ONR
Marine Mammal & Biology Program Review

22 - 25 April 2024

Abstract Book

Start Time	End Time	Duration	MMB Topic	PI Last Name	Institution	Award Number	POP	Title
Monday 22 April 2024								
30-35 Minute talk - Progress Review or External Peer Review								
8:30	9:00	30 min	Mike - Welcome / Program Overview / Update					
9:00	9:30	30 min	EoS-BRS	Southall	Southall Environmental Associates, Inc.	N000141912572 N000142312497	8/19 - 7/23 6/23 - 9/25	Behavioral and physiological response studies (BPRS) with social delphinid cetaceans using operational and simulated military mid-frequency active sonar
9:30	10:00	30 min	EoS-BRS	Thomas	University of St. Andrews	N000141812807	9/18 - 3/23	Double MOCHA: Phase II Multi-study Ocean acoustics Human effects Analysis
10:00	10:30		Break					
10:30	11:00	30 min	Mon and Det	Roch	San Diego State University	N000142112567	9/21 - 9/24	Machine learning detection of cetacean tonal calls without human annotations
11:00	11:30	30 min	Mon and Det	Nosal Co-PI : Henderson (NIWC)	University of Hawaii	N000142212772	10/22 - 9/24	MAMBAT (Multiple-Animal Model-Based Acoustic Tracking): Improving underwater passive acoustic multi-tracking methods
11:30	11:45	15 min	Mon and Det	Baumgartner	Woods Hole Oceanographic Institution	N000141812810	7/18 - 2/23	The wide-band detection and classification system
11:45	12:00	15 min	Mon and Det	Zitterbart	Woods Hole Oceanographic Institution	N000142212832	10/22 - 9/25	Impact of the Beaufort lens on the bowhead whales' acoustic communication space density estimates for the Chukchi and Beaufort seas
12:00	13:30		Lunch					
1:30	2:00	30 min	Mon and Det	Zitterbart	Woods Hole Oceanographic Institution	N000141912669	8/19 - 7/23	Development of the next generation automatic surface whale detection system for marine mammal mitigation and distribution estimation
2:00	2:30	30 min	Mon and Det	Mansfield (Murnane) / Wackerman	US NRL	N0001423WX01668	12/31/23	Automated Processing of Marine Mammal Satellite Imagery Detection Maritime Domain Awareness (MDA) Systems
2:30	3:00	30 min	Mon and Det	Carroll (Presented by McCarthy)	University of Auckland (University of Copenhagen)	N000142112712	9/21- 9/24	Developing a universal beaked whale genotyping panel for assessing population level impacts of anthropogenic activities
3:00	3:30		Break					
3:30	3:45	15 min	Mon and Det	Harris (Presented by Wilcock)	University of St. Andrews (University of Washington)	N000142112564	9/21 - 8/25	A comparison of ranging methods using Ocean Bottom Seismometers
3:45	4:00	15 min	Mon and Det	Schick	Duke University	N000142312562	6/23 - 9/25	Process based data fusion for Marine mammal science
4:00	4:20	20 min	Speed Talks EoS - BRS, Mon and Det	Thomas Co-PI: Watwood (NUWC)	University of St. Andrews	N000142112613	5/21 - 1/24	Improving estimates of Cuvier's beaked whale sonar response by linking satellite tag and range acoustic data
				Freitag (Presented by Baumgartner)	WHOI	N000142212600	8/22 - 9/24	DMON Portable Range Engineering
				Bonnel (Presented by Baumgartner)	WHOI	N000142212568	8/22 - 7/25	Passive Acoustic Monitoring of Ocean Sounds, Meteorology, and Marine Mammals from Long-endurance Expendable Profiling Floats
				Wilcock	University of Washington	N000142312442	8/23 - 7/25	Automated Detection and Localization of Fin and Blue Whale Calls Recorded with Distributed Acoustic Sensing on the Ocean Observatories Initiative Cables
4:20	5:00	40 min	Discussion					

Tuesday 23 April 2024								
30-35 Minute talk - Progress Review or External Peer Review								
8:30	9:00	30 min	Mon and Det-eDNA	Baker (Presented by Archer)	Oregon State University (NOAA)	N000142112713	8/21 - 11/24	Environmental eDNA metabarcoding for estimating haplotype diversity and population differentiation of social odontocetes
9:00	9:15	15 min	Mon and Det-eDNA	Iken (Presented by Galaska)	University of Alaska Fairbanks	N000142212792	9/22 - 8/27	AMBON – linking biodiversity observations in the Arctic
9:15	9:30	15 min	Mon and Det-eDNA	Parsons	North Gulf Oceanic Society	N000142012569	7/20 - 9/24	Using targeted environmental DNA (eDNA) sampling to generate population-level sequence data for a species frequently detected but rarely sampled in Naval OpAreas: Gervais' beaked whale, Mesoplodon europaeus
9:30	10:00	30 min	Mon and Det-eDNA	Thielen	Johns Hopkins University	N000142112610	5/21 - 7/23	Advancing environmental genomics for marine mammal characterization
10:00	10:30		Break					
10:30	11:00	30 min	Mon and Det-eDNA	Kelly	University of Washington	N000142212719	08/22 - 07/25	MURI: MMARINeDNA: Marine Mammal Remote detection via INnovative environmental DNA sampling
11:00	11:30	30 min	Mon and Det-eDNA	Thielen	Johns Hopkins University	N00024-22-D-6404 N00024-13-D-6400	9/30/24	Defining a Path to Autonomous Environmental DNA Sequencing (Platform development for autonomous DNA sequencing)
11:30	12:00	30 min	Mon and Det-eDNA	Jensen	HJ Science & Technology, Inc.	N6833522C0119	01/22 - 12/25	SBIR: Automated technology for rapid marine eDNA sampling and detection
12:00	12:15	15 min	Discussion					
12:15	1:45		Lunch					
1:45	2:15	30 min	IER	Visser	Kelp	N000142012702 N000142212605	7/20 - 7/24 9/22 - 9/25	Off-range beaked whale integrated ecosystem study Cuvier's beaked whale integrated ecosystem study: Combining deep-sea optics and acoustics for high-resolution recording of Cuvier's beaked whale predator-prey interactions
2:15	2:45	30 min	IER	Baumann-Pickering	Scripps Institution of Oceanography	N000142014000	7/20 -9/24	Fine-scale foraging behavior of marine mammals in relation to oceanography, prey and mid-frequency active sonar
2:45	3:15		Break					
3:15	3:45	30 min	IER	Schorr	Foundation for Marine Ecology and Telemetry Research	N000142012755	8/20 - 12/24	Cuvier's beaked whales at Guadalupe Island, Mexico: A comprehensive assessment of demographics and behavior in an undisturbed area
3:45	4:15	30 min	IER	Sirovic	Norwegian University of Science and Technology	N000142112611	6/21 - 1/25	Relationship between blue, fin, and beaked whales and their prey in Southern California
4:15	4:30	15 min	IER	Visser	Kelp	N000142212735	9/22 - 2/24	Quantifying the effect of anthropogenic noise sources on cetacean fine-scale diving biomechanics and its energetic and physiological implications
4:30	4:45	15 min	Speed Talks IER	Baumann- Pickering	Scripps	N000142312435	05/23 - 9/25	Cetacean behavior in relation to oceanography, prey, and mid-frequency active sonar
				Schorr Co-PI: Watwood (NUWC)	Foundation for Marine Ecology and Telemetry Research	N000142312622	7/23 -6/26	SOARing for data: Assessing the body condition of Cuvier's beaked whales on a Navy sonar range using aerial photogrammetry
				Visser	KELP	N000142312753	9/23 - 8/26	Cuvier's beaked whale integrated ecosystem study: Systematic use of novel eDNA methodology to characterize and compare beaked whale populations and their associated prey community across regions
4:45	5:00	15 min	Discussion					
5:00	5:30		Break					
5:30	7:00		Poster Session					

Wednesday 24 April 2024

30-35 Minute talk - Progress Review or External Peer Review

8:30	9:00	30 min	IER - S&TD	Holland	Wildlife Computers, Inc.	N000142112632	9/21 - 9/24	Improvement of CTD satellite tags for use on large baleen and odontocete whales
9:00	9:15	15 min	IER - S&TD	Shorter	University of Michigan	N000142112612	5/21 - 5/23	Training, data archive integration, and analysis tools for high-resolution tag data
9:15	9:30	15 min	IER - S&TD	Gomez	National Marine Mammal Foundation	N000142112619	6/21 - 2/24	HemoTag, a Minimally Invasive Remote Blood Collection Device for Cetaceans
9:30	10:00	30 min	EoS- Phys	Williams	University of California - Santa Cruz	N000142012762	8/20 - 7/24	Physiological resilience in mammalian Divers: Assessing the role of vascular conflict and control in recovery from anthropogenic disturbances
10:00	10:30		Break					
10:30	10:45	15 min	Ed - Outreach	Cox	Savannah State University	N000142112600	08/21 - 9/24	Internships for Diversity and Inclusion in Marine Mammal Research
10:45	11:15	30 min	EoS - PCAD	Christiansen	Aarhus University	N000142112601	9/21 - 9/24	Developing a bioenergetic model for right whales to assess population consequences of exposure to multiple stressors
11:15	11:45	30 min	EoS - PCAD	Falcone	Foundation for Marine Ecology and Telemetry Research	N000142112563	5/21 - 9/24	Vital rates of Cuvier's beaked whales: A Multi-regional comparative assessment
11:45	12:00	15 min	EoS - PCAD	McHuron	University of Washington	N000142312492	7/23 - 9/24	Identifying predictive relationships for daily lactation costs in marine mammals
12:00	1:30		Lunch					
1:30	2:00	30 min	EoS - PCAD	Bejder	University of Hawaii	N000142012624	8/20 - 10/23	Dolphin population photogrammetry (DoPoGram): Assessing feasibility of generalizable method to determine delphinid population health
2:00	2:15	15 min	EoS - PCAD	Claridge	Bahamas Marine Mammal Research Organization	N000142012756	8/20 -8/24	Quantifying key vital rates in Blainville's beaked whale reproduction, including age at weaning, pregnancy rates and calf survival to inform Population Consequences of Disturbance models
2:15	2:30	15 min	EoS - PCAD	Takeshita Co-PI: Kellar (NOAA SWFSC)	National Marine Mammal Foundation	N000142212706	9/22 - 1/25	VESOP II: Developing broadly applicable models to predict vital rates from remotely sampled health measures including epigenetics
2:30	2:45	15 min	EoS - PCAD	Claridge Co-PI: Watwood (NUWC)	Bahamas Marine Mammal Research Organization	N000141812778	7/18 - 7/24	Assessing nutritional stress and pregnancy in Blainville's beaked whale at the Atlantic Undersea Test and Evaluation Center (AUTEC)
2:45	3:15		Break					
3:15	3:45	30 min	Speed Talks IER - S&TD; EoS - PCAD	Goldbogen	Stanford	N000142312624	7/23 - 6/26	Engineering and design to enhance heart rate detection in cetacean-borne tags
				Irvine	Oregon State University	N000142312561	7/23 - 4/26	Developing a robust satellite-linked smart tag for long-term monitoring of large cetacean behavior
				Shorter	University of Michigan	N000142312496	5/23 - 7/26	Advancement of Attachment of Biologging Tags
				Beltran	UC Santa Cruz	N000142212707	10/22 - 9/25	Targeted management approaches for minimizing Navy activity impacts on long-lived vertebrates
				Beltran	UC Santa Cruz	N000142312527	5/23- 04/26	YIP: Identification of Navy-relevant oceanographic hotspots guided by ethical practices and experiential learning
				Bejder (Presented by Gough)	University of Hawaii	N000142212721	8/22 - 8/25	Developing Hawaii-based capacity to analyze accelerometry tag data to evaluate responses of marine mammals to disturbance
3:45	4:30	45 min	Discussion					

Thursday 25 April 2024

30-35 Minute talk - Progress Review or External Peer Review

8:30	9:00	30 min	EoS - PCAD	Read (Presented by Allen)	Duke University	N000142012642	7/20 - 9/24	Determining the energetic cost of locomotion in pilot whales and killer whales
9:00	9:30	30 min	EoS - PCAD	Tyack	University of St. Andrews	N000142112096	1/21 - 9/25	Towards an understanding of the cumulative effects of multiple stressors on marine mammals - an interdisciplinary working group with case studies
9:30	10:00	30 min	EoS - PCAD	Torres	Oregon State University	N000142012760	7/20 - 7/24	Finding patterns within the noise: Modelling baleen whale response to multiple stressors through replicate physiological sampling of gray whales
10:00	10:30	30 min	EoS- Hearing	Cranford	San Diego State University	N000141912682	8/19 - 11/24	Investigating bone-conduction as a pathway for mysticete hearing
10:30	11:00		Break					
11:00	11:30	30 min	EoS- Hearing	Houser Co-PI: Finneran (NIWC)	National Marine Mammal Foundation	N0001420C2022	1/20 - 9/24	Collection of auditory evoked potential hearing thresholds in minke whales (Balaenoptera acutorostrata)
11:30	11:45	15 min	Discussion					
11:45	1:15		Lunch					
1:15	1:45	30 min	EoS- Phys	Fahlman	Global Diving Research, Inc.	N000141912560	8/19 - 7/23	Neurobiological and physiological measurements from free swimming marine mammals
1:45	2:00	15 min	EoS- Phys	Tift (Presented by Keenan)	University of North Carolina Wilmington	N000142212721	6/21 - 5/24	Central nervous system lymphatic structure in marine mammals: A morphological comparison of shallow and deep divers
2:00	2:15	15 min	EoS- Phys	Somarelli	Duke University	N000142112365	10/22 - 9/25	Defining the molecular physiologic impacts of stress on beaked whale hypoxia tolerance: Implications for behavioral response
2:15	2:30	15 min	EoS- Phys	Fahlman	Global Diving Research, Inc.	N000142112652	9/21 - 9/24	Modeling gas dynamics in shallow and deep diving cetaceans: Accounting for changes in metabolic rate and blood flow changes
2:30	3:00		Break					
3:00	3:15	15 min	EoS- Stress	West	University of Hawaii	N000142112642	6/21 - 6/25	BALEEN: Balaenopterids – A Layered Exposures and Effects Nexus
3:15	3:30	15 min	EoS- Stress	Burgess	New England Aquarium	N000142112903	9/21 - 9/24	Development of blow hormone quantification for Blainville's beaked whales to provide physiologic data to better inform PCoD models
3:30	4:00	30 min	EoS- Stress	Romano	Sea Research Foundation	N000142112568	6/21 - 5/24	Next steps for utilizing cetacean blow or exhaled breath condensate to monitor the physiological response to stressors and to aid in determining "biologically significant" disturbance
4:00	4:20	20 min	Speed Talks EoS- Stress; EoS-Phys; Mod - Data	Khudyakov Co-PI: Kellar (NOAA SWFSC)	University of the Pacific	N000142312444	06/23 - 5/25	Molecular Indicators of Physiological Stress and Its Health Effects in Cetacean
				Fahlman	Global Diving Research, Inc	N000142312002	1/23 - 12/25	Bioimpedance and Electrical Impedance Tomography for Mammals in Water
				Houser	National Marine Mammal Foundation	N000142212833	10/22 - 9/26	Metabolic costs and immune system impacts from chronic cortisol elevation and decreased energy acquisition
				Bierlich	Oregon State University	N000142312422	05/23 - 12/24	Developing an R package and workshop for robust and standardized drone-based photogrammetry
4:20	5:00	40 min	Discussion					

Behavioral and physiological response studies (BPRS) with social delphinid cetaceans using operational and simulated military mid-frequency active sonar

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Background

Social dolphins are generally not endangered or observed in mass-stranding events involving Navy mid-frequency (3-4 kHz) active sonar (MFAS). They occur in groups of many hundreds of small-bodied individuals that travel quickly, change behavior ephemerally, and are not amenable to conventional tagging methods, posing challenges in quantifying noise impacts. Because they commonly occur within Navy testing and training ranges in large numbers, whether and how they respond to MFAS is a very important compliance and management question. To date, behavioral responses have been inferred from laboratory measurements or anecdotal, uncontrolled contexts. No direct experimental studies of the potential behavioral or physiological responses of these animals to MFAS in controlled field conditions exist.

Objectives

(1) Apply integrated remote sensing methods to simultaneously track group movement behavior and physiological parameters for social dolphins. (2) Obtain direct measurements in known conditions to better characterize baseline parameters and identify group behavioral changes and stress hormone responses resulting from controlled MFAS exposure. (3) Expand and enhance analytical methods for quantifying and tracking movement and social behavior for multiple delphinid species using data from current and previous projects.

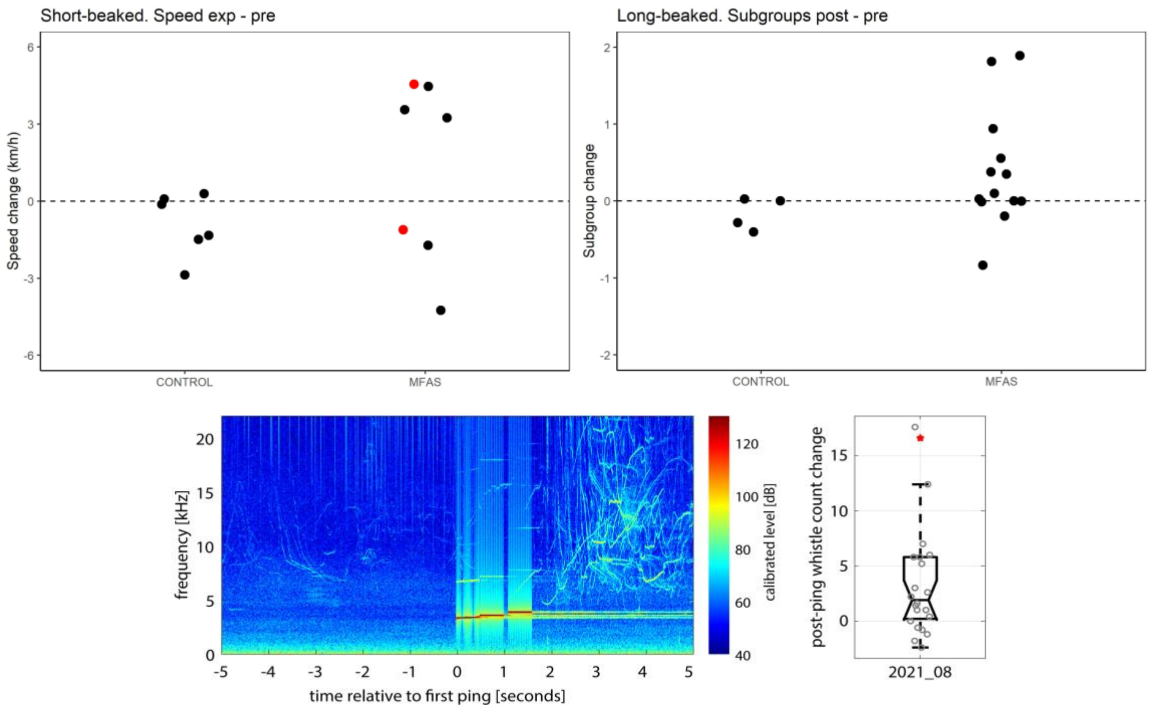
Methods

We integrated complimentary sampling approaches to measure behavioral and physiological responses of short- and long-beaked common, bottlenose, and Risso's dolphins to MFAS. Controlled exposure experiments (CEEs) with sequential 10-min pre-exposure, exposure, and post-exposure phases included signals from simulated MFAS or operational helicopter-dipping MFAS sources, or no signals during control CEEs. Directionality and velocity of focal sub-groups was measured with drone-based photogrammetry. Group acoustic behavior was recorded with strategically-positioned listening sensors. Broad-scale visual observations provided tracking and sub-group configuration of the entire group. Biopsy sampling was conducted at strategic times following MFAS and control CEEs to examine physiological stress hormone response. We fit Bayesian hidden Markov models to the number and movement of focal sub-groups and group whistle counts, in 5-s periods, describing these response variables through two possible states: baseline or enhanced. Posterior distributions of enhanced state probability were calculated for each CEE phase to evaluate response probability in exposure and post-exposure phases. Given very rapid transitions expected and observed in delphinid whistle production, much finer temporal windows were applied in a separate analysis to provide more fine-scale resolution to evaluate potential acoustic responses to MFAS.

Results

Large datasets were obtained on baseline acoustic (Oswald et al., 2021) and physical/physiological parameters (Leander et al., 2021; Keller et al., in prep.). Field and analytical methods were developed for evaluating responses during CEEs (Durban et al., 2022) and enhanced tracking of individuals (Boulil et al., 2023). Fifty CEEs were conducted across all species, yielding hundreds of hours of acoustic and group sampling data, hundreds of thousands of spatially-explicit photogrammetry measurements, and nearly 200 biopsy samples in known conditions. Our response analyses focused first on common dolphins. Changes in sub-group movement and aggregation between CEE phases were more pronounced in MFAS but not control CEEs (Fig. 1). Vocal behavior was highly ephemeral in MFAS and control CEEs using 10-min windows, but distinct responses to MFAS were identified within finer analysis windows. Responses, particularly movement changes, were evident in short-beaked common dolphins (n=14 CEEs), and a direct relationship between response probability and received level was observed. Long-beaked common dolphins (n=18) showed less consistent movement responses, although contextual differences may have limited which responses could be detected. However, group aggregation changes were consistently observed in MFAS CEE post-exposure phases, typically including segregation into more sub-groups. These are the first controlled experimental behavioral response data for these abundant species to directly inform impact assessments for any military active sonars.

Figure 1. Responses of common dolphins for different behavioral parameters. Summary examples are shown for movement speed in exposure ("exp") phases relative to pre-exposure phases ("pre") for MFAS and control CEEs for short-beaked common dolphins (top left; operational MFAS sources in red) and relative changes in the number of discrete sub-groups of individuals within the entire group in post-exposure ("post") vs. pre-exposure for long-beaked common dolphins (top right). Fine-scale (5s pre/post ping windows) analyses of acoustic responses are shown for an example MFAS CEE with long-beaked common dolphins with the distribution of the change in whistle activity following relative to just before a ping with the first ping in red (bottom; Casey et al., in press).



Project collaborators: John Durban, Fleur Visser, John Calambokidis, Holly Fearnbach, Nick Kellar, Caroline Casey, Hannah Clayton, James Fahlbusch, Kiirsten Flynn, Selene Fregosi, Ari Friedlaender, Vincent Janik, Sam Leander, Julie Oswald, Machiel Oudejans, Henry Scharf, Kristin Southall, Alex Vanderzee.

References:

Boulil, Z.L., Durban, J.W., Fearnbach, H., Joyce, T.W., Leander, S.G., & Scharf, H.R. (2023). Detecting changes in dynamic social networks using multiply-labeled movement data. Journal of Agricultural, Biological and Environmental Statistics, 28(2), 243-259.

Casey, C, Fregosi, S., Oswald, J. N., Janik, V.M., Visser, F., & Southall, B.L. (In press). Common dolphin whistle response to experimental mid-frequency sonar. PLOS One.

Durban, J.W., Southall, B.L., Calambokidis, J., Casey, C., Fearnbach, H., Joyce, T.W., Fahlbusch, J., Oudejans, M.G., Fregosi, S., Friedlaender, A.S., Kellar, N.M., Visser, F. (2022). Integrating remote sensing methods during controlled exposure experiments to quantify group responses of dolphins to navy sonar. Marine Pollution Bulletin. https://doi.org/10.1016/j.marpolbul.2021.113194

Leander, S.G.M., Durban, J.W., Danil, K., Fearnbach, H., Joyce, T.W., and Ballance LT. (2021). Sexually dimorphic measurements from stranded and bycaught specimens contribute to the characterization of group composition in free-ranging common dolphins (Delphinus spp.) from aerial images. Mar Mam Sci,1–7. https://doi.org/10.1111/mms.12804.

