, DL, Buckland, ST and Zucchini, W (2002). *Estimating Animal Abundance.* Springer, New York.

Harris, D, Matias, L, Thomas, L, Harwood, J and Geissler, WF (2013). Journal of the Acoustical Society of America 134, 3522-3535.

Küsel, E. T., Mellinger, D. K., Thomas, L., Marques, T. A., Moretti, D. & Ward, J. (2011). Journal of the Acoustical Society of America 129**,** 3610-3622.

Behavioral Context of Blue and Fin Whale Calling for Density Estimation

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Background

The ability to estimate marine mammal densities is crucial for estimating the impacts of US Naval activities on their populations. Since many marine mammals regularly produce a variety of sounds for mating and foraging, passive acoustic monitoring has proved to be a useful, novel method for monitoring their populations (Mellinger *et al.* 2007). More recently, point transect theory has been successfully applied to density estimation from fixed passive acoustic data (*e.g.*, Marques *et al.* 2011). To estimate population density of calling whales based on point transect methodology, we need to estimate the following: (1) average call production rate, (2) call detection range, and (3) the probability of call detection within that range.

During this project, we are focusing on the development of call production rate models for density estimation. Whale call rates vary with season, region, and animal's sex and behavioral state (Clark 1990; Oleson *et al.* 2007a; 2007b). Thus it is crucial to have an understanding of the behavioral context of calling and factors that affect calling rate before meaningful call rates can be applied to density estimation for any set of passive acoustic data. Specifically, we are using blue whales (*Balaenoptera musculus*) and fin whales (*B. physalus*) as our model species because, as endangered species that are common in many areas of naval activity, they are of interest to the Navy.

Objectives

We will use previously collected and new acoustic tag data to determine the context-specific call production rates for blue and fin whales. In addition, we will elucidate gaps in the data which may affect our ability to develop a comprehensive behavioral model of calling; develop a strategy for filling those gaps; and conduct field data collection to address some gaps in the data coverage for these species.

Methods

We deployed acoustic tags in 2015 and 2016 on blue and fin whales. We analyzed for presence of whale calls on these tags and those deployed during other studies like the SOCAL-BRS from 2000 to 2015, including B-probe, Acousonde, and D-tags. Time of all detected calls were stored, along with location of recording, type of call, and behavioral setting. Behavior of whales was analyzed during all dives from pressure (depth) data, and classified as: shallow dive (<50 m) with lunges, shallow dive (<50 m) without lunges, deep dive (>100 m) with lunges, and deep dive (100 m) without lunges. The maximum depth of each dive, bottom time, and start and end time, as well as total duration spent in each behavioral state were measured.

To address geographical sampling bias in the blue whale tag data and evaluate the calling preference of inshore and offshore animals off Southern California, we analyzed recordings from four High-frequency Acoustic Recording Packages (HARPs) deployed inshore and offshore of Channel Islands during 2009-2010. Time periods available year-round at all four sites were identified and A, B, and D blue whale calls were logged during those times. We calculated percentages of different calls, as well as types of song at each site over this time.

Results

We have completed the analysis of both acoustic and dive profile data collected from all tags deployed before 2016. A total of 37 of the deployments contained blue whale acoustic activity, with a total of 1940 calls detected during 383 dives. When linked to behavioral state, 63% of all dives containing calls were shallow dives without lunges (240 dives), and another 27% of calling dives were categorized as either deep or shallow dives with lunges (88 and 17 dives, respectively). The average depths at which A, B, and D calls were detected were 28, 25, and 24

m, respectively. Although these statistics are generally informative, the data from only 3 deployments contained 85% of all calls, with one tag in particular comprising 63% alone. All three of these tags contained repetitive A-B calling bouts representative of blue whale song, and during these singing bouts 76% of dives were shallow dives without lunges (Figure 1).

On 23 May 2016, we had the first successful long-term deployment of the Acousonde acoustic tag on a blue whale. The tag remained on the whale and collected acoustic data until 2 June (10 days). This record is a valuable contribution to our data and will be an important first step in developing a more detailed understanding of daily variability in blue whale calling behavior. Initial analysis of the data showed presence of a large number of D calls in the acoustic record.

A combined total of 18,125 blue whale A, B and D calls were detected between September 2009 and August 2010 at four sampled HARP sites. Seasonal differences in A and B calls between the four sites were significant, with a fall peak in AB calling that continued into the winter months inshore. Overall, the total number of call detections at the two offshore sites (2335 and 6038 calls) was lower than detections at each of the two inshore sites (4369 and 5383 calls).

Interestingly, during four months of peak calling, blue whale A calls constituted a higher percentage of all detections at the inshore sites (30 and 34%) than offshore (24 and 22%; Figure 1). Different song types also dominated at these two geographic locations, with a higher proportion (64% and 55%) of all song bouts at the offshore sites of type ABB (single A followed by two or more B calls), whereas about 61% of song bouts at the inshore sites were AB-type (alternating A and B calls). In contrast, average D call rates showed no statistically significant seasonal variation between the four sites. The shared seasonality in D call rates between all four sites suggests that D calls, rather than the commonly reported and widely studied A-B calls, may be a more robust proxy for density estimation using passive acoustics.

Notes:

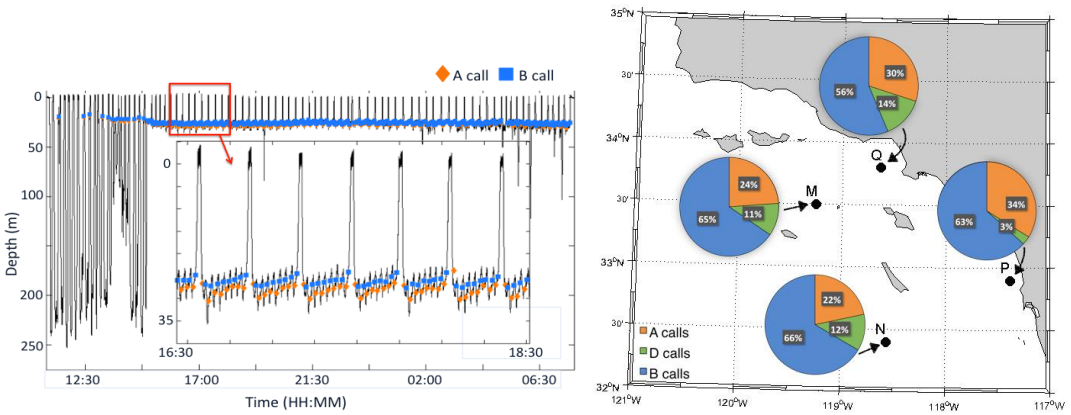

Figure 1 (left): Example blue dive profile from an Acousonde tag deployed on 16 August 2011, with the majority of all A and B call (marked) occurring during shallow dives without lunges. (right): Overall percentage of each call type detected at the inshore and offshore HARP sites from September 2009 – August 2010.

References:

Clark CW (1990) Acoustic behavior of mysticete whales, in *Sensory abilities of cetaceans*, eds J Thomas & R Kastelein (Plenum Press, New York), pp. 571-583.

Marques TA, Munger L, Thomas L, Wiggins S, Hildebrand JA (2011) Estimating North Pacific right whale *Eubalaena japonica* density using passive acoustic cue counting, *Endangered Species Research* 13, 163-172.

Mellinger DK, Stafford KM, Moore SE, Dziak RP, Matsumoto H (2007) An overview of fixed passive acoustic observation methods for cetaceans, *Oceanography* 20, 36-45.

Oleson EM, Calambokidis J, Burgess WC, McDonald MA, LeDuc CA, Hildebrand JA. (2007a) Behavioral context of Northeast Pacific blue whale call production, *Marine Ecology Progress Series* 330, 269-284.

Oleson EM, Wiggins S, Hildebrand JA (2007b) Temporal separation of blue whale call types on a southern California feeding ground, *Animal Behaviour* 74, 881-894.

A framework for cetacean density estimation using slow-moving autonomous ocean vehicles

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Background

Autonomous underwater vehicles such as ocean gliders and vertical profiling floats have the potential to play a key role in future marine mammal monitoring efforts. When equipped with hydrophones, these vehicles can collect passive acoustic data from marine mammals. The major advantage of gliders and other autonomous vehicles over existing passive acoustic monitoring approaches is their ability to provide both broad spatial and temporal coverage of a survey area due to their slow movement. This is in comparison to moored stationary instruments that provide good temporal coverage but limited spatial coverage, or towed hydrophones from vessels that can have extensive spatial coverage during a relatively short timeframe. However, while a variety of methods have been developed to estimate animal density from acoustic data collected by fixed or moving platforms, methods for estimating cetacean density from autonomous ocean vehicles do not currently exist.

Objectives

The overall goal is to develop a general framework for estimating cetacean density from data collected by autonomous vehicles such as ocean gliders and profiling floats, taking into account species' acoustic and behavioral differences and environmental variation. There are five primary objectives:

1. Evaluate whether ocean glider data can be analyzed in a design-based framework using existing data from surveys completed in three areas of naval interest: Gulf of Alaska (GOA), Mariana Island Range Complex (MIRC), Hawaii Range Complex (HRC). This objective does not apply to profiling floats as they cannot be steered along designed survey tracks.

2. Quantify ocean glider/profiling float survey effort and evaluate encounter rates of example species at the same three areas of naval interest. The example species will include at least one deep diving odontocete (sperm or beaked whale), one shallow-diving delphinid, and one baleen whale.

3. Develop a methodology for estimating the probability of detecting cetacean vocalizations on ocean gliders/profiling floats using data collected as part of an existing project at the Southern California Offshore Range (SCORE).

4. Estimate animal densities (or call densities if average call rates are needed but not available) of example species at the three areas of naval interest using ocean glider data.

5. Develop an experiment that does not rely on a large Navy range (such as SCORE) to estimate a species' probability of detection by an ocean glider. Perform a test at or close to SCORE and compare results with detection probability estimates derived using the SCORE array.

Methods

All deployment tracks from the GOA, HRC, and MIRC sites have been evaluated to assess the degree to which the planned track lines were adhered to. Cetacean detection records for all three sites have also been produced as part of other projects. Using these results, example species will be selected and encounter rates for these species will be calculated at the GOA, HRC, and MIRC sites. A literature review will be conducted to see whether encounter rates of the example species could be compared with encounter rates from previous visual surveys in these areas. Finally, using the estimated encounter rates, the required glider survey effort in these naval areas of interest to achieve density estimates with a reasonable level of precision will be estimated.

Data collected at SCORE will be used to develop a method to estimate the probability of detecting vocalizations on an ocean glider/ profiling float. For some species, the range hydrophones at SCORE can be used to localize individuals, effectively setting up detection trials for the ocean glider/profiling float, which can be modelled using a logistic regression approach (e.g., similar to Marques *et al*., 2009). Alternatively, in the event that localization is not successful for a given species, the range can likely be used as an array for spatially explicit capture-recapture methods (e.g., Borchers, 2012) or a simulation approach can be implemented using existing acoustic tag data and propagation modelling (similar to the approach used in Küsel *et al*., 2011). If deemed suitable, the detection function generated for the ocean glider will be used to estimate call densities from the surveys in the GOA, MIRC, and HRC. For species for which suitable call rate data are available, animal densities will be estimated.

A small field effort was also conducted (in July/August 2016) that will attempt to estimate detectability of cetaceans from a glider without using an instrumented Navy range. A glider and profiling float were co-deployed near Catalina Island, California, with autonomous non-profiling drifting hydrophones. The

drifting hydrophones will be used in the same way as the range hydrophones – either to localize vocalizations, which can be used to set up trials for the glider/profiling float, or to use the drifting hydrophones, glider and profiling float in a spatially explicit capture-recapture analysis, which would allow estimation of detection probability for each type of instrument.

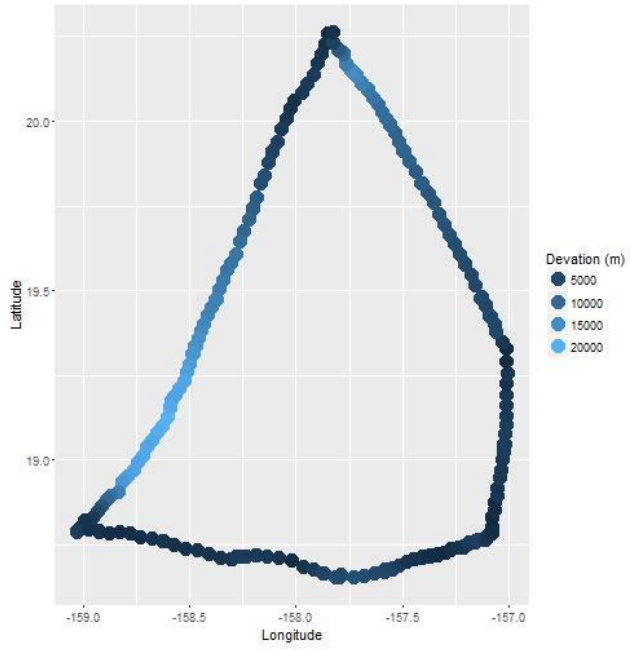
Results

Results will be presented from the four objectives that are currently ongoing (Objective 4 is not scheduled until FY2018).

Comparing GPS surface locations with the straight-line track between planned waypoints showed that the GPS locations could deviate up to 20.2 km from the planned track (Fig. 1), though the median deviation was only 1.6 km.

Based on the cetacean encounters at GOA, MIRC and HRC, and the encounters from both SCORE and the Catalina Island experiment, our provisional target species are fin whales, beaked whales (both Blainville's and Cuvier's), and Risso's dolphins. The choice of target species will be confirmed once all acoustic data from SCORE and the Catalina Island experiment have been fully analyzed. Localizations of baleen whales and beaked whale groups (identification to species level is ongoing) at SCORE have been provided by project partners and further analysis of those data is ongoing.

Cuvier's beaked whale encounters were recorded by the drifting hydrophone array during the Catalina Island experiment. Furthermore, the three-dimensional localization algorithm that will be used to locate as many beaked whales as possible from the drifting hydrophones has been demonstrated using data from the Catalina Island experiment.



Notes:

Fig. 1 Plotted GPS locations showing the spatial pattern of deviation from the planned track line for the HRC 2014 deployment.

References:

- Borchers, D.L. (2012) A non-technical overview of spatially explicit capture recapture models. Journal of Ornithology 152 (Suppl 2), S435-444.
- Küsel, E. T., Mellinger, D. K., Thomas, L., Marques, T. A., Moretti, D. & Ward, J. (2011) Cetacean population density estimation from single fixed sensors using passive acoustics. *Journal of the Acoustical Society of America* **129:** 3610-3622.
- Marques, T. A., Thomas, L., Ward, J., DiMarzio, N. & Tyack, P. L. (2009) Estimating cetacean population density using fixed passive acoustic sensors: an example with Blainville's beaked whales. *The Journal of the Acoustical Society of America* **125:** 1982-1994.