Oswald, J.N., Walmsley, S.F., Casey, C., Fregosi, S, Southall, B. and Janik, V.M. (2021). Species information in whistle frequency modulation patterns of common dolphins. Philosophical Transactions Royal Society B 376, 20210046, https://doi.org/10.1098/rstb.2021.0046

Double MOCHA: Phase II Multi-study Ocean acoustic Human effects Analysis

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Background

Behavioral Response Studies (BRSs) aim to directly quantify the relationship between potential anthropogenic disturbance and their effect on marine mammals. The US Navy is making a substantial investment in BRSs, aimed at understanding the effect of active sonar on species of concern. BRSs can be broadly divided into those that rely on a formal experimental design (Controlled Exposure Experiments, CEEs), and those that make opportunistic use of real-world naval activities (observational studies). CEEs on wild swimming animals are difficult and costly to perform and thus sample sizes are necessarily small, and the resulting multivariate datasets are complex to analyze. These and other issues led to the formation of a Navy-funded working group, MOCHA (for Multi-study Ocean acoustic Human effects Analysis, ONR award number N000141210204), which developed and implemented analysis methods that made most effective use of the available data. The outputs of the MOCHA project substantially enhanced our ability to quantify the response of marine mammal species to Navy sonar and other acoustic stimuli. However, as the BRS field studies have evolved so have the analytical requirements. In particular, the collection of data across multiple spatial and temporal scales from a variety of different platforms presents new analytical challenges.

Objectives

The overall aim of this project was to develop new quantitative models and analytical methods for inferring behavioral response of marine mammal species to Navy sonar. Our focus was on studies estimating the response to mid-frequency active sonar, but the methods developed are widely applicable. The results are directly applicable to current Behavioral Response Studies (BRSs) operating on multiple species in multiple oceans and will support future Navy Behavioral Response Criterion development. The objectives were addressed by three project tasks:

Task 1. Develop analytical methods for estimation of behavioral response and subsequent recovery from controlled exposure experiments (CEEs) that are applicable to data collected across a range of spatial and temporal scales.

Task 2. Develop recommendations for effects analysis of long-term passive acoustic data.

Task 3. Develop next-generation models for behavioral response based on our understanding of marine mammal signal detection and the evolutionary drivers of response.

Methods

Task 1. The evolution of BRS field studies whereby data are now collected across multiple temporal and spatial scales and resolutions raises many analytical challenges: inferring location in the face of measurement error; dealing with intermittent time series; reconciling the mixed resolution of horizontal and vertical data. These challenges were addressed by developing continuous time models that are capable of simultaneously handling data from multiple temporal resolutions, measurement error, intermittent observations and random effects arising from multiple individuals. These models include estimation of behavioral state and will therefore be capable of inferring behavioral response as well as recovery to baseline conditions, given adequate data. Model development was approached in stages and through multiple routes.

Task 2. The original proposed task was to investigate the utility of two different analytical approaches, Generalized Estimating Equations (GEEs) and Hidden Markov Models (HMMs), for analyzing data from passive acoustic monitoring. It was realized that HMMs are unsuitable for this purpose given the size of PAM datasets, so efforts focused on comparing regression modeling approaches, specifically variants of generalized additive models (GAM) including one implemented within a GEE framework. A case study approach was used for the comparison, using beaked whale (*Ziphius cavirostris*) click detections made from High-frequency Acoustic Recording Packages (HARPs) deployed at one site within the SOCAL Navy testing range.

Task 3. This task focused on synthesizing knowledge generated from studies of animal audition, perception, energetics and population consequences of disturbance with the ultimate goal of creating more biologically-based models for behavioral and physiological response of marine mammals to Navy sonar. The task was approached in a series of linked stages: 1) a literature review of the potential factors affecting behavioral and physiological responses; 2) a workshop with experts from the fields of marine mammal hearing, attention, stress physiology, energetics, behavioral ecology, and statistical ecology to consider the potential for creation of a quantitative, mechanistic model for behavioral response; 3) the development of a conceptual model framework and model code to enable quantitative exploration and sensitivity analysis on how the model inputs (contextual variables) relate to outputs (responses).

Results

Task 1: A number of different approaches were developed to allow for the detection of behavioral changes in both DTag and satellite tag data, in diving behavior and horizontal movement behavior. Approaches ranged from an exploratory "pre-post" test to identify changes in diving behavior (Hewitt et al. 2022), to a varying-coefficient stochastic differential equation framework to detect changes in x, y and z dimensions (Michelot et al. 2021, 2023), hierarchical continuous time movement models to characterize dive behavior from satellite tags (Hewitt et al. 2021), and continuous-time discrete space models to improve estimates of animal location (Hewitt et al. 2023).

Task 2: The focus was on analysis methods for detecting a change in cue detection rate caused by a potential disturbance, using PAM data from single acoustic sensors or sparse arrays. Full details of the methods comparison are provided in a CREEM Technical Report (Oedekoven et al. 2023). In summary, three different modeling tools were investigated in the statistical software R for fitting GAMs which each employ different methods for fitting smooth functions and for dealing with correlation as well as using different model selection criteria. Overall it was found that each approach has limitations, which are discussed in Oedekoven et al (2023).

Task 3: A new conceptual framework, the Functional Dose-Detection-Response" (FDDR) framework, has been developed and case studies considered. A mechanistic simulation model was developed to explore the framework quantitatively and examine the influence of the proposed mechanistic components on measured dose-responses curves in a sensitivity analysis. The simulation model has been used to address generic questions but has also been applied to case studies to better understand its applicability. The FDDR framework demonstrates why disturbance responses are highly context-dependent and provides tangible mechanisms to capture variability in the next generation of statistical models for disturbance.

References:

Hewitt et al. (2021) Continuous-Time Discrete-State Modeling for Deep Whale Dives. Journal of Agriculture, Biological and Environmental Statistics, 26, 180–199 (2021).

Hewitt et al. (2022) Kernel density estimation of conditional distributions to detect responses in satellite tag data. Animal Biotelemetry, 10:28

Hewitt et al. (2023) Time-discretization approximation enriches continuous-time discrete-space models for animal movement. Annals of Applied Statistics, 17(1): 740-760

Michelot et al. (2021) Varying-Coefficient Stochastic Differential Equations with Applications in Ecology. Journal of Agriculture, Biological and Environmental Statistics.

Michelot et al. (2023) Continuous-time modelling of behavioural responses in animal movement. Annals of Applied Statistics, 26, 446-463

Oedekoven et al (2023) Methods comparison for analysing PAM data to detect a change in cue detection rate of cetaceans related to a potential disturbance. (CREEM Technical Report No. 2023-02) University of St Andrews

Machine learning detection of cetacean tonal calls without human annotations

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Background

Deep learning has shown great potential in species-level detection and classification of marine mammal vocalizations. Recent studies suggest that training a neural network that performs well on unseen data often requires a large amount of analyst-annotated data, which are expensive and time-consuming to obtain for marine mammal species.

Objectives

This work focuses on tonal calls in odontocetes and mysticetes. Unlike most work which detects call presence or absence over a fixed time window, we focus on reliable extraction of detailed time-frequency information that has the potential to provide improved recognition of species as well as track changes in acoustic behavior that may be indicative of population trends such as the year-over-year decline of pitch in blue whale song (McDonald *et al.*, 2009). Fine-grained measurements of calls can identify populations (Van Cise *et al.*, 2017) and may contain signals that that can be linked to individual identity (McCordic *et al.*, 2016).

Methods & Results

We published three approaches to develop tonal annotations. In Li *et al.* (2020), we showed that deep neural networks coupled with a graph-search whistle extraction algorithm could be used to annotate a set of multi-species whistles from delphinids when there was sufficient analyst training data (precision .96, recall .82) and proposed several techniques to synthesize labeled data using few to no human annotations. When learning from these synthesized annotations, precision was generally good (≥.90) although recall was inferior to learning from analyst-labeled data (0.60-0.69). We showed that this method could generalize to data collected on different species in different geographic regions and published an open-source tool for the bioacoustics community to use (Conant *et al.*, 2022).

Encouraged by these results, the second and third methods that we developed focused on ways to improve the precision and recall when learning from limited (or no) human annotations. We used multi-stage generative adversarial networks (GANs) trained on a small subset of labeled data to learn how to generate training data (Li *et al.*, 2023b). In all cases, using GANs produced gains of up to 1.69 points in the harmonic mean of precision and recall over learning from labels alone.

The final method used models trained with pseudo-labels (Li *et al.*, 2023a). Our pseudo-

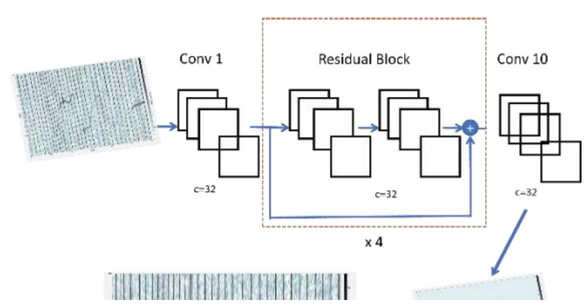

Figure 1 – Demonstration of Li et al. (2020) on an auditory scene with whistles and echolocation clicks. A neural network learns to produce large outputs where it is confident that whistle energy exists, and a graph-search algorithm extracts tonal contours.

labels consisted of approximative tonal contours that were generated without human supervision using techniques that required either no training data (Roch *et al.*, 2011) or minimal training data (Gruden and White, 2020). We showed that naïve learning from these pseudo-labels produced tonals with high precision, but recall that was substantially lower than the performance of the systems used to produce the pseudo-labels. Recognizing that most classifiers that could be used to produce pseudo-labels are likely to have a bias towards better precision or better recall, we introduced a new loss function that encourages the neural network to focus on learning to improve either the precision or the recall from inaccurate labels. We found that the new loss function was able to learn to produce results nearly as accurate as if we had trained the network with a large number of human analyst annotations. Our baseline model trained with human analyst annotations on a difficult data set had a precision of 0.90 and recall of .86. When learning from pseudo-labels, a precision of 0.89 and recall of 0.86 was obtained, nearly as accurate as the model trained with labels that required months of human effort.

In the final year of this project, we are focusing on baleen whale calls and using these tonal annotations to classify calls to species.

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MAMBAT (Multiple-Animal Model-Based Acoustic Tracking): Improving underwater passive acoustic multi-target tracking methods

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Background

Passive acoustic localization and tracking methods are well established in cases of single calling animals or when call classification and association can be readily achieved. However, cases with multiple calling animals with calls that cannot be associated still pose a significant challenge for tracking algorithms. The problem is further complicated by false alarms and missed detections. Proposed approaches for these cases rely on source separation/call association steps, require ad-hoc or manual steps, and are computationally demanding. The overall goal of MAMBAT is to improve model-based passive acoustic methods for tracking multiple marine mammals, and to develop a fully automated, computationally efficient workflow that incorporates missed detections, false alarms, source appearance and disappearance in the problem formulation. While we aim to develop platform agnostic tools and methods, our work is motivated by and will be tested on application to large aperture bottom mounted arrays (AUTEC and PMRF). We work directly with collaborators at NIWC Pacific (Elizabeth Henderson and team).

Objectives

1) Implement and test MAMBAT for a single animal case (Single Target Tracking STT): a) Set up overall signal processing workflow; b) Identify the best pre-processing and measurement extraction approach; c) Develop and/or modify tracking models
2) Implement and test MAMBAT for multiple animal case (Multi Target Tracking MTT): a) Evaluate STT signal processing workflow and adjust for MTT as necessary; b) Adjust pre-processing and measurement extraction approach if necessary; c) Evaluate and adjust tracking models if necessary
3) Test MAMBAT on more complex scenarios: a) Different species; b) Larger array of sensors; c) Implement on Navy Ranges (in collaboration with NIWC Pacific)

Methods

Objective 1: The MAMBAT framework consists of two main parts. In the first part, a "track-before-localize" strategy is employed to track Time-Difference-Of-Arrival (TDOA) and Direct-Reflected-Time-Difference (DRTD) information based on cross- and auto-correlograms, respectively, from multiple pairs of sensors. In the second part, a "localize-then-track" strategy is used to track sources based on ambiguity surfaces that account for depth-dependent sound speed profiles [1,2] in the 3-D spatial domain. Tracking is achieved with a modified version of the GM-PHD-SA filter [3], a multi-target Bayes filter formulated within the random finite set framework. Parameters of the filter are trained on a small set of annotated data. Technical details on the GM-PHD-SA filter are given in our MAMBAT project poster/abstract.
Objective 2: In the multiple animal case the MAMBAT framework needs slight modification in the construction of cross- and auto-correlogram step to account for different SNR between sources on the same receiver. For clicks, cross- and auto-correlograms are constructed based on click maps that are created using a binary mask based on the energy of the signal. Parameters of the GM-PHD-SA filter are

trained on a small set of annotated data.
Objective 3: Multiple datasets from the Pacific Missile Range Facility Barking Sands (PMRF) test range are being investigated and consist of an array of multiple sensors (between 18-60) and four different species that produce broadband and narrowband signals: pilot whales, sperm whales, Blainville's beaked whales, and killer whales. Some of the animals were tagged and comparison between acoustic tracks and tag data can be made. Ongoing effort is in collaboration with NIWC Pacific.

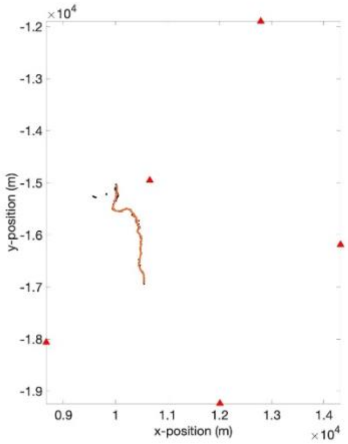

Fig. 1: 2-D view of the automatically extracted spatial track of a sperm whale with MAMBAT. Black dots denote extracted measurements from the combined ambiguity surfaces; colored line denotes extracted track; triangles denote receiver positions. The fully-automated results compare well to Fig. 6 in Ref. [5] and Fig. 3 in Ref. [2].

Results

The MAMBAT framework was applied to "bench-mark" data from the 2nd International Workshop on DCLDE [4], that were prepared by NUWC and consist recordings of a sperm whale(s) recorded at the AUTEC test range [4].

Objective 1: The results (Fig. 1) show that MAMBAT successfully tracks a single sperm whale in 3-D spatial domain. The results compare well with previously reported trajectory in Refs.[2,5]. In contrast to other methods applied to this dataset, no explicit detection of the echolocation clicks, or pruning was performed.
Objective 2: Results from the multiple-animal DCLDE dataset show four whales successfully tracked through false alarms and missed detections (Fig. 2). Work is still in progress to improve measurement extraction, especially on the noisy z-coordinate. The results compare well with previously reported trajectories in Ref. [6]. The most impactful and important contribution of the MAMBAT framework is full automation.

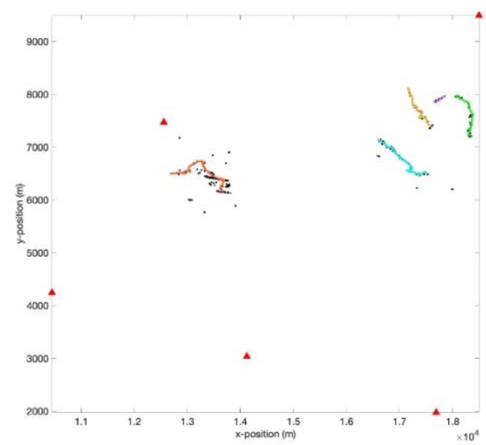

Fig. 2: 2-D view of the automatically extracted spatial tracks of four sperm whales with MAMBAT. Black dots denote extracted measurements from the ambiguity surfaces; colored lines denote extracted tracks; triangles denote receiver positions. The fully-automated results compare well to Fig. 5 in Ref. [6]. Note that track fragmentation occurs for the whale located between ~1.7-1.9 km x and 7-8 km y.

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The Wide-Band Detection and Classification System

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Background

The development of passive acoustic detection and classification methods for marine mammals has traditionally focused on one species at a time, resulting in a myriad of algorithms that must be applied one at a time to detect several species of interest in a single recording or real-time audio stream. Some recent detection systems aim to detect and classify classes of marine mammal calls, such as clicks, buzzes, or tonal calls, which allows the detection of many species simultaneously in a single processing stream. The low-frequency detection and classification system (LFDCS) is one of these systems. It is designed to detect and classify low-frequency tonal calls, such as those produced by baleen whales, using pitch tracking from a spectrogram (call contouring), sound characterization (attribute extraction), and discriminant function analysis. It uses spectrograms based on the short-time Fourier transform (STFT), which allow pitch tracking over a roughly 4-octave frequency band; however, marine mammal tonal calls span 12+ octaves (14 Hz to over 60 kHz). Hence, pitch tracking with traditional spectrogram creation methods allows characterization of tonal calls in only a narrow band of frequencies, thus limiting the number of species that can be simultaneously detected.

Just as the tonal sounds of marine mammals occur over a wide range of frequencies, so too do pulse sounds, such as echolocation clicks. Algorithms developed to date focus solely on a single, relatively narrow frequency band optimally chosen for the target signal. No work has been done to develop a single wide-band pulse detection algorithm that will allow any pulse, regardless of frequency band, to be detected and classified based on extracted features. The long-term goal of this project is to develop a system that can detect both tonal and pulse sounds over 12+ octaves, allowing the efficient detection of multiples species ranging from blue to beaked whales in a single processing run. This generalized detection system would be used both to analyze archival audio and to detect marine mammals in real time from autonomous platforms.

Methods

Tonal call identification with the wide band detection and classification system (WBDCS) was developed using a constant-Q (CQT) based spectrogram, which estimates spectra on a logarithmic frequency scale instead of the linear frequency scale of the STFT. Pitch tracking, attribute extraction, and discriminant function analysis of tonal calls was accomplished using the same approach as in the existing low-frequency detection and classification system (LFDCS; Baumgartner and Mussoline 2011). The WBDCS was also intended to have a time-domain front end that takes advantage of the CQT pre-processing to detect and classify pulses as well, but this capability was not implemented during the project.

Objectives

The objective of the project was to develop the CQT-based spectrogram and associated tonal call detection method both on a desktop computer for analysis of archival audio and on the digital acoustic monitoring (DMON2) instrument for real-time detection on autonomous platforms. Given the complexities of programming the DMON2 instrument and running the algorithm in real time (e.g., limited on-board memory, fixed-point processing, and a slower processor), this implementation was considered a priority to complete.

Results

Considerable effort was expended to streamline individual processing jobs used by the DMON2's priority-based real-time job scheduler. To assess each job, new diagnostic capabilities were created that utilized the DMON2's extended memory to log processing and memory-use events while the firmware runs. Using these new capabilities, all of the jobs involved in LFDCS/WBDCS processing were assessed. Several jobs were identified that took up too much processing time and therefore delayed real-time processing of incoming audio. These jobs were re-written in such a way that the original functionality was completed in multiple shorter-duration jobs that did not prevent higher priority jobs from running. These changes improved efficiency considerably, allowing much faster processing throughput than before.

The CQT-based spectrogram is created by sampling the mid- and low-frequency hydrophones of the DMON2 at 128 and 8 kHz, respectively, and then each of these waveforms are successively low-pass (i.e., anti-alias) filtered and decimated by a factor of 4 to produce waveforms of 128, 32, 8, 2, 0.5 and 0.125 kHz. Each of these 6 waveforms are used to produce spectrum values over a double octave band in the CQT-based spectrogram using the methods of Brown and Puckette (1992). Each double octave band consists of 42 logarithmically spaced frequency bins (21 bins per octave); thus, 42 bins per double octave for 6 double octave bands yields a CQT-based spectrogram consisting of 252 frequency bins that span 15 Hz to 59.5 kHz. As with the LFDCS, the CQT-based spectrogram is smoothed using a kernel filter, equalized using an exponential running mean, and transient broadband noise is identified and removed from the spectrogram. The pitch tracking algorithm uses this conditioned CQT-based spectrogram to identify tonal sounds.

To test and demonstrate the system, previously recorded audio was delivered to the low- and mid-frequency audio inputs on the DMON2 board using a National Instruments USB-6363 data acquisition (DAQ) unit. Figure 1 shows results using audio combined from two disparate sources: recordings of dolphin whistles and recordings of fin and sei whales. The CQT-based spectrogram (Figure 1a) clearly shows the dolphin, fin whale and sei whale vocalizations, and the pitch tracks (Figure 1b,c) faithfully represent the frequency and amplitude modulation of those vocalizations.

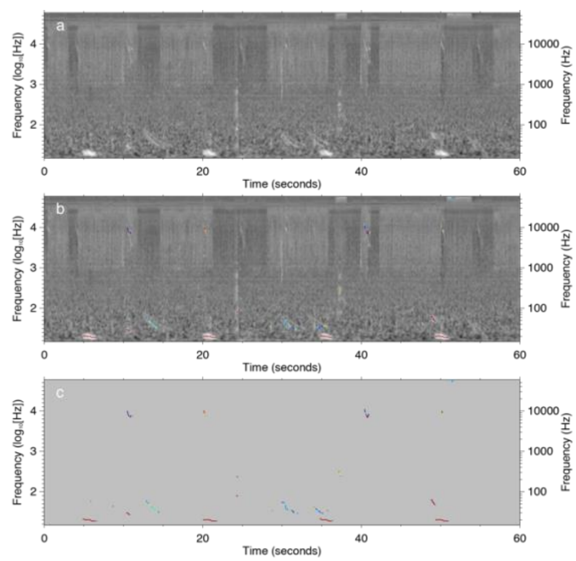

Figure 1. (a) Conditioned CQT spectrogram produced in real time by the DMON2 when presented with combined audio of dolphin whistles and sei and fin whale calls. (b) Same CQT spectrogram as in Figure 1a shown with pitch tracks produced by the DMON2 in real time. (c) Pitch tracks without the associated CQT spectrogram. High amplitude sounds are shown as brighter cells in the spectrogram or as warmer colors in the pitch tracks, while low amplitude sounds are shown as darker cells in the spectrogram or cooler colors in the pitch tracks.

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Impact of the Beaufort lens on the Bowhead whales' acoustic communication space density estimates for the Chukchi and Beaufort seas

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Background

The long-term scientific goal of this project is to provide the basis to calculate the dynamic distribution of marine mammal population density and to map its spatial heterogeneity by exploiting all available acoustic information, especially the soundscape information describing the baseline statistics, as well as the variability, of the acoustic environment. Towards this goal, in the previous proposal, we successfully developed a deep-learning approach to optimize detection of dispersed Bowhead and Right whale gunshot calls for localization (Goldwater et al., 2020) and studied the impact of the environmental conditions (specifically the Beaufort lens, a recurring warm water intrusion in the Arctic Ocean) on the Arctic Ocean ambient noise (Bonnel et al., 2021).

Objectives

The specific aim of this study is to evaluate the impact of the Beaufort lens on bowhead whale acoustic communication space and subsequently bowhead whale density estimates. To this end we study if bowhead whales change their source level depending on the prevalent propagation conditions, as well as perform a density estimate for the 2016 – 2017, on data collected during the CANAPE ONR Ocean Acoustics experiment, that is now available for analysis.

Furthermore we aim to provide density estimates for several seal species, including endangered ringed seals if encountered acoustically in this area.

Methods

In a first step we will finish automatic and manual annotation of the CANAPE dataset. The resulting call occurrence dataset will be used to localize bowhead whales and estimate their density throughout 2016 and 2017. Localization will be accomplished with classic time-difference-of-arrival algorithms if whale calls can be detected and associated at least across three hydrophone stations, or using backpropagation if calls are only available on one vertical line array.

To estimate the source level we will model the sound propagation along the path from whale to receiver and retrieve its source level. All SHRU Hydrophone arrays are calibrated and allow for source signal reconstruction and source level estimation (Lin et al., 2012). Acoustic propagation will be conducted using either locally available oceanographic data from the CANAPE experiment or using the Hycom 1/12° data assimilated ocean model.

Results

Our dataset to be used was collected during the Canada Basin acoustic propagation experiment. The Canada Basin acoustic propagation experiment was conducted in 2016 and 2017 in the Canada Basin and on the Chukchi Shelf. Many acoustic assets, both receivers and sources, were deployed as part of the experiment. We worked on a year-long passive acoustic dataset collected by Woods Hole Oceanographic Institution (WHOI) on the Chukchi Shelf (CANAPE). Five "Several Hydrophone Recording Unit" (SHRU) moorings were deployed on the Shelf edge. Each mooring consists of a vertical line hydrophone array (4 channels) with temperature and pressure sensors. Unexpectedly, this dataset only become avaliable to be analyzed after a data embargo was lifted in October 2020. We so far annotated a total of 5 Months of the entire dataset manually which summed up to over 10000 calls across a network of 5 hydrophones arrays. We detected an overall of 81643 Calls between October 2016 and February 2017. Out of this we found 7753 Bowhead calls, 73359 bearded seal calls and 399 ringed seal calls in the CANAPE acoustic dataset.

To perform localization and density estimation we developed an Time-different-of-arrival based localization algorithm and could localize over 3000 whale and seal calls. Acoustic data of manually annotated time-stamps are first cropped, bandpass filtered, cross correlated, and the resulting time differences are used to localize the whale calls. This approach works across marine mammal species, so we localized Bowhead whales, Bearded seals, endangered Ringed seals as well as unknown seal calls.

Density maps show that bowhead whales predominantly use the Southwestern area, closer to shore, of the CANAPE Array, while Bearded seals are spread out over the entire area of the shelf, and less on the shelf break. We were able to localize a few ringed seals, which clearly show that they are acoustically much less present than bearded seals. The current amount of analyzed data does not suggest any area preference within the CANAPE Array area.

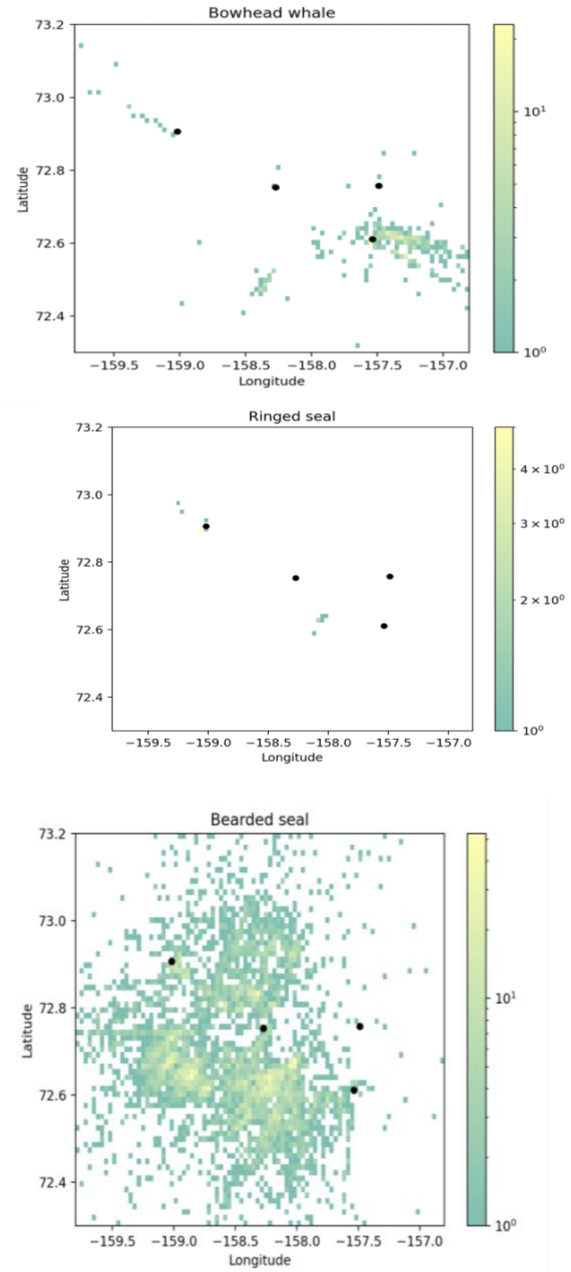

Figure 1) Density maps of Bowhead whales, Ringed and Bearded seals in the CANAPE Array

Development of the next generation automatic surface whale detection system for marine mammal mitigation and distribution estimation

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Background

The threat of vessel strikes is one of the significant dangers to whale populations worldwide, especially for species such as the North Atlantic right whales, which are on the brink of extinction. The year 2017 was marked by a tragic reminder of this threat when 17 right whales perished, with vessel collisions accounting for many of these deaths. This unfortunate occurrence underscored the urgent need for effective detection and mitigation technologies to prevent ship-whale collisions, particularly for autonomous vessels that cannot rely on human observers. Current detection methods, which predominantly rely on human observation, are not suitable for round-the-clock, all-weather monitoring and are infeasible for autonomous operations.

Objectives

The overarching goal of this research project was to conceive and develop a hardware and software system that enables the highly reliable detection of marine mammals on the water's surface. Ultimately, the system is intended to serve a dual purpose: to act as a protective measure against ship strikes and as an autonomous tool for marine mammal surveys, estimating populations and distributions within their natural habitats.

The key objective of the study was to engineer a prototype for a state-of-the-art thermal imaging-based automatic whale detection system. This system integrates several data streams, including high-resolution thermal cameras, standard marine radar, real-time meteorological data, and advanced electro-optical cameras with telephoto lenses for definitive species identification. This data-fusion approach was used to refine the system's ability to discern marine mammals from other objects, significantly decreasing the rate of false alerts.

Methods

The approach taken was multipronged: initially, design and develop the prototype (completed in 2020), followed by extensive data collection using the prototype systems (conducted in 2021), and finally, the application of data fusion algorithms to synthesize the collected data. Particular emphasis was placed on enhancing the system's capability to detect the critically endangered North Atlantic right whale. Moreover, the project sought to model ship-strike risk by considering various factors, such as ship speed, vessel height, and whale behavior, through observed data.

Results

Throughout 2022 and 2023, long-term field tests of two prototype systems were conducted aboard Canadian ferries. The first prototype, a low-cost whale detection system, was deployed on MV Bella Desgagnes and provided a 22° field of view in front of the vessel. The second prototype, represents an advancement in the detection and identification technology. Installed on MV Blue Puttees, the WhaleID system features an array of ten thermal cameras yielding a 120° field of view and a high-resolution color camera with a long-range lens for capturing detailed images of detected whales.

The whale detection system on MV Blue Puttees was operated throughout from 06/15/2022 until 05/31/2023. We experienced problems with condensation during this first phase of operation, because the material used for the camera housing print was hygroscopic and retained too much humidity during operation.

The detection system on MV Blue Puttees detected a total of 128 whales with the majority of detections below 1500m range and a maximum detection range of 4 km on its way between North Sydney and Port aux Basques Whales were detected on 37 days of a 158 days of operation. The whale detection system on MV Bella Desgagnés has been operated between from 09/09/2021 until 03/31/2023 without any major problems. Throughout the duration of the experiment we detected a total of 168 whales along the route of MV Bella Desgagnés distributed over 33 days.

We quantify the performance of the whale detection system with a range-dependent detection function. The range dependent detection function describes the probability to detect a whale at a given distance from the vessel, assuming whales are equally distributed in the ocean. The range of the peak of the detection function can be interpreted at the reliable detection distance up to which "all" cues can be detected. We find that the reliable detection range on MV Bella Desgagnés is at 1.5km, with detections out to 7 km range. Those ranges are by far enough to allow for evasive actions of a vessel such as MV Bella Desgagnés.

The implications of this research are profound. The prototypes, now fully developed and refined, hold the potential for widespread adoption in mitigating vessel strikes and conducting autonomous marine mammal surveys during continuous operation.

Figure 1) Whale detection system installed on MV Blue Puttees

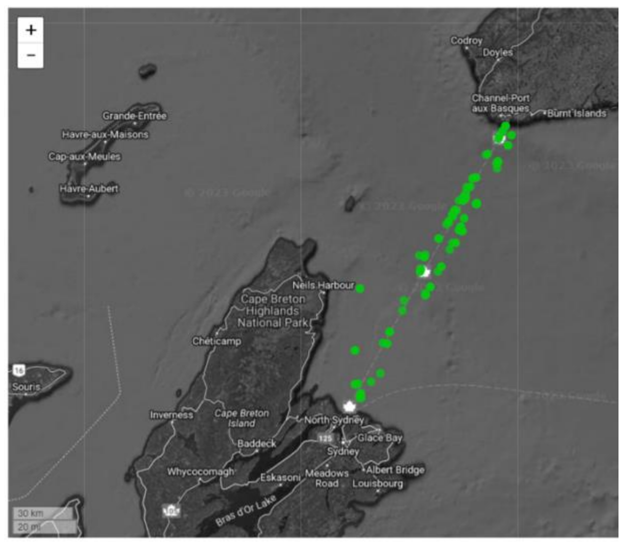

Figure 2) Automatic whale detections along the path of MV Blue Puttees

Automated Processing of Marine Mammal Satellite Imagery Detection Maritime Domain Awareness (MDA) Systems

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Background

NRL Spaces Systems Development a Department, Mission Analysis Section (Code 8114) is contributing the long-term vision to operationalize marine mammal satellite detection by developing software and toolsets. Previous work has demonstrated that whales that are on or near the surface can be visually detected in commercial, high-resolution, multispectral (MSI) and panchromatic satellite imagery. Pixels containing the whale are visually distinct from surrounding water. This can be observed manually through geospatial analysis tools (e.g. QGIS). However, this approach is meticulous and prone to error. Commercial imagery contains thousands of pixels representing hundreds of squared kilometers and it is apparent an automated approach for approximating pixel statistics and spectral signatures of objects within the image scene is needed.

Objectives

NRL has a well-established history of detecting vessels in high-resolution satellite imagery and has applied this expertise to the detection of marine mammals in satellite imagery. NRL has integrated two independent detection algorithms into an automated system architecture such that commercial imagery is automatically queried, extracted, processed, and chipped for whale-like objects within the images. NRL is continually assessing, modifying, and integrating these methods to optimally accommodate whale detection capabilities in system architectures currently designed for detecting vessels. The approach consists of four tasks: 1) create a framework for pulling satellite imagery around known whale locations, 2) build a database architecture for storing whale-like chips detected in imagery, 3) integrate two independent NRL algorithms into the architecture – namely Wack and CTAR, and 4) prototype user tools to allow users to search, view, download, and analyze whales detected in imagery in a Common Operating Picture (COP).

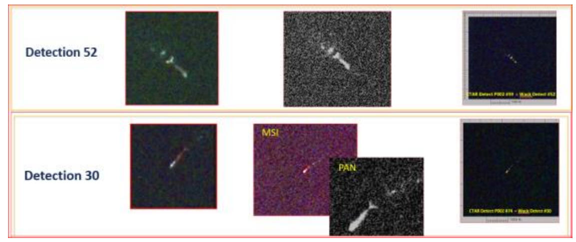

Figure 1: Two whale-like detections found automatically by Wack, manually in QGIS, and automatically in CTAR (following left to right columns)

Methods

A WorldView3 (WV3) was downloaded from two independent, open-source image repositories Maxar's GEDG and NGA's iSPY. The image selected was a Cape Cod 24 April 2021 image (Catalog ID =1040010067D36B00; acquisition time 15:40:33Z). This image was an ideal location, as whales are known to aggregate to feed there during late winter and early spring. The strip consists of 24 panchromatic (PAN) and 24 multispectral (MSI) tiles (8 rows, R1-R8 by 3 columns, C1-C3). The exemplar tile we selected was 21APR24154033-M3DS_R4C2 (R4C2 for short). The image contains 8 bits per pixel, representing 8 individual spectral bands: Coastal, Blue, Green, Yellow Red, Red Edge, and two near infrared bands. WV03 PAN resolution is 31cm GSD and 8-band MSI resolution is 1.24 meters. Two different methods were used to detect whales within this image, namely Wack and CTAR.

NRL Seafloor Sciences Branch (Code 7350) developed a detection algorithm, "Wack", for the detection of whale-like objects in satellite imagery. Code 8114 adopted this algorithm to integrate into their automated system architecture. The Wack approach combines a feature vector (with either an optimal linear combination of feature elements or a neural net) to compare against a known whale signature. The feature vector is a combination of 1) normalized statistics, 2) statistics designed to determine if the image values within some target box are statistically different from those in the local background, and 3) a set of metrics designed to measure image properties unique to whale signatures in panchromatic imagery. The aspiration was also for the automated algorithm to perform better on smaller test data sets than the traditional deep learning CNN approaches. Wack has a human-in-the-loop feature: detection thumbnails are presented to the user during processing to allow them to decide whether the detection is whale-like. Wack summarizes user feedback in a grid of thumbnails during output. Wack also provides a text file of all automated detections.

The CTAR detection method was developed under NRL's Space Systems Development Department, Advanced Systems Technology Branch (Code 8120). CTAR implements subspace Reed-Xiaoli (SSRX), a classic anomaly detector based on calculating the Mahalanobis distance for each pixel to the average image pixel after the principal components (or background pixels) are removed. CTAR applies a land mask and a user-defined coastal buffer to ensure only open-water pixels is processed. CTAR MSI tool provides a folder of JPEG pansharpened detections found by the anomaly detection algorithm, 3) a CSV summary of detections, 4) HTML views of detections and 4) a KML to layer of anomalous detections for display on Google Earth.

Results

NRL maintains the Maritime Analysis GEOINT Environment (MAGE) Analysis Suite, whose capabilities include an vessel track to image chip correlator (Trackcon), automated image retrieval and processing, Convolutional Neural Network (CNN) training data sets and performance models, manual and semi-automated analysis workflow, database storage of imagery products, and analytics to generate periodic performance statistics. MAGE is a tool designed for vessel detection and has adapted its architecture to embrace whale detections. Wack and CTAR methods were integrated into the MAGE generate non-vessel, whale-like detections. These detections are then chip and stored in database for either track correlation, or manual analysis on a Common Operating Picture (COP)

MAGE automatically extracts imagery from open-source database provided the user has defined an area of interest (AOI). These AOIs can be static or dynamic. For the dynamic whale AOI, NRL used Woods Hole Oceanographic Institution's (WHOI) Robots4Whales locations. NRL built an API to pull near real time passive sonar whale locations reported on WHOI's Robots4Whales website. The API pulls data daily, stores locations in a database table, and retrieves, imagery that overlaps with the location in time and space. For the static whale AOI, a shapefile containing Cape Cod Bay was used. All imagery that meets the dynamic and static AOI criteria is stored on NRL's Image Archival Storage server for future training and testing. Wack and CTAR are then applied to imagery. As detections are generated, the detection is chipped from the original image, 64-bit encoded and stored in a PostgreSQL database (Fig. 1). Trackcon combines global SPIRE AIS, WHOI's Robots4Whales detections, image frame metadata and whale chips to evaluate the scene. Trackcon outputs a KMZ of the track scene (Fig. 2), a JSON file to the Kafka queue, a pdf-formatted results aggregator report, which includes associated track details, track ambiguity resolution, and vessel/whale correlation to track for the image tile. Logs of Trackcon and image processing within MAGE are also available. Both the Wack and CTAR mechanisms also produce tip sheets but these have yet to be integrated into the MAGE framework.

Wack produced 1524 detections from this image, 94.2% of which was deemed clutter. This was determined through Wack's human validation feature: the analyst took 9.8mins to analyze 1524 detections presented in uniformed, tip sheets. The analyst concluded 89 detections to be whale-like. Another analyst took twice the amount of time and deemed 34 detections to be whale-like. CTAR algorithm resulted in 187 detections and does not have a human validation feature. However, the same 187 CTAR detections were contained within the 1524 Wack detection set.

This work supports the utility in modifying and enriching MAGE's architecture to yield an automated mechanism for ingestion of commercial imagery and processing, analysis and labeling of whales-detections, accessible to a user via a COP interface.

Developing a universal beaked whale genotyping panel for assessing population level impacts of anthropogenic activities

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A comparison of ranging methods using Ocean Bottom Seismometers

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Background

A comparison of ranging methods using Ocean Bottom Seismometer (OBS) data has been performed, including a variety of instrument configurations and acoustic propagation conditions. Ranging is a key element of many density estimation methods. We are also refining the development of a particular density estimation method that relies on modelled acoustic propagation and received energy band levels to estimate whale densities where only chorusing animals are detected, and it is not possible to measure ranges to detected animals. This project is part of a larger project co-funded with the Living Marine Resources (LMR) program entitled 'CORTADO: Combining global OBS and Comprehensive Nuclear Test Ban Treaty Organization recordings to estimate abundance and density of fin and blue whales.

Objectives

The overall goal of this project is to demonstrate and refine methods and to develop open-source software tools that can be used to estimate blue and fin whale density from worldwide OBS and Comprehensive Nuclear Test Ban Treaty Organization International Monitoring System (CTBTO IMS) data, which can be transitioned into use by stakeholders. In the larger CORTADO project, there are three primary research phases, each with specific objectives. In this project we focus on Phase 1 and the first task in Phase 2.

Work in Phase 1 compares ranging methods on OBS case study datasets. Under Phase 2, the first task is to refine the existing received energy band level method for the selected datasets.

Methods

In Phase 1, existing OBS datasets around the world were reviewed and a set of case studies containing blue whale and/or fin whale calls that represented the variability in conditions among OBS deployments were identified. Case study choice was conducted in consultation with ONR and LMR.

Case study OBS datasets were then used to compare available signal processing methods to estimate range to detections. Three existing range estimation approaches were compared: using multipath arrivals at a single OBS (Weirathmueller et al., 2017), using multiple OBSs with time difference of arrival (TDOA) methods (Soule & Wilcock, 2013) and using particle velocities at a single OBS (Matias & Harris, 2015). Results were evaluated and method refinement was conducted where necessary. Finally, signal processing code for each ranging method is being prepared for stakeholder use.

In Phase 2, the energy band density estimation method for chorusing animals is being refined to account for different instruments, bottom types, and sound propagation environments. The method is also being extended to include distributions of the data inputs, such as call source levels, durations, and inter-pulse intervals, as well as variability in poorly-known propagation loss model parameters.

Results

The multipath, TDOA and particle velocity methods have been compared using six sites (e.g., Fig. 1). The multipath ranging method can generally range to greater distances than the particle velocity method. In general, the particle velocity method was best suited to soft sediment conditions. A paper detailing the multipath method has been submitted to the Journal of the Acoustical Society of America.

Method development for the particle velocity method has focused on determining which estimated ranges are likely within a maximum valid range and are not spurious range estimates. A generalised additive model was used to investigate whether a given range estimate being inside, or outside, the maximum valid range was a function of various parameters. The parameters that presented significant effects were linearity of the signal, horizontal channel amplitude, the ratio between horizontal and vertical channel amplitudes, and the quality of the vertical channel data. The multipath ranging method has also been automated for use in density estimation tasks.

Software is also being developed for the energy band level density estimation method, which requires information about call production, propagation loss modelling, and receiving sensors (hydrophones/seismometers). Specific information being incorporated includes 1. the distribution of average amplitudes of calls; 2. the distribution of source levels; 3. the distributions of inter-call interval(s), sequence lengths, inter-sequence intervals, and bout durations; 4. the distribution of propagation losses given distributions of parameters used in propagation loss modelling; 5. information about the various sensor types used; and 6. combining these sources of information to estimate the distribution of acoustic energy received from a calling whale at a sensor.

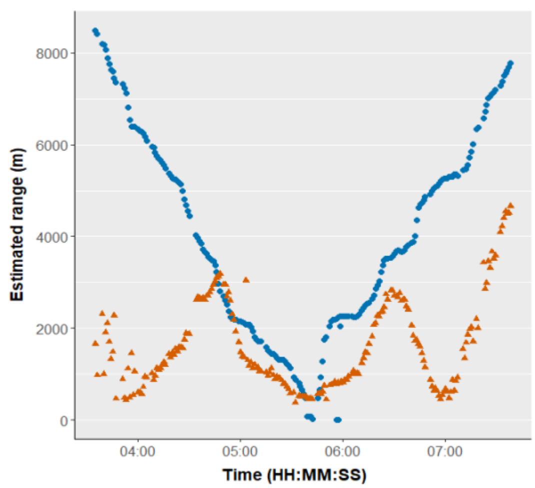

Figure 1: Estimated ranges (in m; particle velocity in orange, multipath in blue) for a fin whale track recorded in the Marianas area.

References:

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Soule, D. C., and Wilcock, W. S. D. (2013). Fin whale tracks recorded by a seismic network on the Juan de Fuca Ridge, Northeast Pacific Ocean, *Journal of the Acoustical Society of America*, **133**, 1751-1761.

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Process-Based Data Fusion for Marine Mammal Science

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Background

For decades in the marine mammal realm, scientists have been using myriad techniques to gather data about behavior, physiology, movement, distribution and abundance, among others. Often each dataset is treated in a stand-alone fashion when researchers build and test inferential models to quantify features in the data. Our research is motivated by a need and desire to bring together disparate datasets to better develop a quantitative understanding of the hidden processes key to these species. In particular, we are motivated by the North Atlantic right whale, an endangered baleen whale, which has been studied for decades, and for which an extensive and diverse dataset exists. We focus on Cape Cod Bay, MA (CCB), where overlapping datasets exist from both aerial and vessel-based surveys, from in situ prey sampling, and from passive acoustic monitoring (PAM).

Objectives

Our goals are 4-fold. First, we wish to incorporate both aerial line-transect data and passive acoustic monitoring data to better understand the true, or latent, abundance surface in CCB. Second, we wish to quantify the amount of information we gain through inclusion of the PAM data to better understand distribution and abundance. Third, using simulation, we want to learn about optimal design for PAM and aerial line-transect surveys to inform abundance and distribution. Finally, we wish to investigate the impact of preferential sampling on our inference.

Methods

To date we have focused on three different features of the project. First, we have built an initial data fusion between aerial line-transect data and PAM data in CCB (Schliep et al., arXiv:2310.08397). Herein, we fuse two data sources: 1) the aerial line-transect data, and 2) the detected up-calls from an array of PAM sensors. In addition, we used ancillary data on call rate and availability from DTAG data in CCB. The model assumes there exists a true spatial point pattern of whales, and that the aerial and PAM data are treated as thinned point patterns. The thinning mechanism for each of the two components is drawn from previously published aerial and acoustic detection functions. Second, we hosted a workshop after the NARW Annual Consortium Meeting in Halifax, Nova Scotia to gather feedback on the fusion. This was a one-day workshop at the Bedford Institute of Oceanography, and which included approximately 20 people in person and remote. Third, to incorporate feedback from the workshop on how NARW calling behavior may change through time, we have built a multivariate Hawkes process model to examine calling and counter-calling behavior of NARW in CCB.