Application of Density Estimation Methods to Datasets Collected from a Glider

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Background

Population density estimation methods have been developed for fixed sensors. Slow moving platforms such as ocean gliders, can provide an inexpensive alternative for marine mammal population density studies. Using gliders it is possible to monitor a bigger spatial area than using a fixed passive acoustic recorder. It is a low-noise, low-speed platform, easy to set up, maneuver and transport on land, deploy, and recover. Furthermore, gliders can sense the environmental conditions of the survey area, which are important for estimating detection distances through propagation modeling. Finally, gliders can be fit with more than one sensor, which allows for tracking animals in azimuth (two sensors) and localizing individuals in range and depth (three or more sensors). The extra information (e.g. bearings to sound producing animals) derived from having more than one sensor can also be used to improve density estimates [1].

Objectives

The main objective of this work is to evaluate the use of moving, unmanned platforms, such as an ocean glider, for population density estimation. Current methodologies developed for fixed sensors will be extended to these platforms. A secondary objective is to test an inexpensive acoustic recording system comprising of two hydrophones connected to an off-the-shelf voice recorder installed inside the glider.

Methods

A first generation Slocum glider (Teledyne Webb Research) named Clyde, was equipped with two High Tech Inc. hydrophones (model # HTI-92-WB) mounted one on each wing at a separation of 3 ft., and connected to a linear PCM recorder manufactured by Tascam (model # DR-07 MKII). The data acquisition system offers a sample frequency of 96 kHz, and is capable of continuous recording of two channels of data at 16-bit resolution for up to 24 hours. An opportunistic experiment conducted in June 2014 in the Mediterranean Sea, off the west coast of the island of Sardinia offered the perfect conditions to test the glider's operation and the acoustic acquisition system. Several gliders were deployed perpendicular to the coast at regular intervals in order to collect oceanographic and/or acoustic data, as part of a major oceanographic and acoustics experiment (REP14-MED).

Following the REP14-MED sea experiment, a second generation Tascam recorder was devised. In order to have more control over the recording schedule, including the start time, a low-power microcontroller was linked to the digital recorder. More data storage was also added to the system, amounting to four 32 GB micro-SD cards, instead of only one in the original design. Data recording time was then increased from 24 hours to 96 hours. The new improved system was tested during two opportunistic sea trials, one in the Tyrrhenian sea, Italy, between August and September 2015 (GLISTEN15), followed by a two-week mission in the Gulf of Mexico, in October 2015.

During the Gulf of Mexico experiment a compact and self-contained acoustic recorder manufactured by Ocean Instruments NZ (SoundTrap 300 HF) was also fitted to the glider and placed externally underneath the science bay. This independent sound recorder offered bandwidths of 20 Hz - 150 kHz (± 3 dB), sampling frequencies between 36 and 288 kHz, and the capability to record data continuously for up to 13 days on internal batteries.

Results

During the span of the three opportunistic experiments major operational issues were observed in the glider and acoustic acquisition systems. Glider specific issues were fixed by sending it back to the manufacturer both in 2014 and 2015, after the experiments in Italy. One of the hydrophones installed on the glider's wings also stopped working during the GLISTEN15 cruise, and could not be fixed in time for the Gulf of Mexico deployment. Consequently, only the 2014 cruise data set contains data from both sensors. The second generation of the acoustic recorder also presented scheduling problems, even after many testing hours. Recording would

often stop for no obvious reasons, in the middle of a mission. Currently, a third alternative, the JASCO AMAR board is being tested with the PSU glider. This board offers higher sampling frequency, more storage capacity and the capability of recording up to 8 channels.

Data recorded during the REP14-MED contained echolocation clicks from sperm whales as well as clicks and whistles from unknown dolphin species. The data was recorded in deep waters (> 2000 m) off of Western Sardinia. A simple energy sum detector was run through the data for the detection of sperm whale clicks. The detected clicks on channel 1 were then cross-correlated with the data on channel 2 for the estimation of the time difference of arrival used to estimate bearing angles to clicking animals [2].

Over one hour and thirty minutes of data containing high activity of sperm whale clicking, were analyzed for bearing estimation. A few tracks could be realized from this analysis and it was also observed that most of them followed closely the deviations in glider heading (Fig.1). Due to left-right ambiguity of bearing estimations, the tracks were not corrected for glider heading. However, by resolving tracks more accurately, possibly using a particle filter, it is expected that the ambiguity can be resolved. A more accurate cross-correlator detector for this data set is currently being developed and its performance bounds are being calculated. Bearing angles are expected to be used as extra information for developing density estimation methods from two-hydrophone slow-moving gliders.

Notes:

[1] Borchers, D.L., Stevenson, B.C., Kidney, D., Thomas, L., and Marques, T.A. (2015). "A unifying model for capture-recapture and distance sampling surveys of wildlife populations," J. Amer. Statist. Assic. ***110****(509), 195-204.*

[2] Küsel, E.T., Munoz, T., Siderius, M., Mellinger, D.K., and Heimlich, S. (2016). "Marine mammal tracks from two-hydrophone acoustic recordings made with a glider," Ocean Sci. Discuss., doi:10.5194/os-2016-48, in review.

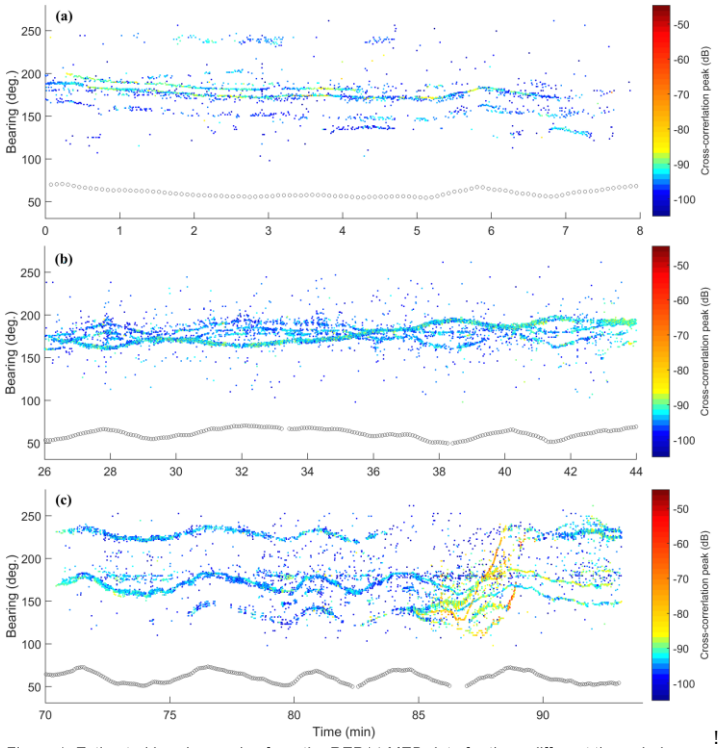

Figure 1. Estimated bearing angles from the REP14-MED data for three different time windows. The glider's heading is shown as the grey circles on the bottom of each plot. The colorbar represents the magnitude of the cross-correlation peak in dB.