Results

For the first fusion, we fit the model to two consecutive days in 2009; we estimated 64 NARW present on 9 April 2009, and 53 whales present on 10 April (Figure 1). We estimated calling rates of 3.86 and 1.2 calls per hour per whale on these two days. The inclusion of the PAM data to the fusion facilitates better estimates of abundance but is less spatially informative than the aerial data.

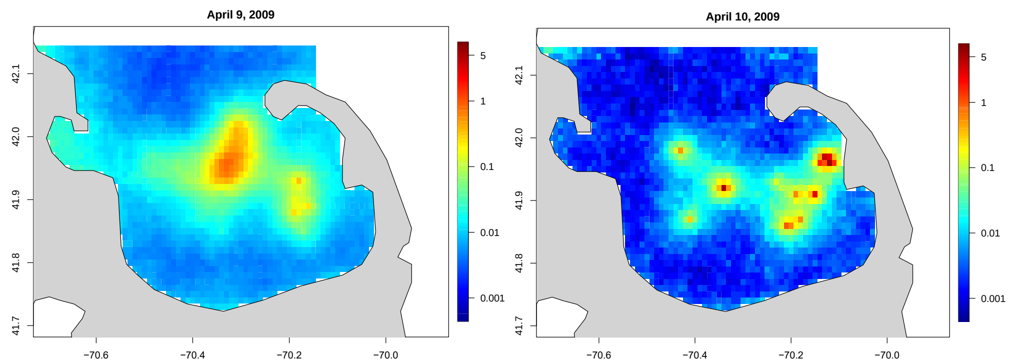

Figure 1. *Posterior mean surface of the intensity of abundance for Cape Cod Bay on April 9, 2009 (left) and April 10, 2009 (right) under the proposed data fusion model.*

Using a Hawkes process model to examine NARW acoustic behavior across the CCB array, we were able to estimate background calling rate, the intensity and the decay of the response to up-calls, as well as the impact of ambient noise on calling rate (Figure 2).

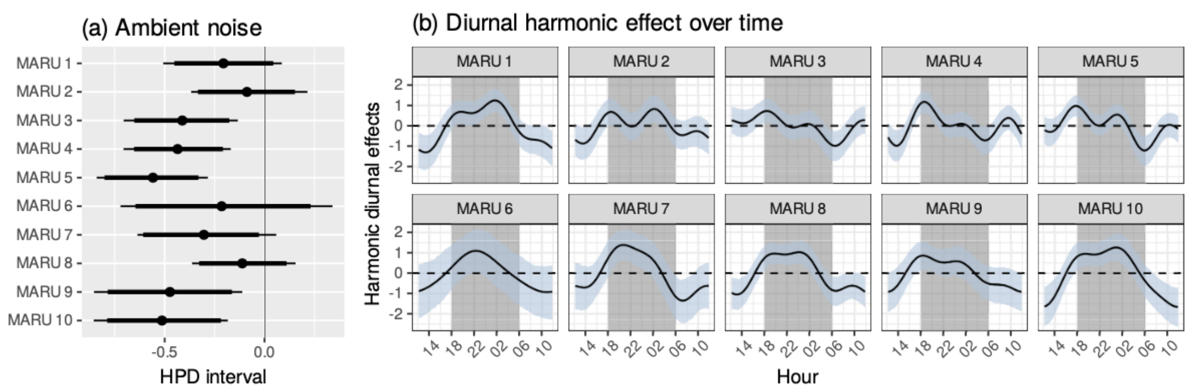

Figure 2. *(a) Estimated posterior means (shaded dots), 95% HPD intervals (thin horizontal bars), and 90% HPD intervals (thick horizontal bars) for the coefficient of the ambient noise per MARU. (b) Estimated diurnal harmonic effects over time for each MARU. Blue shades are 95% credible bands.*

References:

Schliep, E. M., Gelfand, A. E., Clark, C. W., Mayo, C. M., McKenna, B., Parks, S. E., Yack, T. M., and Schick, R. S. (2023). Assessing marine mammal abundance: a novel data fusion. arXiv preprint arXiv:2310.08397. *In review* at Annals of Applied Statistics.

Improving estimates of Cuvier's beaked whale sonar response by linking satellite tag and range acoustic data

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Background

Short-term movement responses of Cuvier's beaked whales to Navy sonar have been documented using animal-borne satellite tags. Hydrophones installed in several Navy training ranges detect Cuvier's beaked whale echolocation clicks, and a processing pipeline (M3R) has been developed that associates detections from nearby hydrophones into Group Vocal Periods (GVPs), representing echolocating groups. This system also detects sonar. The two data sources are complementary: tag data gives movement patterns and sonar response of a small number of individuals over timescales up to a few months while hydrophone data gives population-level acoustic patterns with and without sonar over several years. This project aimed to bring the two together, using data from the Southern California Anti-Submarine Warfare Range (SOAR).

Objectives

The long-term goal of the project is to enhance understanding of the population-level responses of Cuvier's beaked whales to Navy sonar. The proximate objective is to unify tag and hydrophone data streams through a common mechanistic model for animal movement and response to sonar.

Methods

Hydrophone data from SOAR were provided for the period 2010-2021 and tag data (fastloc GPS or ARGOS tags) collected between 2008-2019. Data was used from the period of overlap.

Hydrophone data were processed by the M3R system to yield the number and location (i.e., closest hydrophone) of GVPs per 30-minute period. The system also noted which hydrophones were on effort (i.e., recording reliable data), and which hydrophones detected sonar by 30-minute period. The spatial configuration of on-effort hydrophones was tessellated to produce a discrete non-overlapping set of spatial tiles around each hydrophone within which GVP and sonar were either present or absent.

The spatial pattern of GVPs under non-sonar ("baseline") conditions was modeled using a spatial smooth, and the change in this pattern during sonar periods was modeled as a second spatial smooth [1].

Tag data were processed using the continuous-time continuous-space movement modelling tool *crawl* [2], which accounts for the intermittent nature of transmitted locations and their positional error, and output locations were produced per 30-minute period.

Exploratory data analysis was undertaken to determine whether locations of tagged animals were associated with GVP detections on hydrophones.

A joint discrete-time discrete-space model was developed, where movement of individuals between tiles over 30-minute periods was a function of underlying spatial habitat preference and sonar response. The model took patterns from the GVP spatial model as input and was fitted to the *crawl* positions using maximum likelihood.

Results

Data from 17 tags overlapped with times when the SOAR hydrophones were on effort, totaling 10,489 30-minute periods. Hydrophone effort varied over time, requiring multiple tessellations (Fig. 1).

The spatial models of GVPs showed acoustic detections varied over space, day, hour and hydrophone depth, and decreased in the presence of sonar.

Exploratory analysis indicated that GVPs were not necessarily detected close to tracks of individual tagged whales (Fig. 2), validating the use of spatial models from GPV data rather than attempting to link individual tracks and GVPs.

The joint model showed evasive movement in the presence of sonar, but the pattern differed from that in the acoustic-only GVP model, indicating both a vocal and movement response.

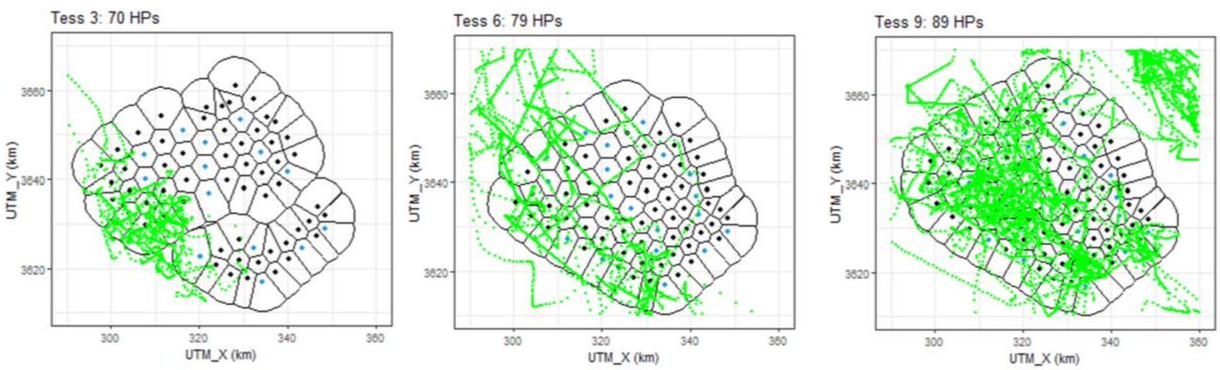

Figure 1: Example tessellation grids for on-effort hydrophones (HPs) at SOAR. Approximate hydrophone locations are shown as dots (blue are bi-directional hydrophones) and tracks of tagged animals are shown in green.

Figure 2: Available online at https://lenthomas.org/soarzc.gif. Animation showing estimated positions (large blue dot) of a tagged beaked whale (ZzTag037) on SOAR at 30-minute intervals, with approximate hydrophone locations (small dots, blue indicating GVPs) and red polygons showing sonar detections.

References

[1] Jacobson, E.K., E.E. Henderson, D.L., et. al. 2022. Quantifying the response of Blainville's beaked whales to US Naval sonar exercises in Hawaii. Mar. Mam. Sci. 38: 1549-1565.

[2] Devin S. Johnson and Josh M. London. 2018. crawl: an R package for fitting continuous-time correlated random walk models to animal movement data. https://doi.org/10.5281/zenodo.596464.

DMON Portable Range Engineering

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Background

The project has as its primary goal the development of methods for monitoring marine mammals and behavior using a portable approach that can be deployed in different areas as required. The program seeks to create (1) a low-cost portable acoustic range that will allow detection and localization after the moorings are collected, and optionally, in real-time by transmitting precisely time-stamped detections via an integrated acoustic modem, and (2) a highly flexible moored autonomous recording system. Previous integration of these components into a basic capability has been demonstrated in Cape Cod Bay. The system takes advantage of detection techniques developed under earlier programs done in collaboration between the WHOI Biology Department and the Applied Ocean Physics and Engineering Department.

Methods

The components that comprise the DMON Portable Range (DPR) and the autonomous recording system have already been developed, including the DMON, WHOI Micro-Modem, an atomic clock, plus an optional small buoy capable of receiving the real-time detection data from the DMON. In this project the effort focuses on utilizing these existing components, and improving the memory storage subsystem, as well as the clock synchronization, to make the system more reliable and user friendly.

Objectives

The objective of this project is to advance methods for studying marine mammal movements and acoustic behaviors by developing an array of acoustic recorders that are time synchronized to enable time-difference of arrival localization of marine mammal vocalizations. The system is designed to be easy to prepare and easy to deploy and recover at sea, and capable of recording time-synchronized recordings for several days to tens of days at a time, depending on the sample rate.

Results

The original DPR DMON2 instruments from a previous ONR-sponsored project were successfully modified to use single-layer cell (SLC) NAND flash chips instead of the original multi-layer cell (MLC) chips. This change allowed the DMON2 to operate with reasonable error correction coding, albeit with a reduced total flash memory capacity. It also brought the DPR DMON2 units into alignment with the many other DMON2 instruments that have been used for several years now for near real-time marine mammal monitoring from autonomous platforms.

DMON2 instruments were programmed to accept the pulse-per-second (PPS) and serial inputs from the chip-scale atomic clock (CSAC), and to “lock” onto this PPS signal to exclude any noise that may occur on the PPS line. The DMON2 sets its internal clock to the time delivered by the CSAC once every 30 seconds; therefore, all audio recordings or detection events are time-stamped with accurate times. Clock setting is reported in the system log, as are times associated with duty cycled recording events (including continuous recording). This timing information is accessible after instrument recovery so that audio recordings can be associated with accurate timing.

To demonstrate this ability on the bench, two DMON2 instruments, each with attached CSACs, entered recording mode after a GPS was used to serially and independently synchronize each CSAC. An impulsive sound was introduced to the hydrophones of each of the DMON2 instruments. The clocks of each DMON2 were independent of one another, but the impulsive sound was aligned in the audio recordings to within a few tens of microseconds (Figure 1).

The DMON DPR is presently nearly ready for at-sea testing and validation with test signals, after which it can be used for marine mammal localization via time difference of arrival. Final assembly of the systems is now completed and documentation is available with instructions for operation.

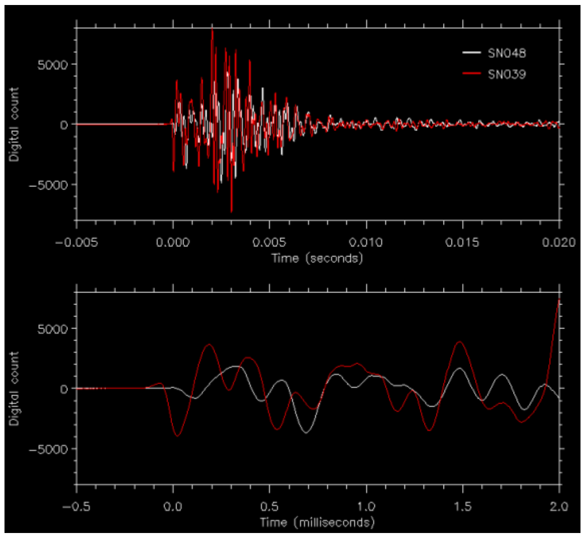

Figure 1. Waveforms of an impulsive sound introduced to the hydrophones of two DMON instruments (s/n 048 and 039) whose clocks are set independently set via attached CSACs. Sample rate is 240 kHz. The waveforms are aligned, indicating the clocks are independently synchronized. The upper panel shows the entire impulsive sound, whereas the lower panel shows a zoomed in view of the first few milliseconds of the impulsive sound.

Passive acoustic monitoring of ocean sounds, meteorology, and marine mammals from long-endurance expendable profiling floats

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Background

The project proposes to pursue, finish and validate the development of a novel long-endurance acoustic profiling float. The system has the capacity to record, process and transmit to shore float (GPS location, instrument status, ...), CTD (Conductivity, Depth, Temperature) and PAM (Passive Acoustic Monitoring) data. It has direct applications for ambient noise monitoring, acoustical meteorology and marine mammal detection. Future applications, not covered under this project, include environmental characterization using acoustic sources of opportunity.

The project leveraged recent work by Bonnel and Baumgartner (co-PIs). Prior to this project, they integrated the digital acoustic monitoring (DMON2) instrument and its software suite (LFDCS), into a commercially available profiling float (ALTO float from MRV) heavily used in the Argo program.

The prototype acoustic float, shown in figure 1, was deployed on the New England Shelf from June 24, 2021 to August 18, 2021 (8 weeks) around (N 41°, W 71°). The float successfully recorded, processed and transmitted data (ambient sound spectra, summary of whale detection, CTD data, as well as instrument status) to shore every ~12 hours via Iridium short-burst data (SBD) messages during the 8-week mission. The float was recovered at the end of its mission.

All of the data have been updated in near real-time on a website developed by the 2 co-PIs [1]. Further, the raw acoustic data was stored inside the float. Since the float has been recovered, these raw data are available for further analysis, to be done during this project.

Objectives

In the context cited above, the specific objectives of the project are as follows:

- Analyze the recently collected acoustic data to 1) identify potential sources of platform noise generated by the float, 2) manually verify the marine mammal detections and 3) implement state-of-the-art methods to estimate wind speed and rainfall rate from the ambient sound spectra;
- Write and submit a peer-reviewed article describing and evaluating our acoustic float system;
- Optimize the existing system to 1) mitigate self noise from the platform and 2) compress DMON2 data transmissions to limit the number of SBD messages to be sent by the float (thus reducing satellite communication cost and time at the sea surface for the float);
- Perform a long-term (multi-year) expendable mission with 2 floats in the North Atlantic.

Technical tasks

To reach the objectives, the work is divided in 4 technical tasks:

Task 1 Data analysis: a) Explore raw acoustic data and identify sources of self noise. b) Evaluate performance of marine mammal detection. c) Infer wind speed and rainfall rate from acoustic data.

Task 2 Software update: a) Reduce the volume of float data sent back to shore. b) Update shore-side server processing to perform acoustical meteorology

Task 3 Hardware update: Mitigate self-noise

Task 4 At-sea trial: Perform a long-term (>1 year) expendable mission with two acoustic floats.

Results

Data analysis: The raw acoustic float data (collected before the start of this project) were fully reviewed by an experienced analyst, both aurally and visually in a spectrogram. The analyst reported presence or absence of sei, fin, right and humpback whale vocalizations.

Both the analyst and the DMON consistently report very little detection/presence for sei, right and humpback whales. Those numbers are deemed to small for a more detailed quantitative comparison. On the other hand, both the analyst and the DMON reported a high number of detection/presence for the fin whales. As in previous DMON performance studies [2], a logistic regression was used to relate the analyst-identified occurrence of vocally active fin whales to the number of fin whale calls (see figure 2). Overall, we demonstrated the capacity to perform automatic marine mammal detection with the DMON-equipped acoustic floats, with performances similar to other DMON-equipped autonomous platforms.

Hardware update: Existing data was thoroughly analyzed and new recording tests were performed in the lab to track the sources of the self noise. Three sources of noise were identified: the DMON recharging circuit, the RS232-TTL converter, and the transducer head.

Solutions to mitigate the self-noise have been found and are implemented:
* the float transducer head will be changed (test performed with a glider transducer head demonstrated this solution to be effective).
* plugging the DMON into a rechargeable Li-Polymer battery (while still leaving the float battery pack plugged in) has been found effective to mitigate self noise from the DMON recharging circuit
* removing the RS232-TTL converter (to convert the float logic-level signals into DMON RS232 signals) and modifying the DMON board to accept TTL signals has been found effective to mitigate self noise associated with float-DMON communication.

Overall, all the major sources of self-noise have been tracked an mitigated. Figure 3 illustrates acoustic data before/after self noise mitigation.

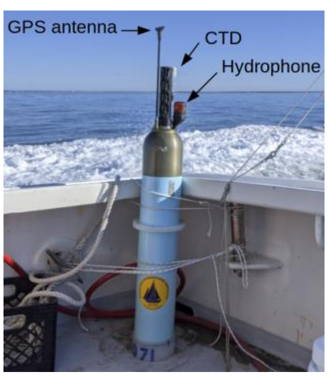

Fig 1: Acoustic float

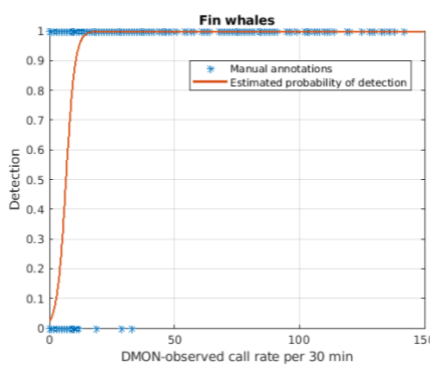

Fig 2: Relationship between DMON-observed call rates and analyst-observed probability of presence. The orange line is the fitted logistic regression $logit(p_a)=b_0+b_1c_r$, with c_r the DMON-observed call rate per tally period (30 min), and b_0 and b_1 are the model parameters.

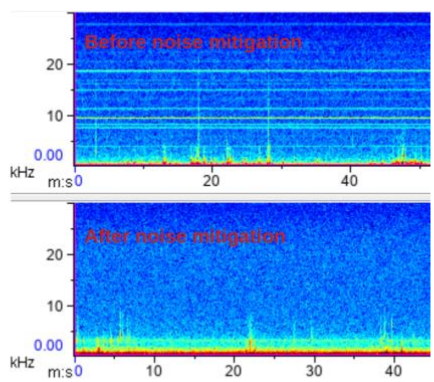

Fig 3: Noise recording test in the lab, before (top) and after (bottom) hardware modification

References

[1] http://dcs.whoi.edu/nesh0621/nesh0621_nesh_html/nesh0621_nesh_summary.html
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Automated Detection and Localization of Fin and Blue Whale Calls Recorded with Distributed Acoustic Sensing on the Ocean Observatories Initiative Cables

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Background

Distributed acoustic sensing (DAS) is an observational technique that interrogates an optical fiber with repeated laser pulses and detects changes in the phase of backscattered light to measure the strain rate along the fiber (Hartog, 2017). The method can work to distances of ~100 km and at this scale has a spatial resolution of tens of meters. DAS has a broad frequency sensitivity, which depending upon the application can extend from <0.001 Hz to >>1 kHz (Lindsey and Martin, 2021). A DAS fiber optic cable behaves like a long line of closely spaced single-axis broadband seismometers that sense deformation in the direction of the fiber, although DAS measures the spatial derivative of ground velocity (or rate of change of strain) rather than ground velocity (Hartog, 2017).

Several studies have evaluated the potential of submarine DAS for low frequency acoustic studies. Rivet et al. (2021) used DAS to track a tanker passing over the MEUST cable at water depths of both 85 m and 2000 m. Matsumoto et al. (2021) compared DAS and hydrophone recordings of airguns using a cable extending off Japan to >3000 m water depth. Both systems were sensitive to airgun signals from 0.1 to tens of Hz, although the DAS had lower signal-to-noise ratios above a few Hz. In a shallow Fjord in Norway, airguns were recorded with similar signal-to-noise ratios using DAS on a cable at 100-400 m water depth and a towed hydrophone streamer (Taweesintananon et al., 2021). Using the same DAS data set, Bouffaut et al. (2022) and Landrø et al. (2022) present recordings of baleen whales and ships at frequencies up to nearly 100 Hz and demonstrate tracking near the cable. DAS has typically required unused (dark) fibers, but new multiplexing approaches will now allow it to run on lit fibers without reducing telecommunications capacity, so it may become more common on commercial submarine cables.

Objectives

The overall objective of this project is to explore a public domain DAS data set that has been collected with the two cables of the Ocean Observatories Initiative Regional Cabled Array offshore central Oregon (Wilcock & OOI, 2023), to understand the capabilities of submarine DAS to record low frequency fin and blue whale calls and to develop automated tools to detect and localize baleen whale calls that can be applied to this and other DAS data sets. The OOI DAS experiment took place during a scheduled maintenance shutdown of the OOI RCA from November 1-5, 2021, and collected ~26 TB of data. The experiment recorded several tens of thousands of fin whale calls as well as call sequences from several blue whales. Wilcock et al. (2023) presented a preliminary analysis of the whale calls and ship noise in the OOI DAS data and demonstrated that the data could be used to localize fin whale calls with manual arrival time picks. To build upon this work, a postdoctoral scholar, Quentin Goestchel, started work in January on this project which will address the following four tasks:

1. Develop approaches to automatically identify fin and blue whale calls in DAS data.
2. Develop techniques to locate fin and blue whales automatically and accurately with DAS data.
3. Analyze the performance of DAS in recording whale calls for different geometries.
4. Estimate cable location using the whale calls.

Methods

For our initial work we are focusing on task 1, comparing techniques to identify fin whales using a 60-s data segment that includes both low and high frequency notes from two fin whales. We have implemented an efficient sparse hybrid band-pass *f-k* filtering to improve the signal-to-noise ratio of the whale calls. This also can be used to achieve compression rates up to 95%. Different filter settings are compared quantitatively by considering their signal to noise (SNR):

$$SNR_{jn} = 10 \times log_{10}\left(\frac{|s_{jn}+i\mathcal{H}(s_{jn})|^2}{\sigma_j^2}\right),$$

with j the spatial index, n the time index, s the strain, σ the standard deviation and $\mathcal{H}$ the Hilbert transform.

We are evaluating two conventional detection methods, matched filtering using hyperbolic chirp call templates that are based on the instantaneous frequency extracted from example calls and spectrogram correlation using templates created by combining the same call frequency characteristics with a hat function. We plan to explore the application of image processing techniques, including edge detection filters, to the amplitude envelope of the DAS data and the use of convolutional neural network encoders. As we develop algorithms, we are contributing them to the existing Python package, DAS4Whales (Bouffaut, 2023) so that they are available to the community.

Results

Filtering comparisons based on SNR estimates show that infinite-speed *f-k* filtering improves the SNR near the apex of the call (the closest point on the cable to the whale) but decreases it elsewhere. Non-infinite *f-k* filtering is thus preferred. The SNR is be optimized for both low and high frequency calls with a 14-30 Hz bandpass filter and a 1350-3450 m/s *f-k* filter (Fig.1). Matched filtering detects calls up to ~35 km from the call apex although detection thresholds that minimize the number of false detections result in missed detection on many channels (Fig. 2). Preliminary results suggest that spectrogram cross-correlation is a more effective detector but is an order of magnitude less computationally efficient. We will next explore image processing approaches.

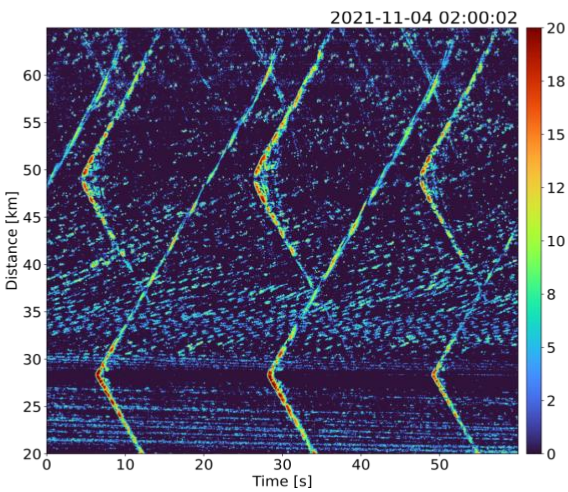

FIG. 1. Estimates of the SNR for fin whale calls in 60s of DAS data between 20 and 65 km from the shore that has been first filtered with a 14-30 Hz bandpass filter and a 1350-3450 m/s *f-k* filter.

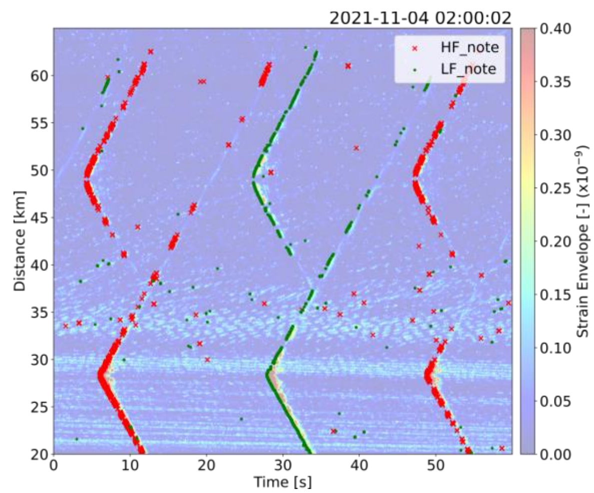

FIG. 2. Strain envelope for the same data as shown in Fig. 1 overlain by matched filter detections for the high frequency (red crosses) and low frequency (green dots) notes.

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Environmental (e)DNA metabarcoding for estimating haplotype diversity and population differentiation of social odontocetes

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Background

Environmental (e) DNA metabarcoding has great potential for characterizing mitochondrial and nuclear diversity from large groups of social odontocetes commonly found on the Navy's training ranges. To date, descriptions of genetic diversity and differentiation of these social groups have been dependent on a relatively small number of biopsy samples from any given social group. In the case of species with high mtDNA diversity and group sizes of several hundred individuals (e.g., common dolphins), this is obviously inadequate. Even with modest group sizes (e.g., bottlenose dolphins), it can be difficult to collect a representative sample without behavioral disturbance. Conversely, some social odontocetes are strongly matrilineal and exhibit very low levels of mtDNA diversity (e.g., killer whales and pilot whales), making it difficult to sample rare variants that might reflect social interchange. The eDNA sampling in the passage of dolphins or whales, and the subsequent next-generation metabarcoding, should provide a more comprehensive estimate of haplotype diversity. Unlike biopsy sampling, eDNA sampling is neither invasive nor intrusive, and requires relatively little training or specialized equipment.

Objectives

1) Work with Primary Investigators of ONR funded project N00014-19-1-2572 (B. Southall and J. Calambokidis), to collect eDNA samples in proximity to social odontocetes near Catalina Island, California;
2) Work with Cascadia Research Collective (R. Baird), to collect samples from other odontocetes along the Kona Coast, Hawaii;
3) Work with Associate Investigator (E. Archer) to develop analytical methods for species identification and for estimating haplotype diversity using eDNA metabarcoding;
4) Investigate the potential for characterizing nuclear diversity of social groups by amplification of microsatellites or Single Nucleotide Polymorphisms (SNPs) (E. Carroll); and
5) Trial a system for automated seawater collection and filtering of eDNA, in collaboration with Center for Coastal Studies, Provincetown, Massachusetts (C. Hudak).

Methods

The workflow of our methodology is summarized in Figure 1. Building on the progress made in our previous projects, we used droplet digital (dd)PCR to quantify eDNA and an Illumina MiSeq to sequence mtDNA haplotypes from seawater collected in the proximity of large groups of social odontocetes. By using a next-generation sequencing platform, such as the Illumina MiSeq, and primers designed to target the variable region of the cetacean mtDNA control region, we can resolve numerous haplotypes from each sample. Referred to here as "eDNA metabarcoding", the identification of unique Amplicon Sequence Variants (ASVs) and their relative frequencies of occurrence will be used to improve estimates of diversity and differentiation of social groups.

Results

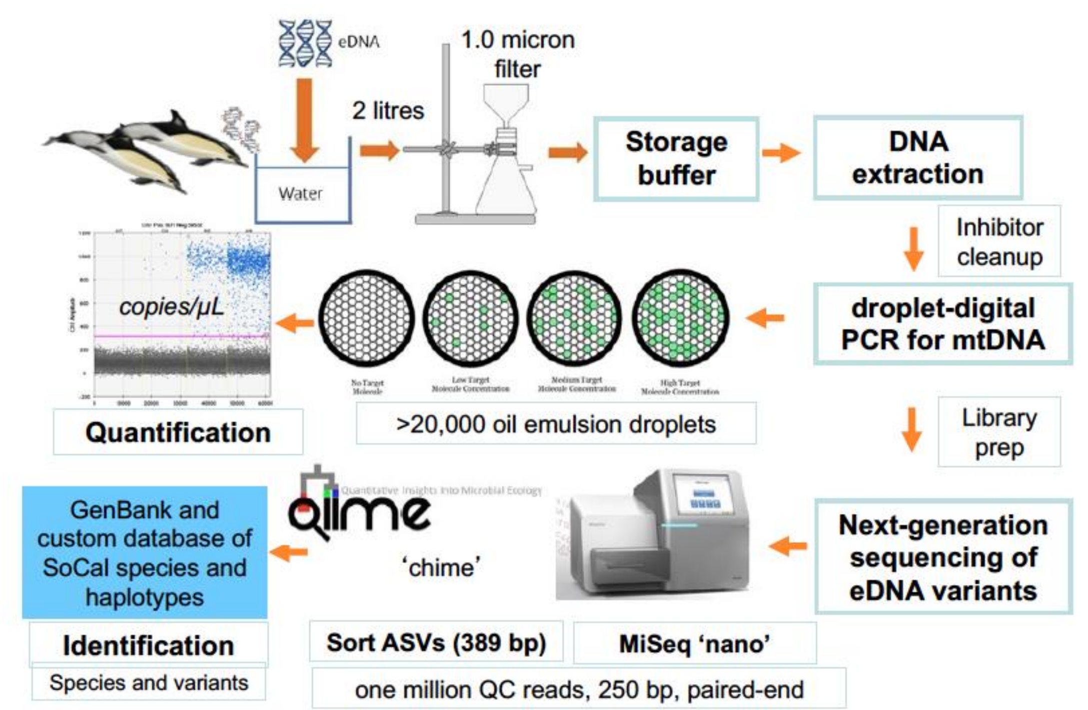
The field component of *Objective* 1 was completed in October and December, 2021. In total, n = 126 paired samples, totaling 2L each, were collected during 12 days of field effort. The field component of *Objective* 2 was completed in October 2022. A total of 45 samples (2L each), including negative controls, were collected during 12 encounters with 7 species.

Objective 3 is ongoing, with a focus on the species and haplotype diversity near Catalina Island. All samples have now been tested for PCR inhibitors and quantified by ddPCR. All samples have been submitted for one round of sequencing on the MiSeq (250 bases, paired-end reads). Each MiSeq run has been de-multiplexed and filtered for unique variants, i.e., haplotypes, using the program QIIME. A customized bioinformatic pipeline and database of reference sequence for cetaceans in the SoCal region has been developed for species and haplotype identification. Results of preliminary analyses confirm the identification of focal species and calculations of diversity that are consistent with expectations from observation and previous organismal sampling. Two measures of diversity have been calculated for each species: 1) Haplotype richness, the simple number of unique ASVs and 2) the Effective number of ASVs, the true measure of diversity, taking into account relative frequency of occurrence. Haplotype Richness and the Effective number of haplotypes were noticeably higher for the two species of common dolphins and noticeably lower for the Risso's dolphin and bottlenose dolphin, as expected from known characteristics of these species. This was confirmed using a resampling procedure to model the relationship of Richness and Effective number of ASVs across all encounters.

Objective 4 is ongoing. Initial efforts to amplify short fragments (less than 200 base pairs in length) of nuclear introns or microsatellites from the eDNA samples have not been successful. The field collection for *Objective* 5 has been completed. Research cruises devoted to North Atlantic right whales were conducted on 11 and 13 April, 2023 as part of a pilot trial of the portable Smith-Root environmental DNA (eDNA) sampler. Laboratory analyses are ongoing.

Our overall results to date confirm the potential for using eDNA metabarcoding to identify species, subspecies and within-species haplotype diversity from large groups of social odontocetes. Further analyses are underway to account for error in haplotype identification and to model diversity and differentiation.

Figure 1: The technical workflow for eDNA quantification by ddPCR, with species identification and estimation of haplotype diversity by eDNA metabarcoding using a next-generation sequencing platform.



AMBON – linking biodiversity observations in the Arctic

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Background

The Arctic Marine Biodiversity Observation Network (AMBON) is an interdisciplinary project aimed at characterizing biodiversity in the US Arctic ecosystem through an extensive array of physical ocean techniques, observers, acoustics, and environmental (e)DNA. With the Arctic undergoing change due to warming temperatures and reduced annual sea ice, this work aims to understand the impacts at all trophic levels, from microbes to critically important marine mammals. Of particular interest is the tracking of marine mammal presence and distribution in the US Arctic; the AMBON project aims to increase marine mammal detection through eDNA as well as compare these eDNA-based detections with those from passive acoustic devices and shipboard observing.

Methods

One-liter eDNA samples are being collected on spring and fall annual cruises throughout the Bering and Chukchi seas, from automated eDNA samplers at long-term moored sites (Fig. 1), and through community science efforts in the Native Village of Kotzebue (NVOK). All the samples are being interrogated using 12S MiFish, Baker D-Loop, Leray COI, Prada 16S, and Machida 18S primers. These suites of markers were selected to capture the broad biodiversity present at each site. Of specific interest to the detection of marine mammals are the 12S MiFish and Baker D-Loop primers, and early findings will guide their utility and continued application in detecting marine mammals.

Objectives

The AMBON project is actively assessing its suite of eDNA markers to evaluate which are the most effective at identifying the presence of the marine mammals in the US Arctic and recovering species-level resolution. Comparative analyses will be performed in the immediate future on the R/V Sikuliaq 2021 cruise samples, which will be presented within this talk.

Results

Initial results from gap analyses and samples analyzed from the US west coast, performed at PMEL, suggest that the 12S MiFish eDNA marker should be ideal for detecting the pinnipeds and cetaceans that occur in the Arctic. These results are going to be compared to sequencing data scheduled to be received during late March 2024 for the 2021 Sikuliaq cruise.

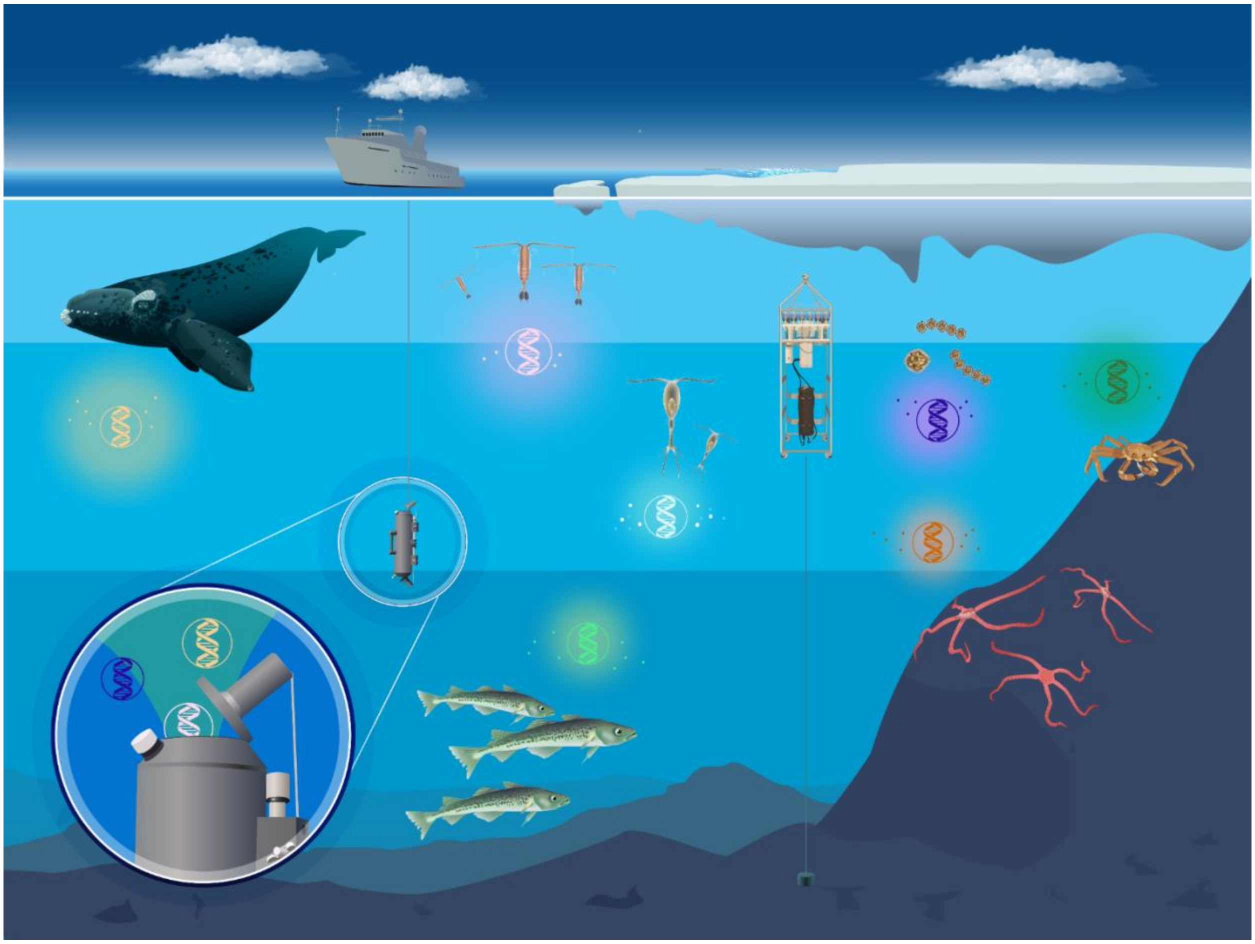

Figure 1. Illustration of eDNA sampling from Arctic species using both ship-based Niskin water samples and the moored McLane PPS automated sampler.

Using targeted environmental DNA (eDNA) sampling to generate population-level sequence data for Gervais' beaked whale (*Mesoplodon europaeus*)

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Background

Atypical mass strandings and behavioral responses of beaked whales (family Ziphiidae) associated with naval sonar have increased concern about the effects of anthropogenic noise on cetaceans and highlighted the vulnerability of this particularly cryptic taxon. Due to shared requirements for deep-water topography, Navy testing areas regularly overlap with beaked whale habitat, highlighting the need for tools to both detect and monitor the status of cryptic populations.

Population genetic assessments provide a valuable approach for identifying biologically meaningful stocks and detecting temporal changes in population structure and patterns of gene flow. However, remote collection of tissue biopsy samples can be challenging for some species and resulting sample sizes are often prohibitively small for the lesser known, cryptic beaked whales. More efficient techniques for large-scale monitoring and genetic sample collection are needed to better characterize and monitor the effects of anthropogenic activities and environmental impacts on these sentinel species.

Detection of aquatic organisms from shed cellular DNA has demonstrated success for genetic species identification and detection of rare, and more recently for generating data beyond species ID alone. Here, we build upon our previous work on beaked whales in The Bahamas supplementing existing archived tissue samples with targeted eDNA samples to generate sequence data that can be used to characterize population genetic structure for Gervais' beaked whales (*Mesoplodon europaeus*).

Methods

Dedicated eDNA and opportunistic biopsy sampling were conducted in known foraging areas for Gervais' beaked whales accessible by small boat (6.8 m RHIB): east coast of Great Abaco Island and southwest Eleuthera Island in 2022 and 2023. Photo-identification was used to document individual identifications, group size and composition. Surface seawater samples (3L) were collected in whale fluke prints to maximize collection of genetic material from target animals.

To isolate the genetic material suspended in seawater samples, 3L seawater samples were filtered onto sterile 1.0μm cellulose nitrate membranes ≤ 8 hrs post-collection. Filter membranes were preserved dry or in a lysis buffer, stabilizing eDNA until shipping. DNA was extracted from eDNA filter membranes in a dedicated pre-PCR laboratory, using previously validated approaches. Mitochondrial sequence data was generated from archived *M. europaeus* tissue samples (n = 20) collected from The Bahamas previously, targeting a region of the d-loop that encompasses the fragment targeted for eDNA amplicons. Custom primers targeting a smaller region of the d-loop specific to ziphiids were developed and used to generate NGS sequences from targeted eDNA samples. Haplotype diversity and metrics of genetic differentiation will be generated from tissue and eDNA sequence data to better understand patterns of population structure for *M. europaeus* in the canyons of central and northern Bahamas.

Objectives

1. Develop an mtDNA assay targeting the mtDNA control region for *M. europaeus*.

2. Collect biopsy, photo-ID, and surface eDNA samples from *M. europaeus* in The Bahamas.

3. Quantify patterns of genetic diversity and population genetic structure for *M. europaeus* in The Bahamas integrating data from both archived tissues & contemporary eDNA samples.

Results

Work during the current reporting period [October 1, 2022 – September 30, 2023] included design and optimization of a custom assay targeting the mitochondrial control region of ziphiid cetaceans, a two-week eDNA survey, and analysis of archived tissues and eDNA samples collected in 2022.

Existing cetacean mitogenome sequence data was mined to support *in silico* design and testing of an mtDNA assay to specifically target an informative and variable region of the d-loop for cetaceans of the Family Ziphiidae. The assay was designed to capture approximately 400bp of the mitochondrial control region known to be informative for both species identification and intra-species genetic diversity. Archived *M. europaeus* tissues (collected during previous ONR and SERDP funded research) were sequenced representing 20 *M. europaeus* tissue biopsies to characterize intra-specific mitochondrial genetic diversity.

Modified primers were designed for compatibility with Illumina sequencing platforms and applied to DNA isolated from 23 eDNA samples collected during June and August 2022 field efforts in waters east and west of Abaco and Eleuthera Islands respectively. eDNA samples collected in the fluke prints of three ziphiid species (*M. europaeus*, n = 18, and *Z. cavirostris,* n = 5) were sequenced on an Illumina MiSeq platform, resolving 13 unique amplicon sequence variants (ASVs). Multiple ASVs were detected in four samples indicating the presence of eDNA from multiple individuals in a single sample. Additional work will be conducted following the analysis of the 2023 eDNA samples to quantitatively define thresholds for identifying unique ASVs and differentiating between rare targets and data contamination due to sequencing artifacts.

During 14 days of fieldwork conducted in August 2023, weather conditions limited surveys to 11 days. A total of 683 km was surveyed for cetaceans using both visual and acoustic search methods. Search areas were defined by weather, topographical features and previous 2022 encounter locations. Eight cetacean groups were encountered including three delphinid species and one ziphiid species. Seventeen eDNA samples (delphinids, n=9 and *Z. cavirostris,* n=5) were collected in or near the fluke prints of target animals. Sampling and filtration modifications to the 2022 protocols included the use of an at-sea portable filtration unit and self-preserving filters, streamlining eDNA collection procedures and eliminating the need for sterile handling of filter membranes. At-sea filtration and sample preservation should also help to reduce the potential effects of eDNA sample degradation due to both elapsed time and ambient temperatures (SST ~31ºC). All samples were shipped to the NWFSC Molecular Genetics Lab in Seattle, WA and await DNA extraction and processing.

Advancing environmental genomics for marine mammal characterization

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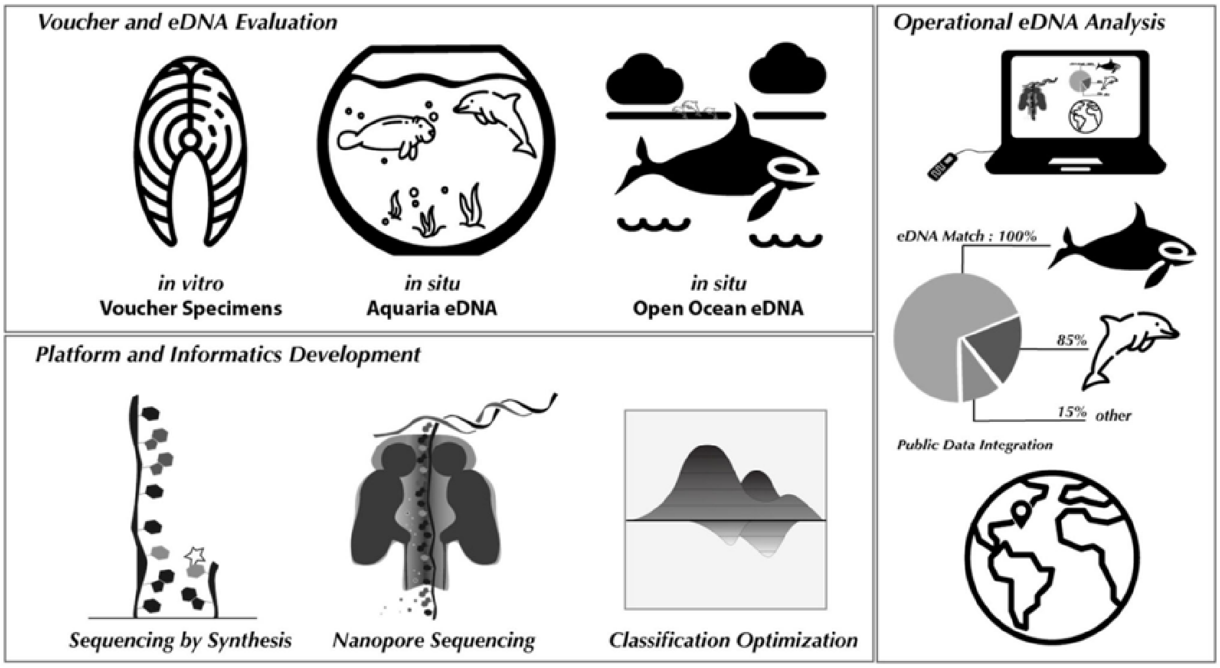
Background: Marine mammal identification is required for many different maritime activities, ranging from routine environmental surveys to identification of marine mammals that may be harmed by commercial or military activity. These surveys are currently performed by field observations or acoustic monitoring that require substantial resources and may be imprecise. Genetic profiling of eDNA from cells sloughed into the water by marine mammals has potential to reduce the time, cost, and equipment required to confidently identify mammal species within a region of interest (Thompson and Thielen 2023).