Comparing manned and unmanned aerial surveys of cetaceans in the Arctic: Preliminary results

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Background

Manned aerial surveys are routinely used to collect data to infer cetacean distribution and density (e.g., Buckland et al. 2001, Garner et al. 1999). Although decades of valuable marine mammal research and monitoring have been conducted successfully from manned aircraft, this approach does have some well-known limitations, including concerns about safety, observer fatigue which can affect data collection, the potential to disturb wildlife, and the high cost of fuel. Unmanned aerial systems (UAS) have been identified as a technology that could augment or replace manned aerial surveys for cetaceans, particularly in remote areas where human observations are challenging due to safety or logistical reasons. To understand whether current UAS technology can be used to collect data to infer distribution and density, a direct comparison of data collected by manned and UAS surveys was necessary. NOAA Fisheries' Alaska Fisheries Science Center led a collaborative effort to conduct and compare manned and unmanned aerial surveys for cetaceans that included the Naval Surface Warfare Center Dahlgren Division (NSWCDD), the Bureau of Ocean Energy Management, U.S. Office of Naval Research, the NOAA UAS Program, the NOAA Fisheries Office of Science and Technology, Shell, and the North Slope Borough.

Objectives

The objectives of our project are to conduct a 3-way comparison of data and derived statistics from human observers in the manned aircraft, digital photographs from cameras mounted in the manned aircraft, and digital photographs from cameras mounted to the unmanned aerial vehicle (UAV).

Methods

Field operations occurred from August 21 to September 7, 2015, north of Barrow, Alaska, using a twin engine Turbo Commander operated by Clearwater Air, Inc. and a ScanEagle® UAS operated by the NSWCDD. The ScanEagle® was operated under a Certificate of Authorization from the Federal Aviation Administration (FAA) that allowed beyond visual line-of-sight flights. Imagery was collected by a Nikon D810 camera system on the Turbo Commander, and by a similar camera system on the ScanEagle®. Flights by both platforms were conducted on pre-determined transect routes at a line density expected to provide sufficient sightings to allow statistical comparisons between survey types. Imagery from both digital camera systems was reviewed manually to search for marine mammals. Preliminary estimates of density and total numbers of whales in the area were calculated.

Results

Operational results: Each platform conducted five flights within a 16,800-km^2 study area; the manned aircraft collected 44,849 images in 26.7 flight hours, and the UAS collected 24,600 images in 21.8 flight hours. The platforms were limited by the same weather; the manned aircraft had the ability to fly through visible precipitation to access improved sighting conditions. The harsh environmental conditions of the Arctic increased the maintenance required for ScanEagle® operations. Technology that directly contributed to the ability to conduct UAS flights in the study area included: software that provided a direct link to the FAA's radar system, which enabled de-confliction with local aircraft; temperature and humidity sensors on the ScanEagle®; software that provided near-term forecasts of cloud cover and precipitation; and a portable weather station.

Evaluation of images and cetacean abundance estimation: Manual image processing and analysis by marine mammal photo analysts required 332.5 total hours, averaging 6.9 hours to analyze one flight hour, which involved reviewing every third image. More whales were seen by human observers than in the images collected from either the manned aircraft or the UAS (Table) because the human observers effectively searched a much larger area than captured in the digital images. The coefficients of variation (CV) for the estimated number of whales in the study area were nearly always lower for human observers than for either

imagery dataset. The CVs for all datasets were higher than expected because of sample size limitations; weather limited the number of hours we were able to fly more than expected based on manned aerial surveys conducted in the study area in previous years. The cost of the UAS surveys and associated data processing was an order of magnitude higher than the cost of the manned aerial survey and associated data processing; we expect the cost of the UAS survey to decline as new, less expensive UAS and automated image analysis tools become available.

Notes:

Table: Number of whales detected on transect, effective area covered, estimated total number of whales, and coefficient of variation (CV) of the estimated total number of whales in each survey area. The numbers of sightings in this table are the subset of the total number of sightings made in the survey area, in Beaufort Sea State ≤ 5 (bowhead and gray whales) or ≤ 4 (belugas), during level flight over the designated transect lines. The human observer sightings were limited to those made by primary observers. The effective strip half-width (ESW) is the distance on one side of the trackline that would contain the same number of sightings if detection probability were equal to 1.0 as were actually detected during the survey.

	West survey area				East survey area			
	Images		Human observers		Images		Human observers	
	UAS	Manned aircraft	2015 dataset for ESW	Historical dataset for ESW	UAS	Manned aircraft	2015 dataset for ESW	Historical dataset for ESW
Bowhead whales								
# of whales on transect	3	2	9	9	6	4	12	12
Area surveyed (km^2)	525.4	646.0	3729.7	5989.8	448.5	645.9	3257.7	5231.8
Estimated total # whales	35	19	40	27	69	32	51	36
CV(whales)	0.77	0.71	0.51	0.46	0.53	0.45	0.41	0.34
Beluga whales								
# of whales on transect		0	0	0	6	11	22	22
Area surveyed (km^2)	525.4	646.0	2046.0	983.5	448.5	645.9	1661.4	798.6
Estimated total # whales	0	0	0	0	69	87	181	138
CV(whales)					1.02	0.67	0.73	0.76
Gray whales								
# of whales on transect	1	0	0	0	2	0	0	0
Area surveyed (km^2)	525.4	646.0			448.5	645.9		
Estimated total # whales	10	0	0	0	23	0	0	0
CV(whales)	1.04				1.01			

Acknowledgements: This project required hard work and dedication from a large team, including Philip Hall, Van Helker, Bob Lynch, Amy Willoughby, Amelia Brower, Janet Clarke, and the flight teams from the Naval Surface Warfare Center Dahlgren Division and Clearwater Air, Inc.

Additional information on the field methods is available at:
http://access.afsc.noaa.gov/pubs/posters/pdfs/pAngliss01_uas-arctic.pdf

Exploring the Limits of Thermal Automatic Whale detection (ETAW)

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Background

Spotting marine mammals for mitigation purposes in the context of naval sonar or air-gun deployments is a difficult and tedious task. Commonly is implemented by visual observations and/or possibly continuous passive acoustic monitoring (PAM). Both methods however suffer from significant constraints, limiting their general applicability. PAM, for example, critically depends on the animal being vocal, while visual observations necessarily depend on day-light.

Independent of such confining factors, infrared (IR) or thermographic imaging (TI) may be employed 24/7 to detect marine mammals on the basis of differences between their body's or blow's thermal radiance and that of the background, i.e. the sea surface (Figure 1).

Utilizing a 360° state-of-the-art thermal imaging sensor, FIRST-Navy, Zitterbart et al. (2013) demonstrated the efficiency of thermal imaging based automatic whale detection in waters cooler than 10°C. Their study focused on the cold water realm, excluding warmer waters and hence corresponding evaluations. Contrastingly, this project set out to explore the sea surface temperature (SST) supremum up to which thermal imaging based whale detection is still feasible.

Objectives

Whether an analyst is capable of perceiving the mammalian cues against the background depends on environmental conditions (e.g. sea-surface temperature), technical parameters (e.g. IR-sensor resolution), and animal specifics (e.g. distance, blow height). *Thermal perceptibility*, and how it is constrained by abovementioned set of co-variates, is hence the fundamental question to ask, when exploring the regional limits of thermographic imaging for marine mammal detection.

With TI providing *per se* a digital video stream, the desire for a computer based, automatic detection algorithm for marine mammals is obvious. Such algorithm should be capable of reliably detecting marine mammal cues while avoiding false alerts from birds, glare, waves, boats, etc.. *Thermal detectability* evaluates the balance between correct alerts (true positives) to false alerts (false positives), which will also depend on abovementioned sets of parameters. Costly mitigation decisions will of course not solely be based on the output of computer algorithms. More realistically, a marine mammal observer will validate the algorithm's automatic alerts in near real-time by reviewing the recorded video footage and conducting additional direct visual observations. Combining the IR-systems unwavering 360° vigilance with the human's supreme analytical capabilities, this approach is expected to minimize false alerts while improving on the number of correct alerts, resulting in traceable mitigation decisions and an increased situational awareness. The joint performance of this *observer validated thermal detection* hence represents best practice in an operational setting as to be encountered during naval exercises or seismic surveys.