Objectives: This work aims to develop and demonstrate fieldable methods for marine mammal identification using environmental DNA (eDNA) from water samples. During the first year of research, an emphasis was placed on development of the tools and methods needed to implement rapid eDNA classification of marine mammals, while the second phase of research emphasized optimization, sample collections, and increased field research to understand method shortcomings (Thielen et al. 2023).

Methods: A suite of hardware and software to enable rapid field-based marine mammal eDNA sequencing were produced for initial evaluation in operational environments (DeHart et al. 2023). These approaches incorporated full-length mitogenomes from all marine mammals, either from public or non-public collections, to produce a custom taxonomic classifier for high-throughput sequencing data. Informatics methods were validated on metabarcoding and shotgun sequencing data, and capabilities were ultimately integrated into low-power NVIDIA compute devices for standalone data analysis on low-power compute platforms. Following field and laboratory observations, these data were additionally integrated into a bespoke fate and transport model to begin evaluating eDNA dispersal and degradation in open-ocean environments.

Results: Upon initial capability development, analytical methods were evaluated using voucher-based and field-collected samples. An initial field test was conducted in conjunction with the Monterey Bay Aquarium Research Institute (MBARI), and generated biodiversity data in near real-time while aboard ships. These observations were later evaluated alongside traditional laboratory-based approaches, and found to be highly consistent with traditional approaches. Open ocean eDNA fate and transport concepts will be highlighted as part of ongoing efforts to better understand marine mammal detectability in real-world settings using physical ocean characteristics.



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MMARINeDNA: Marine Mammal Remote detection via Innovative environmental DNA sampling

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Background

To help ONR better characterize marine mammals and environments that are difficult or impossible to monitor with acoustic or other traditional means, we aim to understand the transport, persistence, and distribution of environmental DNA (eDNA) in marine environments from marine mammal targets. eDNA provides details on individual species as well as an assessment of marine ecosystem processes and species composition.

Objectives

Three project modules focus on a different spatial scale and leverage large-scale sampling efforts across the West Coast: Module 1 links oceanographic modeling with field observations to detail the behavior of DNA at scales of 0.1-10km. Module 2 joins eDNA data with acoustic and visual mammal sightings on larger-scale transects, complementing these with time-series analysis of eDNA from archival samples. Module 3 uses samples from an ongoing west-coast-wide NOAA survey to create a 3D map of the abundance of marine mammal + prey eDNA.

Methods

Module 1: Space and time. Uses qPCR, ddPCR and multi-species amplicon sequencing (metabarcoding), coupled with oceanographic models to explore the fate and transport of eDNA and eRNA molecules in marine waters using a non-native species (*Tursiops truncatus*) in Hood Canal, WA.
Module 2: Integrating acoustic, visual, and genetic monitoring. Uses metabarcoding assays to characterize cetacean species diversity across samples collected by cruises over the past decade. By incorporating visual, acoustic, and eDNA datasets from these cruises, we aim to develop an integrated model of cetacean occurrence in the southern California bight.

Module 3: Scaling up. Uses thousands of samples taken along the west coast during NOAA research cruises, and joins qPCR and metabarcoding to inform predictive models and joint species distribution models that map the distribution and co-occurrence of mammal and non-mammal species along the coasts of California, Oregon, Washington, and British Columbia.

Results

Module 1: Space and time
- Downscaled oceanographic model (10-20m resolution) completed, used for predicting DNA spread from point source; guides real-world sampling (Xiong et al in revision; **Fig1A**).
- Observations from transects in space closely match predictions from oceanography; eDNA behaving like a passive particle.
- Draft biophysical model explains observed hour-to-hour variation in eDNA concentration as a function of tide, number of animals, and shedding rate (Brasseale et al. in prep).
- Next: Depth sampling for 3D model calibration, hindcasting.

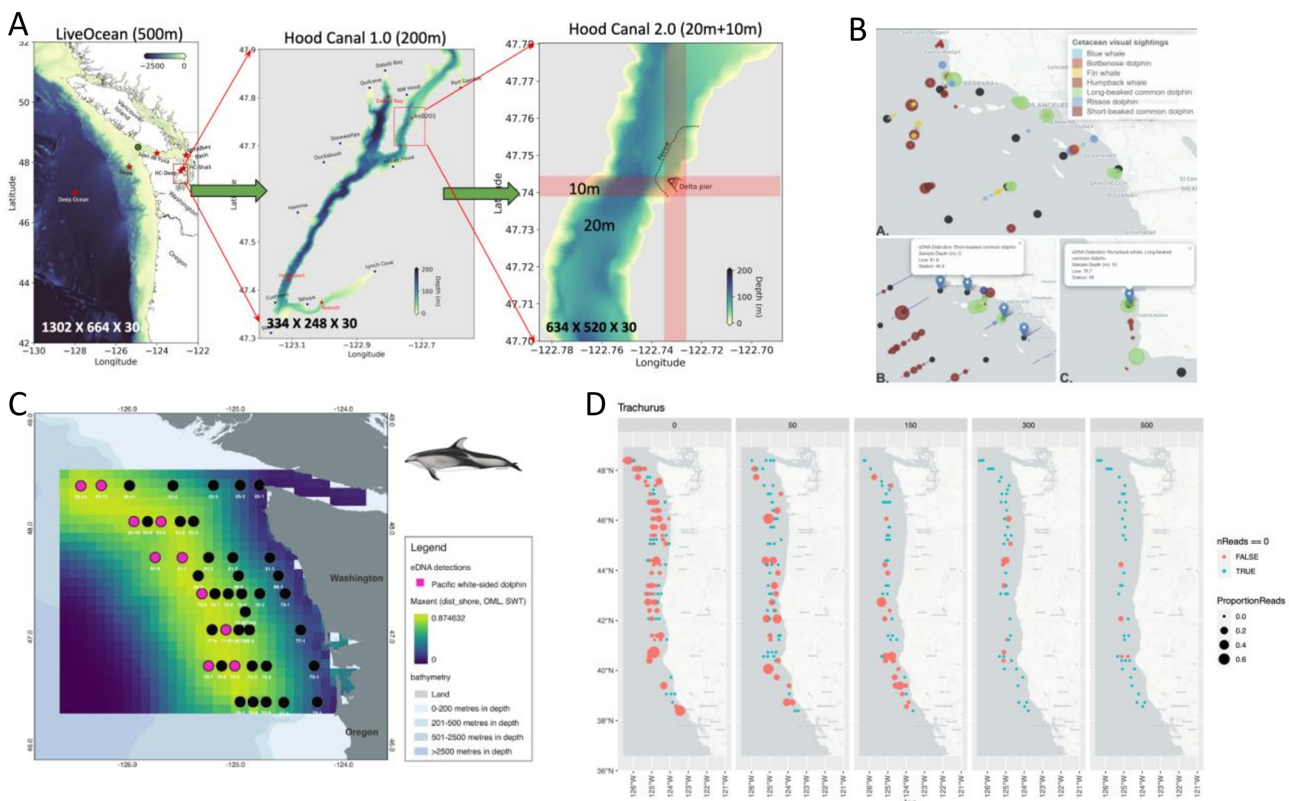
Module 2: Integrating acoustic, visual, and genetic monitoring

- Completed 2014 acoustic data processing for blue, fin, and humpback whales.
- Built prototype spectrogram classification model for blue whale calls in 15 second windows. Needs to be fine-tuned for sonobuoy data. Gathering more diverse training examples.

- Built Shiny app for comparing visual and acoustic detections.
- DNA sequencing runs (12s, dloop) on archival samples from Southern California, originally targeting microbes, yielded detections of various mammals (humpback, delphinids; **Fig1B**) as well as fish, although off-target amplification rates are high. Optimizing sampling and PCR protocols now.
- 1600+ samples from intercalibration cruise presently being sequenced (mammals, fish, invertebrates).

<u>Module 3: Scaling up</u>

- Thousands of samples in hand from NOAA research cruises; DNA extracted, and 17 sequencing runs have so far produced ca. 200 million reads of mammals, fish, and other species. Theoretical model and methods optimization complete (Shaffer et al in revision).
- Cetaceans detected (dloop + 12s) include 2 beaked whales (*Z. cavirostris* and *B. beardii*) and at least 10 others. Many humpbacks, and dolphins, and fin whales.
- Developed preliminary spatial model for three species (Valdivia et al, in prep; **Fig1C**)
- Fish are rich dataset for analyzing mammal prey fields in three dimensions (**Fig1D**)
- Next: dloop primer redesign for better mammal specificity; large-scale sequencing of mammals; spatial-statistical modeling for mammals and prey



Project leads: Kim Parsons (NOAA), Brice Semmens + Simone Baumann-Pickering (UCSD), Len Thomas (Univ. St. Andrews).

Platform Development for Autonomous Environmental DNA Sequencing

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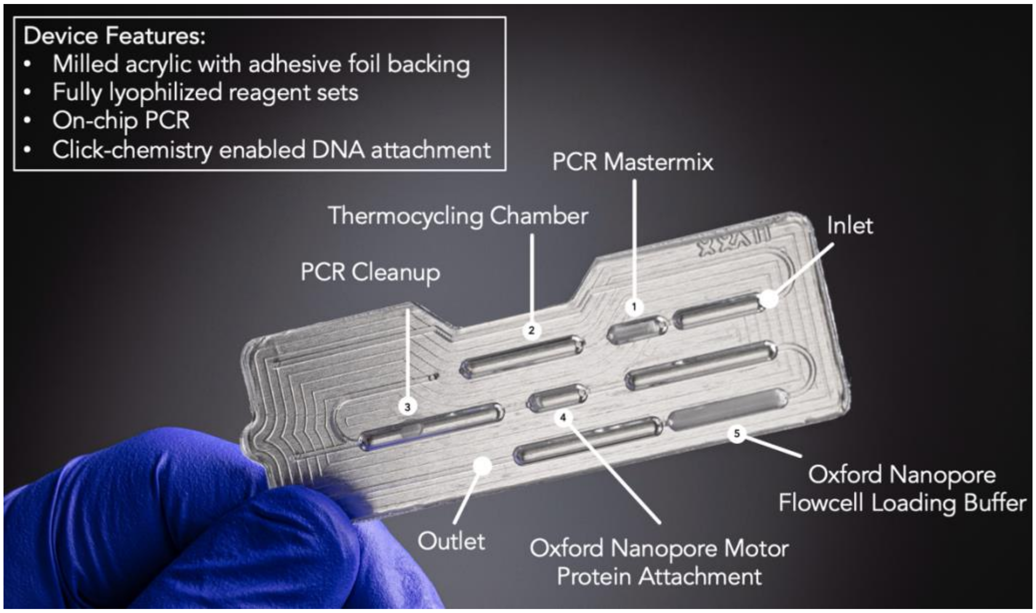
Background: Developing efficient, standardized means of evaluating marine biodiversity is widely recognized as an urgent frontier in ocean observing. The rapid advance of marine environmental sensor systems has revolutionized ocean science, with systems in place to monitor physical and chemical properties of air and water from coastal areas, open water, and satellite imagery. In contrast, tracking of ocean life has lagged far behind due to its spectacular diversity, which presents formidable challenges to both standardized sampling and informatics processing. Complete automation of environmental DNA analysis would enable a new capability for biosurveillance that expedites time to data delivery while paving the way for autonomous biosensing capabilities.

Objectives: This work aims to develop fully autonomous capabilities for marine eDNA sample collection, onboard sample processing, DNA sequencing, and integrated data analysis. The proposed platform requirements have been defined with the objective of deployment within a long-range autonomous underwater vehicle (LRAUV). Initial research has focused on production of a consumable microfluidic device capable of performing all molecular sample processing that would traditionally occur by a person in a laboratory, including PCR, subsequent sample purification, and attachment of the DNA:Protein complex required for sequencing on an Oxford Nanopore platform.

Methods: A low-cost plastic (PMMA) consumable capable of performing PCR, subsequent sample cleanup, and click-chemistry attachment of the DNA:Protein complex required for Oxford Nanopore sequencing is under development. Methods for reagent lyophilization on-chip have been identified, enabling pneumatic operation of the device with microliter-scale fluid movement precision using commercially available microfluidic pumps and valves.

Results: The device has shown significant promise for integrated PCR and fluid movement, with individual components demonstrated as the entire chip is integrated into a complete device. Comparisons of on-chip vs. laboratory PCR amplification are nearly identical, and resulting data show little or no difference between approaches. Design and manufacturing considerations for the device are increasingly under consideration as a low-cost and easy to produce consumable, and the approaches take to date have indicated advantages of the design while highlighting areas for improvement in others. Future platform integration plans for unmanned systems will be highlighted as next steps in capability development.


Device Features:
• Milled acrylic with adhesive foil backing
• Fully lyophilized reagent sets
• On-chip PCR
• Click-chemistry enabled DNA attachment
PCR Mastermix
Thermocycling Chamber
Inlet
PCR Cleanup
1
2
3
4
5
Oxford Nanopore
Flowcell Loading Buffer
Outlet
Oxford Nanopore Motor
Protein Attachment

Automated technology for rapid marine eDNA sampling and detection

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Background

The overall objective of this SBIR project is to automate eDNA sample concentration, rapid nucleic acid amplification, and detection for operation in an autonomous underwater vehicle (AUV). Remote monitoring of eDNA enables tracking of biologic inhabitants such as marine mammals and the study of the ecological impact of human activities.

Objectives

Phase II: Develop miniaturized instrument for autonomous marine mammal eDNA detection compatible with AUV platform.

Phase II Option: Integrate instrumentation into AUV platform.

Methods

We have developed a miniaturized cartridge and instrument (Figure 1) that automates: 1) Concentration of marine mammal eDNA from up to 1 L ocean water 2) Extraction of intracellular eDNA, 3) Isothermal eDNA amplification, and 3) semi-quantitative lateral flow eDNA detection. We performed field testing of the automated system at Pier 39 in San Francisco in the proximity of California sea lions.

Results

In Phase II, we successfully validated end-to-end marine mammal eDNA concentration, amplification and detection using the cartridge and instrument in both a laboratory setting and in the field. Field testing resulted in fully automated detection of California sea lion eDNA at Pier 39 in San Francisco (Figure 2)

Figure 1: 16”X13”X7” portable instrument with attached eDNA analysis cartridge.

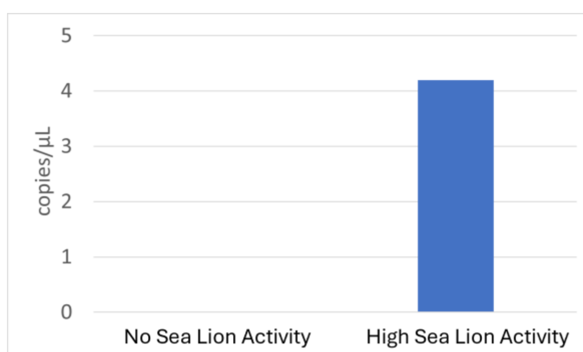

Figure 2: Results of field testing performed at Pier 39 in San Francisco.

Sponsor: ONR
Status: ONGOING

Off-range beaked whale integrated ecosystem study: Foraging ecology, prey and demography of Cuvier's beaked whale at the Azores (North Atlantic)

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Background

Beaked whales can be highly sensitive to naval sonar, posing significant risks at population-level. A major limitation in our understanding of sub-lethal effects is the near-absence of knowledge of beaked whale prey fields and their relation to foraging success, energetic return and loss incurred from impeded foraging. This limits our ability to interpret and quantify the energetic consequences of behavioral responses to naval sonar exposure, at individual- and population-levels. In this study we aim to advance our understanding of the prey fields of CBW help enable assessment of the biological significance of naval sonar responses, fill key knowledge gaps and provide a better scientific basis for Navy monitoring and mitigation measures.

Objectives

1) Characterize Cuvier's beaked whale foraging strategy and gain in relation to its deep-sea prey community; 2) Assess Cuvier's beaked whale prey species, community, density and distribution; 3) Enable comparative analysis of foraging ecology, prey, behavior, individual health and population metrics across on- and off range populations (collaborative effort); 4) Contribute to efforts modelling population consequences of disturbance.

Methods

In a long-term study site of CBW at the Azores, we combine CBW biologging and surface echo sounders with two deep-sea technologies: 1) deep-sea multi-sensor mooring equipped with echo sounders and a passive acoustic recorder (to document deep-sea prey distribution and density in relation to foraging location and quantify Cuvier's beaked whale foraging year-round; lead: Scripps) and b) eDNA sampling across the water column to characterize cephalopod and fish prey communities directly inside CBW foraging habitat (the mooring location) and in two adjacent non-foraging habitats (lead: GEOMAR). This matched approach enables the assessment of prey diversity, density and distribution in and outside of CBW foraging habitat.

Results

Overall, 33 cephalopod and 49 fish taxa were detected with eDNA across the three sampling locations. Cephalopod richness and epi/mesopelagic biomass (0-700 m) were highest in CBW foraging habitat, while fish richness was homogeneously distributed across the three sites. Highest cephalopod richness was detected above the deep scattering layer at depths between 200 - 400 m, while fish richness was highest in surface waters (50-200 m). Active and passive acoustic data from 2021-2023 revealed that CBW were present throughout all years, with lower presence during the winter and spring. First statistical analysis indicates that CBW presence increases with elevated prey backscatter. Prey concentrations, quantified as volume backscatter strength between 1000-1350 m depth, varied annually, with some seasonal trends. There appears to be a delay of approximately four weeks between high mixing events, especially anticyclonic eddies, and subsequent increase in putative prey backscatter at depth. Our ongoing analysis suggests that CBW habitat holds higher cephalopod diversity, a pattern not observed for fish, higher biomass in the deep-scattering layer and increased CBW foraging activity during periods with enhanced putative prey biomass in the foraging zone.

Project collaborators*: Dr. S Baumann-Pickering* (co-PI, Scripps Institution of Oceanography, UCSD), *Dr HJ Hoving* (co-PI, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany), *Dr E Hazen* (co-PI, NOAA, SWFSC Environmental Research Division), *Dr V Merten* (GEOMAR), *S Golan* (SIO UCSD), *Dr M Guilpin* (KMR), *M Oudejans* (KMR) and *J Stefanschitz* (GEOMAR).

Cuvier's beaked whale integrated ecosystem study: Combining deep-sea optics and acoustics for high-resolution recording of Cuvier's beaked whale predator-prey interactions

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Background

Beaked whales can be highly sensitive to naval sonar, posing significant risks at population-level. A major limitation in our understanding of sub-lethal effects is the near-absence of knowledge of beaked whale prey fields and their relation to foraging success, energetic return and loss incurred from impeded foraging. This limits our ability to interpret and quantify the energetic consequences of behavioral responses to naval sonar exposure, at individual- and population-levels. In this study we build on multi-sensor CBW prey studies at the Azores with the aim to advance our understanding of the prey characteristics which drive their foraging decisions and energetic consequences of impeded foraging, and provide a better scientific basis for Navy monitoring and mitigation measures.

Objectives

We conduct direct observations of prey and predator and their interactions in the physical environment of the foraging zones aiming to test the hypothesis that local prey characteristics, notably individual prey size/reproductive state in combination with density, drive foraging decisions in Cuvier's beaked whales.

Methods

In a long-term study site of CBW at the Azores, we combine high-resolution optic and acoustic observations of 1. CBW deep-sea prey, to record prey size, maturity and behavior and of 2. CBW foraging behavior (foraging success, 3D-foraging trajectory). The study is integrated with existing sampling of CBW prey diversity, density and distribution across the water column. Hence, combined, we are moving towards a holistic characterization of the prey scape and foraging decisions, in CBW foraging habitat. In collaborative, multi-disciplinary effort, we apply a combination of deep-sea *Nautilus* cameras and advanced multi-sensor moorings, deployed inside and outside of CBW foraging habitat.

Results

During the first field season (2023) we deployed the Nautilus drift cameras 15 times, at depths between 335-730 m, resulting in 89h of video recordings, holding 28 squids of the family Ommastrephidea (flying squids). We were able to identify the taxa based on morphological characteristics, including arm and tentacle number and morphology and shape of the body and fins. Individuals were between 19->80 cm long, with most (50%) in the size range of 36-70 cm. Most observations were made under red illumination, which squids are unlikely to be able to see, which provide a good observation technique but challenges the capacity of identification to species level. These data represent rare and unique observations on highly cryptic deep-sea squids, enabling assessment of size-distribution with increasing depth. The deployed mooring is planned for recovery in July 2024.

Project collaborators: *Dr. S Baumann-Pickering* (co-PI, Scripps Institution of Oceanography, UCSD), *Dr HJ Hoving* (co-PI, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany), *Dr E Hazen* (co-PI, NOAA, SWFSC Environmental Research Division), *S Dolan* (SIO UCSD), *Dr M Guilpin* (KMR), *M Oudejans* (KMR) and *J Stefanschitz (GEOMAR).*

Fine-scale foraging behavior of marine mammals in relation to oceanography, prey, and mid-frequency active sonar

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Background

Cetaceans, particularly beaked whales, are of concern to the US Navy regarding noise impacts during naval training exercises using mid-frequency active sonar (MFAS). The dynamic nature of the pelagic environment presents a challenge for management and impact mitigation. The goal of this project is to contribute towards an understanding of behavioral impact of naval exercises using MFAS on beaked whales and to provide ecosystem-based solutions for management.

Objectives

The project objective is to investigate the dive behavior of goose-beaked whales (*Ziphius cavirostris*) in relation to measured prey distributions and environmental variables at a highly sonar-impacted site and a minimally disturbed site. We hypothesize that an individual's behavioral response will be dependent on the underlying prey conditions and their current behavioral state.

Methods

Long-term passive acoustic data was collected at four monitoring sites: E, H, N, and W since 2006, 2007, 2009, and 2021, respectively. Additionally multi-channel tracking array data was collected within this project effort at H and W since 2021. *Ziphius* echolocation detections and 3D tracks were extracted automatically using the Matlab-based software packages *Triton*, *DetEdit*, and *Where's Whaledo* (Solsona-Berga et al. 2020, Frasier 2021, Snyder et al. in review). Autonomous active acoustic data was collected simultaneously with upward looking echosounders (70 kHz) from the seafloor. Analysis was conducted using *EchoView*. Concurrent long-term satellite tags were deployed on *Ziphius* in Southern California.

Results

Initial data analysis focused on a principal understanding of how oceanography modulates prey and *Ziphius* presence. Modeled temperature and salinity data were used to identify and quantify source waters: Pacific Subarctic Upper Water (PSUW), Pacific Equatorial Water (PEW), and Eastern North Pacific Central Water (ENPCW). The interannual and seasonal variability in *Ziphius* acoustic presence was related to the variability in El Niño Southern Oscillation events and the fraction and vertical distribution of the three source waters. *Ziphius* acoustic presence was highest during the winter and spring and decreased during the late summer and early fall. These seasonal increases occurred at times of increased fractions of nutrient-rich PEW in the California Undercurrent and decreased fractions of nutrient-poor ENPCW in surface waters. Interannual increases in Cuvier's beaked whale presence occurred during El Niño events. These results establish a baseline understanding of the oceanographic characteristics that correlate with goose-beaked whale presence in the Southern California waters on inter-annual and seasonal scales.

Prey measurements at recording sites E and W identify possible squid targets below 1100 m water depth. There appears to be higher prey density at site W than E which coincides with higher *Ziphius* acoustic presence at W (Figure 1). At site E, most foraging time is spent between 1200 and 1300 m water depth, where individual prey targets are identifiable in the active acoustic data (Figure 1).

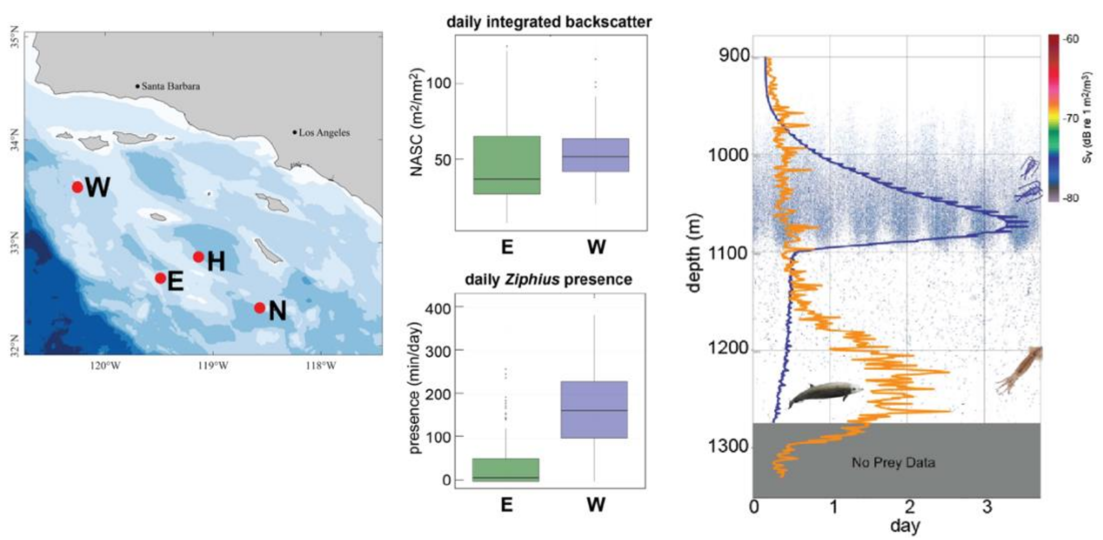

Figure 1: (Left) Tracking sites W, H, E, and N. (Center) Daily integrated backscatter below 1100 m depth at sites E and W document more biomass and higher Ziphius presence at W than E. (Right) Four-day echogram showing presumed zooplankton pray layer at 1000-1100 m and likely squid layer below 1100 m depth (blue line: proportion of integrated backscatter, 1 m bin). Ziphius foraging depth (orange line: proportion of Zc dive depth, 1 m bin) coincides with potential available prey depths.

References:

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Goose-Beaked Whales at Guadalupe Island, Mexico: A Comprehensive Assessment of Demographics and Behavior in an Undisturbed Area

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Background

The Southern California Offshore Range comprises the Southern California Anti-Submarine Warfare Range (SOAR), one of the most heavily used mid-frequency active sonar (MFAS) ranges globally. Despite this, Goose-beaked whales (*Ziphius cavirostris;* hereafter *Zc*) are present there year-round. Approximately 400 km south of SOAR lies Guadalupe Island (GI), around which resides another *Zc* population with an unprecedented encounter rate and relatively little anthropogenic disturbance, thus providing a unique opportunity to collect sizable samples of behavioral and life-history data from this species within a timeframe, and at a level of fidelity, unseen at other established study sites.

Objectives

The goal of this project is to collect comparative data from a relatively undisturbed population of *Zc* to inform and validate population consequences of disturbance models being developed for *Zc* at SOAR. Photo-identification and biopsy data support demographic assessments and documentation of population interchange. We collect photogrammetric images to assess age class, growth rates, and general body condition (including pregnancy). Deploy SMRT and LIMPET tags to allow for assessment of movements and diving behavior. Year-round passive acoustic monitoring in Bahía Norte using a high-frequency acoustic recording package (HARP) provides insight into seasonality, relative abundance, and anthropogenic sound sources. Collectively, these efforts will enhance understanding of the impacts MFAS may have on *Zc* around SOAR.

Methods

Field data collection was carried out via 'directed' and 'leveraged' efforts using large vessels and pangas. Effort was focused on the east side of GI where the highest density of animals occurs. We attempted to collect oblique photographs and photogrammetric videos for all the animals encountered. SMRT and LIMPET tags were programmed and analyzed for direct comparison to ongoing studies at SOAR. We conducted a mtDNA analysis from biopsy samples. Photographs were processed into the catalog, matched against the SOAR catalog, and scored for age-sex classification to facilitate demographic comparisons. Video stills from photogrammetry flights were matched to LIDAR altitudes to facilitate measurements of whale morphology. Acoustic data from HARPs and SMRT tags were analyzed for the presence of *Zc* echolocation pulses and anthropogenic signals.

Results

From September 2021-December 2023, we conducted 9 surveys, sighted 275 *Zc* groups including 11 mom/calf pairs, conducted over 425 drone flights, deployed 11 tags, collected 12 *Zc* genetic samples, and the HARP was refurbished for continuous year-round recordings. The GI *Zc* catalog now contains 105 unique individuals, thirteen of which have sighting histories spanning more than a decade (max = 16 years). Sixty-one individuals (58% of the catalog) have been sighted in multiple years, and 41 individuals have been documented on ten or more different days (max = 65 days), including mom/calf pairs. The catalog includes 20 known-age individuals who were born during the study, 15 of which have multi-year sighting histories. These findings suggest we are approaching the ability to census most of the population each year. Drone photogrammetry flights resulted in 50 distinct whales (7 distinct calves), 28 of which (including 3 calves) have been measured across multiple years. Preliminary growth rate estimates suggest calves grow fastest earlier in their development. There were two measured female whales presumed to be pregnant when sighted based on the presence of a young calf when sighted on the subsequent field effort, and these pregnant females exhibited wider mid-sections relative to adult females of similar body length. SMRT tags recorded 13.5 days of acoustic, kinematic, and positional data. All deep dives contained foraging activity. Despite deep, presumed foraging dives being shallower and shorter at GI compared to SOAR, GI *Zc* tend to exhibit longer IDDI durations. Of the five haplotypes identified, two were shared by *Zc* sampled at SOAR, two have not been previously reported. HARP analysis revealed year-round presence of Zc, as well as the presence of ultrasonic antifouling systems in the white shark cage diving season of 2018 and 2019. The presence of MFAS and low frequency active sonar signals were also detected at least one time via HARP and SMRT, for a total of less than 5 hours

Year	Effort days	Sightings	Tags (LIMPET/SMRT)	Unique ID	Biopsies	Measured whales (calves)	Days of HARP Recordings
2021	10	51	3/2	39	3	28 (2)	
2022	32	132	3/2	53	9	33 (5)	
2023	28	92	0/1	43	0	28 (3)	

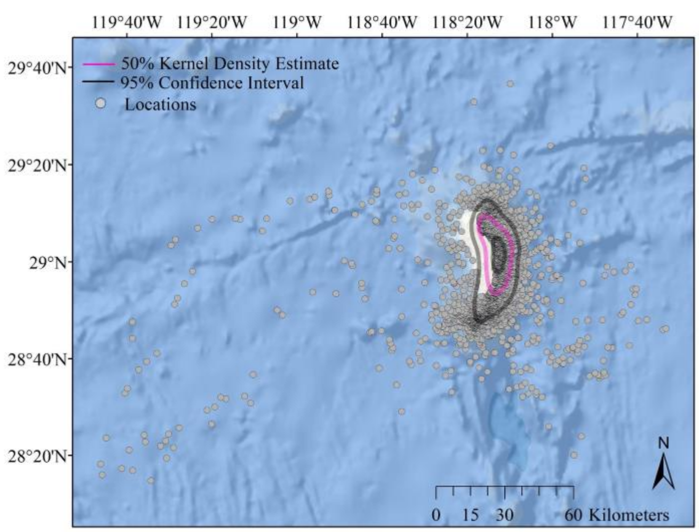

Figure of filtered Argos and GPS positions from LIMPET tags on GI *Zc* overlaid by a kernel density home range estimate, highlighting the preferential use of the leeward side of GI.

Relationship between blue, fin, and beaked whales and their prey in Southern California

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Background

The Southern California Bight (SCB) is a highly productive system due to the seasonally strong upwelling conditions, which attract diverse megafauna including a large variety of cetaceans. Blue whales are known to seasonally inhabit this region, while fin whales and beaked whales are present here year-round. All of them use this region for foraging. It is also an area of high importance to the U.S. Navy with training ranges occupying a large fraction of the SCB.
Passive acoustic monitoring for marine mammals has been ongoing in the region since 2000. Previous work funded by the Office of Naval Research (ONR) enabled investigation of habitat use by blue and fin whales, as well as beaked whales in the SCB, combining passive acoustic monitoring with remotely sensed and modeled environmental data, due to the lack of concurrent in situ oceanographic and other environmental data. Those research efforts have highlighted the need for including measures of cetacean prey into the descriptive and predictive models of cetacean occurrence. The goal of the work presented here is to address the need for understanding the coupling and dynamics of cetacean-prey relationship on fine time scales in this important region.

Objectives

By coupling passive and active acoustic data collection on an oceanographic mooring, we will be able to place the dynamics of baleen and beaked whale habitat use in a broader ecological context of their prey, as well as environmental variables. We will accomplish this by collecting long-term, concurrent whale acoustic occurrence and echosounder backscatter data at different locations in the SCB, allowing for comparative study of cetacean-prey interactions at multiple SCB locations on a fine timescale.

Methods

We deployed two passive-active acoustic data moorings between October 2021 and 2022 in the SCB. One was deployed at at site E, to extend the previous data collection at this site for the full year, wile the other was deployed for six months at site A, and another six months at site T (Figure 1). These deployments were conducted in collaboration with Dr. Baumann-Pickering (N00014-20-1-4000, “Fine-scale dive behavior of marine mammals in relation to oceanography, prey and mid-frequency active sonar”), who deployed two additional, comparable moorings during the study period at sites H and W (Figure 1). These sites were chosen because they cover areas with variable abundance of blue and fin whale calls (Širović et al. 2015), as well as beaked whale density (Baumann-Pickering et al. 2014).

Figure 1. *Locations of acoustic mooring deployments in the Southern California Bight covered under this project (yellow pins) as well as collaborative project led by Dr. Baumann-Pickering (green pins). Moorings at sites E, H, and W were deployed for a period of one year, while moorings at sites A and T were deployed for 6 months each.*

In addition to the mooring data collection, we also collected net tows during mooring deployments in conjunction with fine-scale shipboard echosounder surveys at all locations up to three times of the course of the field work, to allow ground-truthing of active acoustic data. These mooring and net data are also going to be combined with previous comparable data collection at these locations to extend our time series.

Passive acoustic data are being processed using automated detection and classification methods to extract times of occurrence of whale calls. Specifically, we are detecting blue whale B and D calls, fin whale 20 Hz call presence (using acoustic index), and beaked whale echolocation clicks. By recording acoustic backscatter at two frequencies, we can use the differences in backscatter between them to distinguish among different scatterer taxa in the water column. Zooplankton and nekton biomass estimates are being calculated for different depths, producing probability or likelihood estimates for different taxonomic and size classes of scatterers.

These data are being combined and modelled in relation to additional oceanographic variables, such as temperature and water mass structure, using Generalized Additive Modeling (GAM) framework, to improve our understanding of the ecological relationships of cetaceans within their environment.

Results

Fin whale and beaked whale data processing has been completed. Generally, beaked whales were detected most commonly at sites E and W. On the other hand, fin whale 20Hz index was stronger at the southern sites, H and E, than at the more inshore site A and northern offshore site W. We are wrapping up the detection of blue whale calls, but preliminary analysis indicates that, as expected, D calls were most common in the early summer and transition to B calls by late summer and into the fall.

In addition to the acoustic data processing, the time-consuming processing of the depth-stratified net sampling has been completed for two cruises. Generally, copepods were the most numerically abundant organism, and highest counts were encountered in 100-200m depth (Figure 2). The exception to that were deep net tows which were conducted at night; during those highest numeric abundance was in the surface layers.

The final step of the project, coming once all the mooring data are processed later this year, will be model development.

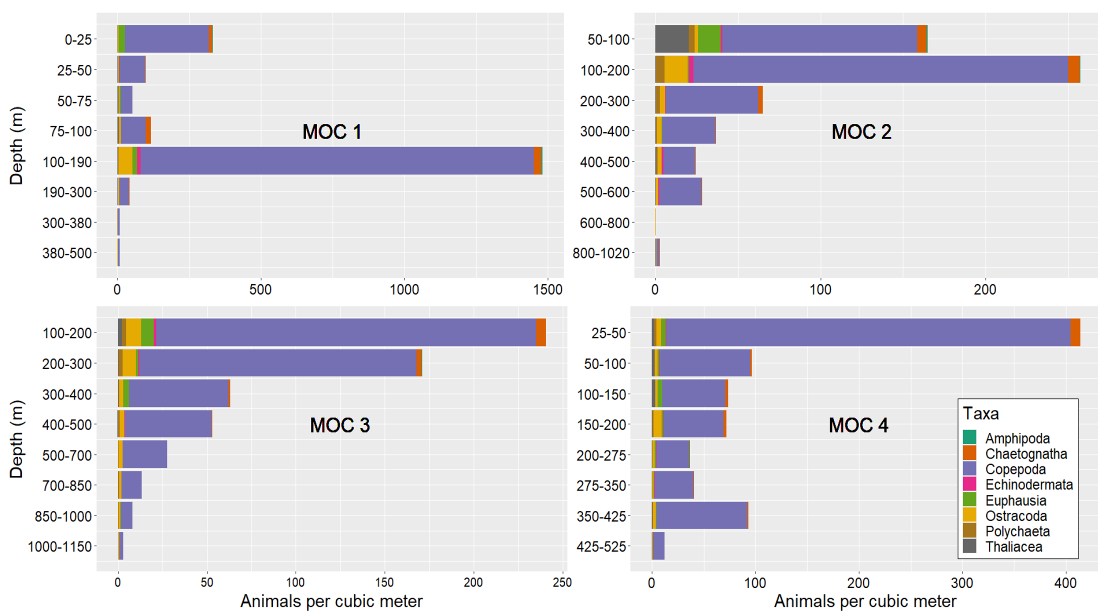

Figure 2. *Small organism (primarily zooplankton) abundance (by taxa type) for four of the six MOCNESS tows collected during the May 2022 sampling cruise. MOC numbers 1-4 correspond, respectively, to sites: A shallow (0-500m), H deep (0-1000m), E shallow, and E deep.*

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Effect of anthropogenic noise sources on beaked whales' fine-scale diving biomechanics and its energetic implications

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Background

Behavioural Response Studies (BRSs) have shown that cetaceans react to anthropogenic sounds such as navy sonar in ways that can lead to increased energy expenditure (EE). Beaked whales are the most sensitive group documented so far. It is hypothesized that sonar can cause individuals to enter an over-exerted physiological state, leading to lethal strandings. Beaked whales use fast and strong strokes (B-strokes) which are hypothesized to be associated with recruitment of fast-twitch fibres when oxygen stores dwindle in long dives. BRS have extensively used the Overall Dynamic Body Acceleration (ODBA) as a proxy for EE, to identify and characterize avoidance responses to sonar. However, ODBA is dependent on body size and activity and can overestimate the EE in large animals, preventing comparative analysis. In addition, quantitative data on how beaked whales manage gas volumes during dives is required to test whether a decompression sickness-like syndrome can occur as a by-product of a strong behavioral response to noise.

Objectives

1) To test whether anthropogenic sound (i.e., simulated 1–5 kHz MFAS) leads to an increase in beaked whale EE (which can occur due to increases in speed, dive duration and/or B-stroke number) and if so, an earlier occurrence of B-strokes**. 2)** Estimate diving lung volume at the start of the dive as a candidate predictor for dive depth, so as to examine if beaked whales change the intended dive depth (e.g., switch from a shallow to a deep dive) due to sonar exposure, and evaluate its consequences.

Methods

We analyzed existing BRS data from four different beaked whale species fitted with DTAG devices. To overcome the limitations of ODBA, here we utilize the DTAG's three axis magnetometer to better separate specific from gravity-related acceleration in order to calculate a more accurate energetic proxy OSA (Overall Specific Acceleration). Dive duration, dive depth, swimming speed, OSA, onset time and number of B-strokes were incorporated into a statistical model testing the effect of exposure condition on EE.
We additionally explored the possibility of utilizing differences in thrust production to differentiate the diving lung volumes of deep foraging dives from shallow non-foraging dives.

Results

Beaked whales responded to sonar by increasing their EE, specifically by increasing their speed, their ascent duration and the number of B-strokes (Fig. 1). Thus, naval sonar exposure resulted in an earlier use of what is thought to be an anaerobic strategy (i.e., the usage of fast and more energetic strokes named B-strokes) when compared to baseline dives. Specifically, B-strokes tended to occur after the animal was exposed, independently of the dive type (deep or shallow) or phase (descent or ascent). Diving lung volume, however did not appear to be responsible for the noted differences in stroke amplitude during deep and shallow descents, instead animal speed and pitch were better determinants of increased stroke amplitude. We were thus unable to find a reliable relationship between dive depth and diving lung volume.

Collaborators: *Dr L Martin-López* (co-PI, KMR), *Dr D Cade* (Stanford University), *Dr S Isojunno* (co-PI, University of St Andrews), *K Colson* (KMR)

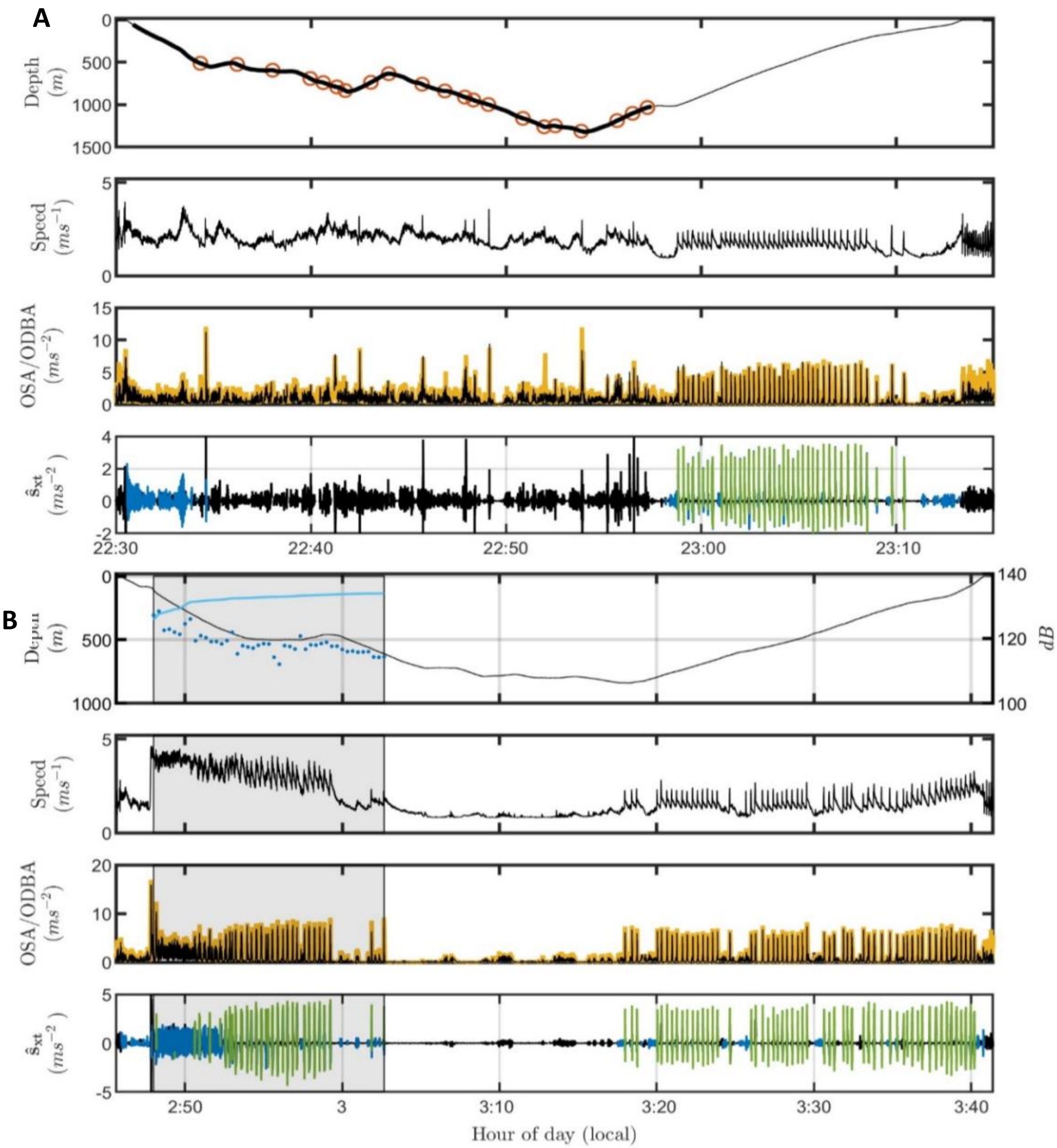

Figure 1. (A) Zoomed view of the dive profile, speed, ODBA/OSA and Sxt during the first baseline deep dive (1320m) of tagged whale ha15_179b. Bolded black line: echolocation click trains, red circles: buzzes; speed in ms^{-1} determined from accelerometer jiggle; ODBA (yellow) and OSA (black) in ms^{-2}; surge specific acceleration (Sxt) in ms^{-2} with normal strokes (blue) and B-strokes (green), during the descent and ascent phase. Bottom phase is colored in black. **(B)** Zoomed view of the dive profile of the same animal when exposed to naval sonar, with the timing of the sonar exposure period lightly shaded and shows received levels of the sonar with ping-by-ping SPL (in dB re 1 μPa) (dark blue dots) and cumulative sound exposure level (in dB re 1 μPa2 s) (solid light blue line).

Cetacean behavior in relation to oceanography, prey, and mid-frequency active sonar

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Background

Cetaceans, particularly beaked whales, are of concern to the US Navy regarding noise impacts during naval training exercises using mid-frequency active sonar (MFAS). To successfully quantify behavioral responses to MFAS and potential short- and long-term impacts, it is essential to understand the behavior of individuals under natural as well as disturbed circumstances. Behavioral responses caused by MFAS will likely be dependent upon the state of an individual. This behavioral state in turn is possibly driven by the underlying environmental conditions that determine prey distributions as a driver for marine mammal presence. The dynamic nature of the pelagic environment presents a challenge for management and impact mitigation. The goal of this project is to contribute towards an understanding of behavioral impact of naval exercise using MFAS on beaked whales and to provide ecosystem-based solutions for management.

Objectives

The project objective is to investigate the fine-scale dive behavior of goose-beaked whales (*Ziphius cavirostris*) in relation to measured prey distributions and environmental variables at a highly sonar-impacted site and a minimally disturbed site. We hypothesize that an individual's behavioral response will be dependent on the underlying prey conditions and their current behavioral state.

Methods

Data collection was conducted through ONR-funded project N00014-20-1-4000. For this analysis, passive acoustic cetacean tracking data from sites E, H, and N (sonar-impacted) and site W (no sonar) in Southern California were used (Figure 1, left). There were a total of 112, 393, 141, and 392 recording days at these sites, respectively.