Methods

Thermographic data were collected using a FIRST NAVY thermal imager at field sites on North Stradbroke Island, Australia (2014) and Kauai, USA (2016). The sensor was mounted on decks overlooking the ocean with fields of view ranging from 90 to 120°. TI data was acquired for a total of 1780 hours (74 days). Observers concurrently scanned the ocean either with the naked eye or binocular for a total of 331 hours. A theodolite was used for a total of additional 170 hours to obtain high-precision localizations of cues. Environmental data was recorded on-site or obtained from nearby weather stations. Further thermographic data was acquired on Kauai using an IRCAM GEMINIS 327k ML infrared dual band camera.

The subtropical/tropical environment required the development of a more generalized detection algorithm exploiting the differential local contrast (DLC) between adjacent image tiles rather than the temporal evolution of the contrast (short-term over long-term). Persistent thermal signatures typical for the costal setting of the experiment (rather than an off-shore environment), such as stand-up paddlers or small boats required implementing a context based classifier, which uses trajectory parameters of thermal anomalies to classify and discard these permanent signatures.

Results

To an informed analyst, *thermal perceptiblilty* of whale groups was more than 90% of the groups sighted visually for tropical and subtropical conditions. A direct comparison between visual sightings and IR sightings reveals a linear relationship between distances and perceptibility (averaged over all environmental conditions and surface displays encountered).

Thermal detectability was reliable up to 2.5 km in subtropical and tropical waters. Detections exceed sightings by up to a factor of 2, probably due to the observer being distracted by manually logging frequent events. However, the absolute performance of thermal detection depends on environmental conditions and recoding setup.

Within a range of 2 km, *observer validated thermal detection* reproduced all sightings logged by (unassisted) marine mammal observers (MMOs). The unassisted MMOs on the other hand, noted only 61% of the pods automatically detected and validated by the (independent) TI-assisted observer.

Conclusion

We found that TI works surprisingly well for marine mammal perception and detection in waters up to 26°C/70°F, which corresponds to all but the equatorial ocean regime (which remains to be tested). Throughout the project, it became clear that SST is not the primary driver of thermal perceptibility and detectability. TI based marine mammal detection benefits from the fact, that the thermal radiance significantly decreases with increasing emission angles (relative to zenith), i.e. a near-surface camera viewing the ocean at a glancing angle receives significantly less radiance from the sea surface than from a body or blow droplets with surfaces perpendicular to the optical path, resulting in an improved signal to noise ratio. Additionally, cloud cover, cloud base temperature, relative humidity, dew point, blow strength and thermodynamics, type and frequency of surface display, and sensor height all influence thermal perceptibility and detectability.

While our data clearly demonstrates good thermal perceptibility in the warm water realm, for thermal detection the challenge prevails to further reduce false alerts to a minimal value, and to make the detection algorithm real-time auto-adaptive to animal specifics and environmental conditions and to ensure an optimal balance of false alerts and missed events.

Notes: 2013, Zitterbart DP., Kindermann L., Burkhardt E., Boebel O. “Round-the-Clock Protection of Whales from Underwater Noise by Thermal Imaging,” PLoS ONE 8(8): e71217. doi:10.1371/journal.pone.0071217 (2013).

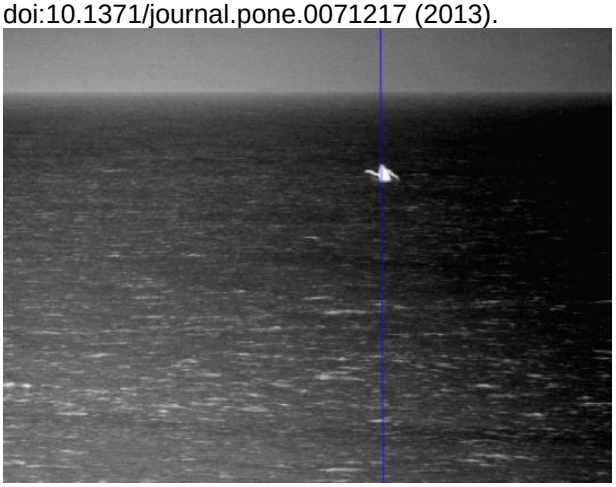

Figure 1: Night-time thermographic image of breaching humpback whale at 750 m distance off Kauai on 04 Feb 2016, 20:01 (sunset 18:28; end civil twilight 18:52) in waters of about 25°C/77°F.

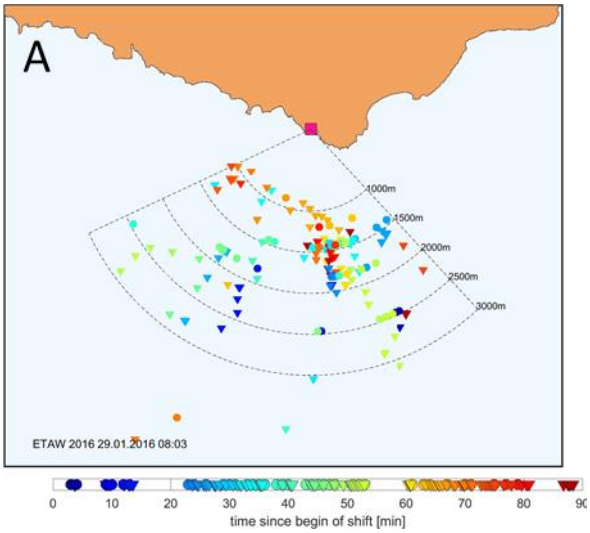

Figure 2: Locations of cues detected off Kauai’s south cost during a single marine mammal watch. IR detections are depicted as filled circles, while visual observations are shown as filled triangles.

eDNA barcoding: environmental DNA for detection and identification of cetacean species

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Background

Molecular identification of species, or 'DNA barcoding', is a powerful tool for routine taxonomic identification of cetaceans, some of which are difficult to identify from appearance or morphology alone (Dalebout *et al.* 2004). Until recently, however, the utility of DNA barcoding as a tool for genetic monitoring of cetacean populations has been limited by the requirement to collect a genetic sample (e.g., a biopsy sample, sloughed skin or feces) from the immediate proximity of the individual. Advances in analyses of environmental (e)DNA now offer an alternative to the limitations of direct sampling of marine and aquatic species in some situations (Taberlet *et al.* 2012). Here we are developing next-generation methodology for detection and species identification of cetaceans using environmental (e)DNA collected from seawater. Referred to here as '(e)DNA barcoding', this new methodology could be used to detect and identify the presence of (non-vocal) cetaceans in the field and identify the species of unknown cetacean vocalizations to improve the accuracy of ongoing passive acoustic monitoring, especially for poorly described species such as beaked whales.

Objectives

1) Develop methods for eDNA sampling and extraction and design PCR primers for eDNA barcoding of cetaceans.
2) Conduct field work to collect eDNA from the vicintiy of killer whales around San Juan Islands, at known distances and times after the passage of the whales.
3) Analyze eDNA samples collected from vicinity of killer whales by extraction, amplification, quantification (by digital droplet PCR) and sequencing.
4) Conduct fieldwork to collect eDNA in an open-ocean environment to be decided dependent on the eDNA thresholds estimated from and in consultation with the Navy.
5) Analyze eDNA samples collected during open-ocean sampling by amplification, quantification (by ddPCR) and sequencing by conventional and next generation metabarcoding.
6) Finalize report on detection and indentification of cetacean species from eDNA and dissemate results by publication and presentation at workshops and conferences.

Methods

We conducted a series of eDNA sampling experiments near San Juan Island in Puget Sound during two weeklong periods of fieldwork in August and September, 2015. We collected seawater from the vicinity of killer whales *Orcinus orca* on 25 encounters. Starting 200 m behind the pod (to comply with local whale watching regulations), we collected serial samples at 15-minute intervals for up to two hours following the passage of the whales. We used a GPS drift buoy to maintain our position in the water mass after the whales passed.

The seawater samples were filtered, on site, through a 0.4 micron track-etched polycarbonate filter using a portable Nalgene™ filter apparatus and low pressure vacuum pump. The filters were stored in Longmire's solution for transport back to a 'clean room'. eDNA was extracted from the filters by conventional phenol/chloroform methods. PCR inhibitors were removed using the PowerClean® Pro DNA Clean-Up Kit(MoBio) or reduced by diluting the extracted eDNA.

We used ddPCR, a powerful new technology for quantifying low levels of DNA by fractionating a PCR reaction into more than 20,000 droplets using a water-oil emulsion (Doi *et al.* 2015). Amplification of the target DNA is quantified by incorporating a fluorescent dye into the PCR reaction or into a molecular probe designed to target a specific sequence. The target-positive and target-negative droplets are individually counted by passing them in a single stream through a flow cytometer. The ratio of the target-positive to the target-negative droplets is used to estimate the number of copies of the target DNA in the sample. To optimize the detection and identification of eDNA from cetaceans, we designed PCR primers using available reference sequences for the mtDNA control region of the killer whale.

Results

We have now quantified the copy number of eDNA from the 77 samples of eDNA collected after the passage of killer whales, with a series of experimental controls (positive and negative), using the BioRad QX200 Droplet Digital™ instrument. All samples are run in duplicate and each has now been run using the Evagreen dye incorporation and repeated using the molecular probe. The results confirm detection of species-specific eDNA but, as expected, show considerable variation. Using the negative controls to establish an initial baseline, we consider that samples with > 0.5 copies/µl are high probability detections. Samples with estimates of > 0.1, but less than 0.5 copies/µl, are lower probability detections. In a few encounters, samples yielded very high estimates of eDNA, e.g., greater than the positive control with 60 copies/µl. Although our analyses are still underway the results to date confirm the ability to detect the eDNA of killer whales, in some cases for up to one hour after the passage of the whales, despite the

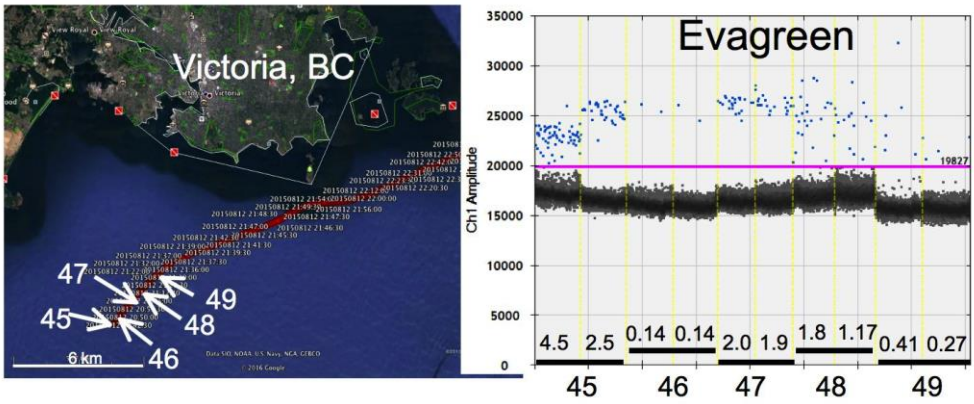
dynamics of tidal movement on the water column. An example of the detection of eDNA across a serial sample of one hour and drift of several kilometers is provided in the **Fig 1**.

To confirm that the eDNA detected by the ddPCR was, in fact killer whale, we attempted to re-amplify and sequence the target fragment by conventional PCR. Although conventional PCR was not successful for all samples that tested positive with ddPCR, there were 6 samples that yielded sequences of sufficient length and quality to confirm the species identity of the killer whales. Two of these samples (#47 and 48) are represented in the **Fig 1**. From one encounter, there were three serial samples (#94-96) of sufficient qualify to sequence over 700 bp of the mtDNA control region. Using available reference sequences to define diagnostic sites for known ecotypes and communities in the North Pacific (Parsons *et al.* 2013), this sequence length allowed us to confirm that the encounter was a pod of the southern resident community. This is promising for identification of not only species, but also ecotype and stocks in other species.

Having successfully completed the first phase of the project (Objectives 1-3), we are now prepared to considering logistics for the second phase - sampling in an open-ocean environment, supported by a fixed or towed acoustic detection array to locate cetaceans. The location and target species for will be decided in consultation with the Navy.

Notes:

Figure 1: The location of samples collected during an encounter with killer whales on August 12, 2015 and the result of the ddPCR quantification of eDNA from serial samples. *Left*) Location of five serial samples (#45-49) as determined by the GPS of the drift buoy deployed initially 200 m after the passage of the whales. *Right*). The visualization and analysis of five serial samples (run as replicate pairs) using the software QuantaSoft™. The estimated copies/µl of eDNA from the killer whales measured by the number of target-positive droplets (shown in blue) above the baseline of target-negative droplets (shown as the dark cloud). The purple line shows the upper threshold of the target-negative droplets calculated from the negative controls. The black bars indicate the pairs of replicate samples. The calculated copies/µl are shown above the black bars for each of the replicate samples.



Literature cited

Dalebout, M.L., C.S. Baker, V.G. Cockroft, J.G. Mead and T.K. Yamada. 2004. A comprehensive molecular taxonomy of beaked whales (Cetacea: Ziphiidae) using a validated mitochondrial and nuclear DNA database. Journal of Heredity 95:459-473.

Doi, H., K. Uchii, T. Takahara, S. Matsuhashi, H. Yamanaka and T. Minamoto. 2015. Use of Droplet Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys. PLoS ONE 10:e0122763.

Parsons, K.M., J.W. Durban, A.M. Burdin, *et al.* 2013. Geographic patterns of genetic differentiation among killer whales in the northern North Pacific. Journal of Heredity: est037.

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. Molecular Ecology 21:1789-1793

Low power, two-channel marine mammal recorder

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Background

The goal of this project is to develop a low power, two-channel, wideband marine mammal recorder (referred to internally as the PAAM Rev. C board) for use in undersea gliders and other energy-constrained sub-sea platforms.

The multi-channel recorder is motivated by a desire to better localize marine mammals in support of density estimation.

Objectives

The primary technical objective for this project is to develop a two-channel hydrophone recorder that can be used by marine mammal researchers to record high fidelity time series for a wide range of marine mammals. The recorder will be capable of recording at sampling rates up to 100 kHz simultaneously on two channels. The second hydrophone and associated signal conditioning/recording electronics is motivated by a desire to better localize marine mammals in support of density estimation. A two-channel recorder with synchronous sampling across the two channels will allow researchers to estimate the direction-of-arrival of signals in the horizontal plane (if the hydrophones are installed with a horizontal offset such as on the wing tips of a glider) or in the vertical plane if the hydrophones are mounted with a vertical offset (e.g., top and bottom of an undersea glider hull).

Low power is also a primary objective for the recorder. Undersea gliders, which are one possible platform for this technology, are capable of staying out for multi-month missions provided their payload is sufficiently low power. For example, Seaglider undersea gliders configured for basic oceanographic work (CTD sensing) routinely operate for ten months. The addition of additional payload modules, such as a marine mammal recorder, reduces the duration. Our goal is to keep the recorder power low enough such that Seaglider missions of duration 2-3 months are feasible.

Methods

Our approach builds on two previous projects, the PAAM Revision B development effort and another recorder project ongoing at APL-US within Dr. Craig Lee's Integrative Observational Platform (IOP) group. The IOP group, under NSF funding, developed a single channel marine mammal recorder board (PMAR) that is capable of sampling rates up to 100 Ksps (kilo samples per second). The PMAR board is based on very low power STMicroelectronics ARM Cortex controller and supports time series storage to a bank of up to seven sdCards.

Our approach is to merge the best of both of the above-mentioned projects. Specifically, the front-end signal conditioning and digitization approach demonstrated quite successfully in the recent NAVFAC program (HDR) and the very low power ARM Cortex processing and storage electronics/software developed by the IOP group.

Key individuals involved in the development include Geoff Shilling and Dr. Jason Gobat. Mr. Shilling and Dr. Gobat are the key developers of both the Seaglider software and the PMAR board.

Results

The board will be tested in a month long deployment of two Seagliders off of the Washington coast.

Notes:

The DMON2: A commercially available broadband acoustic monitoring instrument

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Background

There is currently an urgent need to autonomously record, detect, classify, and report marine mammal calls for both research and mitigation applications. For marine mammal research, such a capability would greatly improve the efficiency of finding animals at sea for study (e.g., for tagging or photo-identification). For the National Oceanic and Atmospheric Administration (NOAA), such a capability would allow improved monitoring of the distribution and occurrence of vocalizing animals for improving our understanding of stock structure and characterizing anthropogenic threats. For both the Navy and some industries that are interested in mitigating their interactions with marine mammals, real-time detection can augment and improve the efficiency of traditional detection methods (e.g., aerial and shipboard surveys), while providing persistent surveillance for marine mammals when traditional methods are ineffective (e.g., at night, during rain, fog, snow, or high winds).

To meet this urgent need, engineers at the Woods Hole Oceanographic Institution developed the digital acoustic monitoring (DMON) instrument, a passive acoustic device capable of recording and processing audio aboard a variety of autonomous platforms. The original DMON was conceived as an open-design programmable passive acoustic instrument, but in early 2010, the DMON was determined to be a defense article by the U.S. Department of State because its "open architecture software and source code allow users to easily tune the device for other purposes, including submarine detection" (U.S. Department of State Commodity Jurisdiction Determination Letter for the DMON, January 21, 2010). This prevented the use of the DMON outside of U.S. territorial waters without an export license, severely restricting its application. Our long-term goal is to make this very capable instrument available to the broader scientific, governmental, and industrial communities for use in mitigation, science, and conservation applications.

Objectives

Our objectives are to develop the DMON2, a new version of the DMON that will (1) allow full spectrum (10 Hz to 160 kHz) recording and processing, (2) be exempt from licensing jurisdiction from the Department of State and Department of Commerce, thus allowing international sales and operation, (3) be much easier to manufacture in commercial quantities, and (4) be much easier to maintain both at the manufacturing facility (WHOI) and in the field. We will obtain a new commodity jurisdiction for the DMON2 that will allow it to be used outside of U.S. territorial waters and exported for international purchase and use. We will change the design to accommodate the new commodity jurisdiction as well as to improve manufacturability, maintenance, and performance of the instrument. Upon completion of this project, we will make the DMON2 commercially available to both domestic and international customers using the same business model as the very successful, industry-standard, acoustic communication device, the WHOI Micro-Modem.

Methods

The DMON2 hardware components will be identical to those of the current DMON, retaining all of the functionality of the original instrument. Features that are prohibited by the DMON2 commodity jurisdiction (e.g., external synchronization of the real-time clock) will either be hardware or software disabled. In essence, we will manufacture DMON hardware that will be sold to the vast majority of customers as DMON2 instruments after minor proprietary hardware and software modifications are made. The DMON instrument (i.e., the manufactured instrument without the modifications) will continue to be available for sale to users with an appropriate export license.

Several changes will be made to satisfy the new commodity jurisdiction. A pressure transducer will be included in the hydrophone head so that the transducer output can be disabled when the system exceeds 1000 m depth. The external PPS timing circuit will be disabled; thus the

instrument's real-time clock cannot be externally synchronized, and it cannot therefore be configured in arrays. Finally, only authorized firmware will be allowed to run on the DMON2; the instrument will not be programmable.

Changes will be made to the design to allow the DMON2 to be much simpler to build and easier to maintain. These changes will not add significantly to the overall size and weight of the device, but are economically essential to making the DMON2 commercially available. We will develop and use a standard, non-oil-filled pressure housing to allow easy and quick access to the card set, internal battery, and hydrophones. We will also make several changes to improve assembly and maintenance, including a modular circuit board design and simpler, more reliable connectors. These changes will make the DMON2 easier to build and bench test (reducing assembly costs) and they will enable field replacement of defective components by the user. The memory capacity will be increased from 32 GB to 128 GB, with the option to include an additional memory board with even more flash memory. The internal battery capacity will be increased from 5 to 10 A-hr to allow for longer deployments. The following is a summary of the DMON2 design:

Overall concept:

- Adoption of a two component system: (1) Main electronics and battery housing and (2) modular (detached) transducer head.
- Manufacturability, ease of assembly and service, reduced cost and export classification all contributed to the need for a new system design.
- Seamless integration of the DMON2 with multiple platforms: Slocum and Wave Gliders, moored real-time buoys and bottom-mounted moorings.

Main electronics and battery housing:

- Use a standard pressure housing with a single end cap for external connections.
- Combine existing main and audio printed circuit boards into a single board design.
- Retain rechargeable lithium polymer for standard operations (increase capacity for longer operations)
- Accommodate add-on battery inside the pressure housing for extended operations.
- Accommodate an additional circuit board with added flash memory.

Transducer head:

- Adoption of a modular 3-hydrophone (low-, mid-, and high-frequency) design.
- Add an analog pressure sensor, LEDs, EEPROM, and serial (I2C) comms.
- Selection of pressure transducer that will operate when encapsulated in urethane.

Results

Commodity jurisdiction request applications were prepared in consultation with the U.S. Naval Undersea Warfare Center and submitted in March 2014. The DMON2 was determined to be outside the jurisdiction of the Department of State (June 2014) and received an Export Control Classification Number (ECCN) of 6A991 by the Department of Commerce (July 30, 2014), allowing it to be exported. The system level design was finalized in 2015, including the circuit board dimensions, battery options, main electronic housing, and separate transducer head. A prototype transducer head was fabricated for a Slocum glider and used in 2 deployments of 1 and 4 months duration during 2016 (Figure 1). The main electronics printed circuit board (PCB), housings, and transducer heads were finalized and fabricated in late 2016.

Notes:

Figure 1. A WHOI Slocum glider with DMON2 hydrophone head installed (brown dome at top/middle of glider). Glider shown after recovery from a 4-month mission in the western Gulf of Maine.

The development of advanced passive acoustic monitoring systems using microMARS technology

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Background

Although the U.S. Navy has good capabilities to monitor the acoustic behaviors of cetaceans in order to examine responses to acoustic disturbances on hydrophone-instrumented ranges, at off-range locations acoustic detection and localization capabilities remain limited. Our work focuses on the development of a portable passive acoustic monitoring and sound localization system, preliminarily named SonarPoint. SonarPoint consists of an array of small, precision time synchronized acoustic recorders.

Objectives

The project has two main objectives:

1. Development of all the components of a compact passive acoustic monitoring system suitable both for shallow water moored configurations including solar power, limited satellite real-time reporting and GPS time synchronization, and self-synchronizing deep water configurations that do not requitre surface equipment. These components including the localization capable recorder, the surface buoy electronics and hardware, an acoustic pinger for deep-water self-synchronization and an acoustic release for data recovery.

2. Field testing and validation of the system through two test series, first targeting the GPS synchronized shallow water and then the self-synchronized deep water configurations.

The project will conclude with the availability a commercialization ready passive acoustic system suitable producing a tightly synchronized multi-channel .wav file suitable for localization of marine mammal vocalizations using standard algorithms.

Methods

The design objectives for this system are ease of use, high reliability and flexibility in deployment configurations. The three main deployment configurations include an acoustically synchronized seafloor array, a surface buoy/moored array, and an ocean drifter array. These flexible configurations are intended to support a variety of use scenarios. For example, the system might be deployed within minutes by small boat when cetaceans are sighted and are recovered after the encounter is completed. Extended endurance deep water deployments is supported by deploying as an acoustically synchronized seafloor array without surface gear, using the associated acoustic releases for recovery. When semi-permanent monitoring is called for in a shallow water environment, a moored configuration with a surface buoy provides GPS synchronization and solar power for operation without a fixed endurance limit.

Results

Progress towards commercial availability, a key objective to maximize the system's value to the community, was accelerated by building SonarPoint on the foundation of the recently developed microMARS acoustic recorders. We incorporated improvements based on lessons learned from that effort, while adding new functionality and capabilities including sound source localization.

We have reached the early field test stages, the results of system tests in a reservoir including operational reliability and results of pinger tracking tests are discussed. We will also discuss the next steps in the validation and commercialization of the system, our progress and outlook in making it readily available to the research and resource management community.

Notes:

Field testing and performance evaluation of the Long-term Acoustic Real-Time Sensor for Polar Areas (LARA)

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Background

Most state-of-the-art passive acoustic monitoring systems are designed to stay submerged for the entire deployment period (for a detailed summary see Mellinger *et al.*, 2007). Deep-moored instruments feature a number of advantages. For example, they are not subject to the wear and tear caused by surface waves, and they are farther from the noise created by those waves. However, with archival instruments it is not possible to access data, gain timely information on the presence of acoustic signals of interest (e.g., marine mammal vocalizations or seismic events), or identify system malfunctions prior to instrument recovery. Furthermore, it is not possible to update the system clock by GPS, which might drift significantly during long-term deployments and hinder accurate localization of sound sources when using multiple instruments (e.g., for tracking vocalizing animals) in an array configuration. A few passive acoustic monitoring systems use a surface buoy to overcome some of these disadvantages, but these cannot be reliably operated in polar areas with potential ice coverage. In addition, surface buoys are exposed to ocean surface waves, which can cause cable strumming (inducing acoustic noise) and can potentially be vandalized or damaged by collisions with vessels.

Objectives

With ONR/DURIP funding, we developed the Long-term Acoustic Real-Time Sensor for Polar Areas (LARA), which combines the advantages of both submerged and surface acoustic observing systems. LARA makes stationary passive acoustic monitoring efforts more effective, and provides maximum flexibility allowing for a wide range of applications in ice-covered polar areas. While the ONR/DURIP award provided funding to design and manufacture the prototype unit, it didn't provide funding for field testing and performance evaluation. The current project is allowing us to conduct initial short-term deployments of LARA in the Pacific Northwest followed by a one-year deployment in the seasonally ice-covered northern Bering Sea in 2017-18 (field work conducted in collaboration with NOAA's Pacific Marine Environmental Laboratory). Throughout the deployment we will closely monitor LARA remotely from Newport, OR and make changes to the firmware if necessary. After recovery, full data analysis and performance evaluation of the recorded acoustic and engineering data will be conducted.

Methods

LARA combines the advantages of both submerged and surface systems. The real-time information system makes stationary passive acoustic monitoring more effective and provides maximum flexibility, allowing for a wide range of applications even in ice-covered areas. In addition, the vertically-moving components of the winch system contain a CTD (conductivity, temperature, and depth) sensor that monitors oceanographic conditions, allowing estimation of the sound speed profile and thus better understanding and modeling of ocean acoustic propagation in the deployment region. LARA is deployed on a typical oceanographic mooring at a predefined depth (current system limited to 300 meters) to detect and record acoustic signals of interest. The control module in the sensor unit runs a sea-ice sensing algorithm based on the temperature and salinity profile in the upper water column. This algorithm has been proven to reliably detect sea ice in the Southern Ocean (Klatt *et al.*, 2007). LARA operates an on-board acoustic event detector (Klinck and Mellinger, 2011) targeting beluga whale (*Delphinapterus leucas*) echolocation clicks in real-time. After an event is detected (or at a pre-defined time interval), a command is sent by the sensor unit to the winch via hydro-acoustic modem to raise the sensor unit to about 10 m depth. During this process a temperature and salinity profile is measured. Based on these measurements, the control module decides whether or not to surface the sensor. If the "no sea ice criterion" is fulfilled, the control module sends a command to the winch to surface the sensor unit. To further reduce the risk of damage by ice and other surface obstacles, only the antenna is raised to the surface; the actual

sensor unit stays at a safe depth of about 10 m. A bi-directional communication link to shore is established via an Iridium satellite connection.

Results

LARA was first deployed from the *R/V* Hayes in Lake Washington, Seattle, WA, USA on 28 September 2016 and recovered after ~18 h of operation to make changes to the firmware. After the firmware modifications were completed, LARA was re-deployed at the same location (N47.669007°, W122.234655°. water depth approximately 60 m.) on 29 September 2016 and operated for ~1.5 days. During this period LARA was programmed to surface a total of 5 times. The communication between the winch and the sensor module worked flawlessly. At the pre-programmed times, the sensor module triggered the winch which raised the sensor module from 30 m depth to 9 m depth. The antenna module which was connected to the sensor module via a 14 m cable surfaced and transmitted engineering data back to shore. The transmissions took on average ~ 5 min. After the transmission was completed, the sensor unit signaled the winch to be lowered back down to 30 m depth again. During the ascent and decent of the sensor unit, temperature and conductivity profiles were recorded by the sensor unit. Temperatures varied between ~18°C and ~9°C at 9 m and 30 m, respectively. Conductivity varied between ~0.0515 S/m and ~0.0450 S/m, corresponding to ~0.38 and ~0.29 PSU (Lake Washington is primarily freshwater), at 9 m and 30 m, respectively. Similar profiles will provide crucial information on sea ice coverage during the deployment in the northern Bering Sea.

References:

- Klatt, O., Boebel, O., and Fahrbach, E. (2007): A profiling float's sense of ice. *Journal of Atmospheric and Oceanic Technology*, 24, pp. 1301-1308.
- Klinck, H., and Mellinger D.K. (2011): The Energy Ratio Mapping Algorithm (ERMA): A tool to improve the energy-based detection of odontocete echolocation clicks. *Journal of the Acoustical Society of America*, 129, pp. 1807-1812.
- Mellinger, D.K., Stafford, K.M., Moore, S.E., Dziak, R.P., and Matsumoto, H. (2007): An overview of fixed passive acoustic observation methods for cetaceans. *Oceanography*, 20, pp. 36-45.