A generalized detector for echolocation signals was run to note presence of toothed whales. Then a neural net extracted *Ziphius* echolocation clicks. Detections were scrutinized and edited using the software *DetEdit* to eliminate false positives (Solsona-Berga et al. 2020). *Ziphius* encounters were fed into the localization software *Where's Whaledo* (Snyder et al. in review). Time-difference-of-arrival (TDOA) localization was used to track the position of *Ziphius*. Tracks were manually cleaned using the *brushDOA* interface in *Where's Whaledo*, which generated azimuth and elevation information for each of two 4-channel arrays. The two array vectors were jointed for each localized point along a track and a smoothing spline was fit to each track. Summary statistics were calculated to identify group size, swim speed, group coordination, and spatial habitat use.

Results

Ziphius are a deep-diving cetacean species known to forage in the submarine canyons of the Southern California Bight. The sites had variable acoustic animal densities, lowest at site N, followed by H, E, and highest at site W. For example, while H had detections on 81% of days and W had detections on 99% of days, the acoustic encounter rate was overall much lower at H than W with 3.6% detection rate (i.e., sum of all detected minutes / recording effort) versus a 14.4% detection rate. There were 84, 873, 433, and 1392 final tracks per site of diving *Ziphius*, respectively (Figure 1, right). The number of individual whales captured during one encounter ranged from 1 to 7, with a mean of 2 across all sites. Tracks including four or more individuals were captured more often during the day. Most acoustic encounters had a duration of 15 to 30 minutes with some shorter and some longer dive captures. This is an indication that typically partial dives were recorded since *Ziphius* are known to undertake longer deep dives of about 1 hour. Three distinct diving behaviors and swim speeds were observed, differentiated by minimum depth, change in depth, and lateral movement: initial descent dive segments (1.7 ± 0.4 m/s), consistent trajectory dive segments (0.9 ± 0.6 m/s), and variable trajectory segments (1.3 ± 0.4 m/s). Comparing spatial use of diving *Ziphius* at the four sites reveals selective use of site-specific bathymetric features. Sites E and W had steep canyon walls and there seemed to be a preference for foraging along the steep walls while site N, and H had a more gently sloping sea floor where there appeared to be less of a preference for a specific area. Median distance to sea floor was 70.3 m and 76.4 m at canyon sites E and W, 146.2 m and 195.3 m at basin sites H and N. Visualizations of 3D dives showed highly coordinated dives within subgroups of 2-4 animals as well as across subgroups.

This long-term monitoring effort and localization approach collected data from *Ziphius* foraging dives to reveal spatial use, group size, and diving behavioral trends at our acoustic monitoring sites. This extensive tracking dataset gives valuable insight into the social and foraging behavior at depth of goose-beaked whales offshore Southern California.

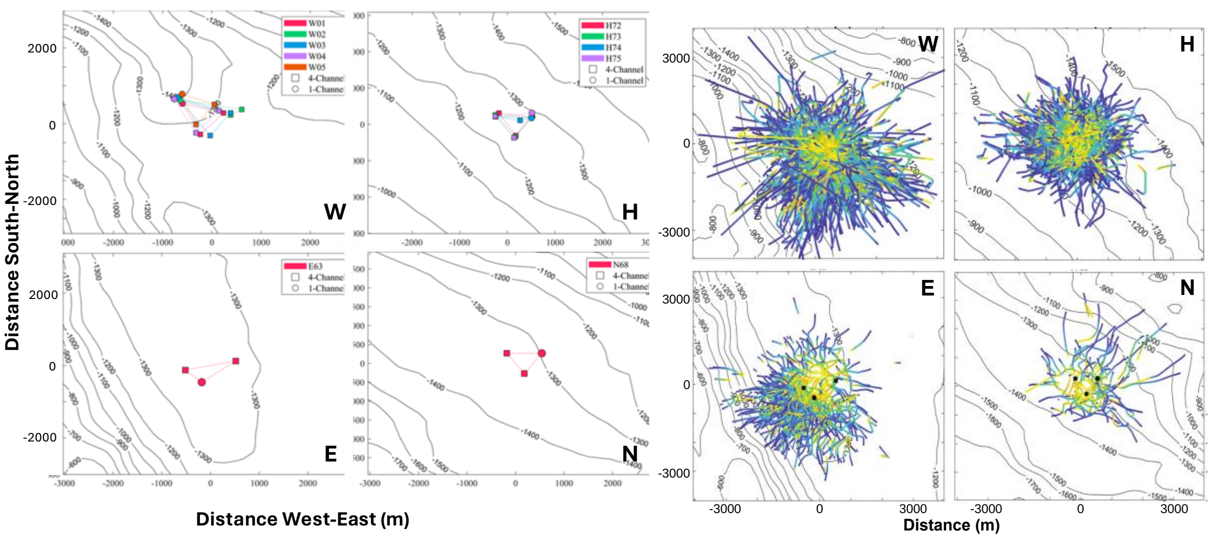

Figure 1: (Left) Tracking sites W, H, E, and N with instrument locations. (Right) Ziphius tracks (blue to yellow = start to end) at each site showing site-specific habitat use.

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SOARing for data: Assessing the body condition of Goose-beaked whales on a Navy sonar range using aerial photogrammetry

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Background

The Southern California Anti-submarine Warfare Range (SOAR) is one of the most heavily used mid-frequency active sonar (MFAS) ranges in the world. Despite this, Goose beaked whales (*Ziphius cavirostris*, hereafter *Zc*) are documented there year-round, with some individuals sighted over the course of sixteen years. In this region, MFAS exposure has been linked to *Zc* foraging disruption, resulting in the cessation of active foraging and extending the time between foraging dives. This well-documented disruption can have significant consequences at both the individual and population level.

Objectives

The goal of this project is to collect photogrammetry images of *Zc* at SOAR and compare them with animals in the relatively undisturbed population at Guadalupe Island (GI), Mexico. Photogrammetry data collection at GI has been ongoing since 2021. Analyses will assess differences in body condition, growth rates, age/sex ratios between the populations, and pregnancy detection.

Methods

Field data collection will be conducted during three weeks of small boat survey effort around SOAR. NDAA certified unmanned aircraft systems (UAS) with onboard LIDAR and a flat, wide-angle lens will be used to image *Zc* and other species of interest. UAS videos and/or images and LIDAR altitude measurements will be collected while flying directly above whales at altitudes typically between 15 and 60 m (never below 7.6 m). Data processing will entail capturing stills from footage where the UAS was directly overhead of a surfacing whale (with body parallel to the water's surface) and the camera pitch was 90 degrees straight down. Whales (example images below) will be measured using the *MorphoMetriX* software, and additional data processing and body condition metrics will be performed using the *CollatriX* software. Final analyses will utilize a Bayesian modeling approach to obtain posterior predictive distributions of measurement uncertainty that will enhance inferences of whale morphology and life history patterns.

Results

A NDAA suitable UAS and associated accessories has been selected and purchased under a DURIP award. With help from ONR, we have obtained a COTS waiver for operating within the Southern California Tactical Training Range and SOAR in particular. Field efforts are anticipated to begin in late June, 2024.

Images (zoomed and cropped for enhanced visualization) show Zc from GI. Top two images show three sets of mom and calf pairs, while the bottom image shows a group of four Zc, one of which has an attached SMRT tag (bottom-right adult male).

Cuvier's beaked whale integrated ecosystem study: Systematic use of novel eDNA methodology to characterize and compare beaked whale populations and their associated prey community across regions

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Background

Some species of beaked whales are highly sensitive to naval sonar. In addition to direct mortality, sub-lethal changes in foraging behavior and habitat-use following sonar exposure may pose significant risks at the population level. A major limitation in our understanding of sub-lethal effects is the near absence of knowledge of their prey fields and the effect of prey patch quality on foraging success, energetic return, and the loss incurred from impeded foraging. This limits our ability to interpret and quantify the energetic consequences of behavioral responses to naval sonar exposure, at the level of the individual and population. A recent ONR-funded study off the Azores pioneered the use of environmental DNA (eDNA) to reconstruct deep-sea prey diversity as a function of depth in the water column. This revealed prey community structure directly in Cuvier's beaked whale (CBW) foraging zones. CBW dive through dense layers holding potentially suitable prey, to target larger, possibly more energy rich individuals of the same prey species, at much greater depths. A combined approach of eDNA analysis of predator and prey, matched with predator biologging, holds great potential to elucidate key data needs in the study of CBW foraging ecology, and its relation to demography. This application may be particularly valuable when applied across populations that are subject to different foraging conditions and exposure to naval sonar.

Objectives

We aim to test 1. whether local prey diversity and community composition drives foraging decisions in CBWs, and 2. the detectability of CBW eDNA across the water column.

Methods

We will conduct a cross-regional collaborative study, matching eDNA sampling to ongoing biologging studies to characterize and compare CBW prey communities in the North Atlantic and Mediterranean Sea. We will systematically develop and apply emerging eDNA methodology to assess prey communities in CBW foraging habitat, matched to the analysis of local CBW population metrics derived from eDNA (CBW eDNA detectability; relative population size, connectivity). Sampling will be conducted in 3 regions (Azores, Cape Hatteras and Ligurian Sea) that support long-term research programs on CBW foraging ecology. These regions are widely dispersed but hold ecologically linked, overlapping deep-sea cephalopod communities. CBW forage at relatively shallow depths in the Ligurian Sea, in comparison to the other two sites (and most of its range). The three regions experience little or no naval sonar exposure (Azores, Ligurian Sea) or very occasional exposure from training activities (Cape Hatteras), further enabling a comparative approach.

Results

This project was recently awarded, with first field effort starting summer 2024.

Project collaborators: *Dr HJ Hoving* and *Dr V Merten* (co-PIs, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany), *Prof A Read, Prof D Nowacek and Dr T Schultz* (co-PIs, Duke University Marine Lab) and *Dr M Rosso* (co-PI, CIMA, Italy), *J Stefanschitz* (GEOMAR), *M Oudejans* (KMR).

Sponsor: ONR
Status: ONGOING

Improvement of CTD satellite tags for use on large baleen and odontocete whales

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Background

Sampling oceanographic data, including salinity (using conductivity), temperature, and depth via instruments attached to diving marine mammals allows for data collection from remote or ice-covered waters where sampling by conventional methods is expensive or impossible (Costa et al. 2008, Laidre et al. 2007). Data collected by marine animals can be incorporated into oceanographic models to monitor trends and variability in ocean conditions over time, they can provide near real-time measurements of oceanographic conditions for operational purposes, and they can be used as proxies for prey availability and habitat use enabling a greater understanding of our ocean ecosystems.

The Wildlife Computers SCOUT-CTD tag incorporates an electrode-based conductivity sensor, and has been successfully deployed on a number of marine species using a variety of tag attachment systems. However, deployments on bowhead whales using a "swing tag" anchor system have yielded unreliable drifts in salinity readings over three field seasons during 2017-2019. Since this unreliable drift appears to be limited to the "swing tag" anchor system, it is suggested that there may not be a problem with the sensor per se but rather with the attachment and the proximity of the tag to the anchor penetration site on baleen whales. Oil leaking from the skin and tissue of baleen whales at the anchoring point may contaminate the electrodes that measure conductivity. Mechanical engineers at Wildlife Computers designed modifications to address this problem by creating separation of the anchor penetration site from the conductivity sensor.

Objectives

1. To redesign the attachment system of the SCOUT-CTD tag for baleen whales. The new design will address the hypothesized contamination of the electrode sensor by foreign substances (e.g., oil from the blubber leaking from the whale).
2. To test modified tags on bowhead and southern right whales over two field seasons to evaluate and optimize the attachment and demonstrate the attachment design solves the observed drift in salinity.
3. To evaluate the reliability and quality of oceanographic data collected in Arctic ice-covered waters by bowhead whales and from the open ocean by right whales.
4. To design the attachment and associated pole deployment system for the SCOUT-CTD such that it can be commercially available to other scientists studying large baleen whales.

Methods

Two methods of preventing oils from the attachment anchor penetration site from reaching the CTD electrode sensor were designed. In 2022, we designed a physical barrier to shield the sensor inlet from potential contamination while maintaining the swing-tag style of attachment. The salinity readings from bowhead and southern right whale field trials yielded mixed results. It was also noted that the barrier did not remain in place during the resighting of the southern right whales. Additionally, there were challenges in deploying the tags with this swing tag attachment system, and resighting showed a bent anchor shaft as it was migrating out of the whale.

In 2023 the attachment system was completely redesigned. The anchor point was moved to be beneath the center of the tag, away from the water inlet points of the conductivity sensor. The cutting tip on the anchor shaft used the triangular (pyramidal) tip used on the Wildlife Computers SPOT-372A tag. The diameter of the anchor shaft was increased to mitigate bending. Two anchor retention systems were designed, retention “petals” similar to those of the SPOT-372A, and innovative retention “cones”. The retention cones are designed to limit the outward migration of the anchor while minimizing tissue damage that can be caused by the edges of the petals. The deployment system was also redesigned to accommodate the new anchoring system, and maximize the force possible for pole deployments.

Results

At the start of this project, we hypothesized that contamination of the conductivity electrodes from oils emanating from the wound at the tag attachment point was a primary cause for drifts in salinity during the 2017-2019 deployments on bowhead whales. In 2022, one tag on a southern right whale transmitted data the suggested a contaminated electrode. This tag was deployed without a barrier in front of the sensor inlet. All other tags deployed that year suggest reliable conductivity data or other types of anomalous readings (sharp increases or decreases in salinity outside the ranges of seawater.)

In 2023 with the center-mount attachment design, we did not observe salinity readings that suggested contaminated electrodes for any of the tags that were successfully deployed. We did see other anomalous readings, however. Review of the conductivity cell’s electronics and onboard software suggest the anomalous readings are due to damage to the cell, or having a foreign body enter the cell changing the volume of water being measured. In-lab testing is being performed to empirically confirm cell damage / foreign body intrusion can cause the anomalous readings.

The retention systems merit further investigation. The transmission duration and battery voltage of the tags using the petal retention suggest the tags were shed far earlier than the battery became exhausted. We believe modifications to the petal shape and stiffness can improve the retention.

The tags using the innovative retention cones proved to be very difficult to insert. Modification to the cone shape, surface and material may reduce the force required to insert the anchor. The potential of the innovative retention cone to increase the duration of tag retention by reducing tissue damage should be explored further.

The deployment and attachment systems, including the pole and cup to hold the tag, are ready for commercial sale.

Training, data archive integration, and analysis tools for high-resolution tag data

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Background

Bio-logging studies, where data are collected using animal-borne devices, continue to grow rapidly in numbers and in scope. But this is a two-part problem, with algorithms used to process data from tags are essential for assessing marine mammal behavior. This is true for both acoustic disturbance, as well as for acquiring baseline behavior for environmental risk models - both critical U.S. Navy interests. However, with the number of users and methods of analysis steadily growing, standardization and verification of the algorithms used for analysis is necessary to ensure the quality of the analysis and to facilitate comparisons of data between studies. Currently, these tools tend to be disseminated by individuals in community driven repositories. Additionally, as the number of tag users increases there is a need to provide structure and instruction to the community to ensure a level of standardization for the resulting analysis. The goals of this work included: 1) Curation and standardization of existing analysis tools used to extract information from high-resolution movement tags in a dynamic centralized repository that can be easily maintained and updated as new tools are developed. 2) The creation of standalone online modules to facilitate the use of the algorithms in the repository. And, 3) data archive integration into the analysis workflow to standardize the resulting data and analysis.

Objectives

1. Online Educational Resources: Translate content that was developed for in person tag tools workshops into freely available online training modules.

2. Dynamic Online Tool Repository: A secondary goal of the project was to verify tool functionality and interoperability on multiple software platforms (R, Matlab, and Octave) and Windows and Mac operating systems; resolving bugs uncovered as the tag tools have been used and tested by researchers; and general updating, maintenance, and reorganization of the animaltags.org project website.

3. Data Archive Integration: Coordinate the integration of the tag tools with data archives, specifically the Animal Telemetry Network Data Assembly Center (ATN DAC) and its Research Workspace. Working to help develop a standard archive format for tag data will ensure output from tagging efforts are accessible and useful to the tagging community, particularly DTAG users. The animaltags tool kit will be available for this integration, and data holders will be explored to enable a workflow for preparing DTAG data for archive submission (attaching metadata and conversion to appropriate netCDF file format).

Work Completed/ Results:

Website: https://animaltags.org/

Objective 1. Online Educational Resources This project has resulted in content and online train modules to cover the following:

- Data Import/export and an archive-compatible netCDF file format for sharing and storing datasets with metadata
- Calibration (from raw data to calibrated data in scientific units)
- Visualization (e.g., time-series plots, multiple events overlaid, long-term spectral averages)
- Data Processing (e.g., event detection, derived metrics like jerk and dynamic acceleration, Dive detection and dive parameter calculation, integrating movement data with other sensors eg acoustic or camera, integrating position data from onboard GPS, visual observations, etc. with movement data

- Statistical Analysis (e.g., track reconstruction, Mahalanobis distance analysis).

The website combines a technical description of the matlab/R data processing tools with educational content to help users better understand the principles behind the signal processing used to identify the signals and features that are important for the characterization of animal movement from tag data. The beginning tutorials include:

- Stroke signal identification from orientation data.
- Complimentary filter design
- Signal processing for pressure data (how to correct and filter pressure data
- Speed estimation from pressure and orientation data
- Techniques to filter high frequency noise from data streams
- Dive segmentation
- Calculating jerk from accelerometry data.
- Converting .Mat Files into .Nc Files

Objective 2. Dynamic Online Tool Repository: Software tools to facilitate calibration, processing, and analysis of data from high-resolution movement-sensor tags are now available in three formats: matlab, pyton and r. These tools facilitate the analysis of sensor data from from tags, typically include accelerometers to measure body posture and sudden movements or changes in speed, magnetometers to measure direction of travel, and pressure sensors to measure dive depth in aquatic or marine animals

Objective 3. Data Archive Integration: Team members maintained regular communication with Megan McKinzie, the ATN Data Coordinator, with Stacy DeRuiter serving as the main point of contact with our team. Under Meg's leadership, project team members participated in several videoconferences to discuss standards for high-resolution biologging datasets, including a larger stakeholder listening session on June 7, 2023. As part of the tag tool kits, we continue to maintain software tools to allow conversion of datasets from high-resolution tags such as DTAGs and SMRT tags to archive-compatible netCDF file formats, and are committed to continued updates to those tools as the process of establishing standards for file format and metadata requirements are defined. As part of this work, we also coordinated and provided support to the SOCAL project and the Tyack laboratory as they negotiate the process of readying existing DTAG datasets for archive submission.

PUBLICATIONS

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2. Fynewever, S., Tejevbo, O., Johnson, M.P., Shorter, K.A., Martín López, L.M., and DeRuiter, S.L. 2021. Training, data archive integration, and analysis tools for high-resolution tag data. 7th International Bio-logging Symposium [virtual event]. 18-22 October 2021.

3. Tejevbo, Oghenekevwe, and Samuel Fynewever. 2022. TagTools: Software Supporting Biologging Research. Grand Rapids, MI: Calvin School of STEM Summer Research Poster Fair, October 21.

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Development of a Minimally Invasive Blood Collection Device for Cetaceans

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Background

Blood sampling is essential for evaluating cetacean health. Blood samples allow researchers to assess, monitor, and answer questions relating to health status, genetics, exposure to microorganisms and toxicants, and physiologic responses to anthropogenic and natural stressors for cetaceans. Veterinarians routinely collect blood samples from the periarterial venous rete (PAVR) in cetaceans under managed care to monitor health. Several studies of wild dolphin populations have used blood samples to assess changes in stress and reproductive hormones and demonstrate adverse effects on organ systems due to chemical contaminants or stressors. However, blood samples are currently only collected when live cetaceans strand or during capture release studies, which are only possible for a limited number of nearshore small cetacean species. It is currently infeasible to perform capture-release health assessments on large whales or most pelagic cetaceans, and therefore the literature on wild cetacean blood biomarkers is focused on nearshore small cetaceans such as common bottlenose dolphins (*Tursiops ssp.*), and to a lesser extent, beluga whales (*Delphinapterus leucas*). We set out to develop a minimally invasive blood collection device for remote application to the skin of small cetaceans, leveraging Tasso Inc.'s medical device technology developed for at-home blood collection from humans.

Objective 1: Using the second-generation HemoLink™ device as an initial prototype, conduct an iterative sequence of testing and engineering modifications to produce a final prototype for cetaceans that will collect at least 200 uL and up to 400 uL of blood on average.

Objective 2: Compare analytes between blood collected with the Hemotag prototype and blood collected via routine venipuncture of the fluke's periarterial venous rete (PAVR).

Objective 3: Evaluate potential engineering options for water-proofing the final Hemotag device or creating a water-proof enclosure around the device.

Methods

Through an iterative engineering process, we developed and tested variants of the original HemoLinkTM device. Blood clotting was a significant challenge, resulting in blood flow obstruction and poor yield. Successful modifications to overcome clotting included coating the blade with anti-coagulant and increasing the amount of anticoagulant in the blood collection channel. Increased sampling depth and warming of the dolphin's skin prior to application also improved sampling volume. A standard location of the dorsolateral body wall cranial to the dorsal fin provided the most consistency with the device with this location ideal for remote placement in the future. The final design reliably collected ~200ul of blood. To determine the comparability of capillary blood collected via the modified

HemoLinkTM device to fluke peri-arterial venous rete blood collected via routine sampling methods, we collected paired blood samples from 29 Navy dolphins. At a minimum, we assessed packed cell volume (PCV), total protein (TP), and a blood smear to perform a manual white blood cell (WBC) count and differential, as well as an iSTAT chem 8.

Results

Preliminary results showed no significant difference between several key parameters, including packed cell volume (Wilcoxon p= 0.76), total protein (p= 0.07), and total white blood cell count (p= 0.34). Future studies will focus on further improvements to the device and testing with cetaceans in various settings. Our ultimate goal is to develop a robust blood collection device for remote application to free-swimming small and large cetaceans, specifically to evaluate blood-based health parameters in individual animals of concern and at-risk populations.

Figure 1: A) Application of the HemoTag device on a dolphin at the U.S. Navy Marine Mammal Program. B) Successful collection of 200 uL of blood using the HemoTag device on a dolphin at the U.S. Navy Marine Mammal Program. C) Application of skin-warming techniques. Photos: U.S. Navy

Physiological Resilience in Mammalian Divers: Assessing the role of vascular conflict and control in recovery from anthropogenic disturbances

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Background

Underwater noise, most notably from shipping, seismic activities and naval sonars, and more recently from offshore wind farm construction, has emerged as one of the most important and controversial stressors on marine mammal populations worldwide (National Academies of Sciences, Engineering, and Medicine, 2016). With geo-spatial and temporal evidence linking anthropogenic noise to mass stranding events by multiple beaked whale species, pilot whales, and melon-headed whales (see Cox *et al.*, 2006; Williams *et al*., 2018, 2022), the deep-diving lifestyle of pelagic dolphins and whales appears to increase vulnerability to injury. Despite this linkage, causal mechanisms have been difficult to identify. The question remains, what factors differentiate when an escape response due to noise exposure leads to recovery or to death in diving cetaceans? Here we have focused on identifying the role of the cardiovascular system in the recovery process of highly-active dolphins and whales to address this question.

Objectives

The overall purpose of this study is to determine the capacity of diving mammals to quickly recover from extreme exercise and extended periods of submergence that occur when avoiding aversive stimuli such as shipping noise, sonar and seismic pulses. Rather than individual dives we are evaluating cumulative physiological costs across time and space as occurs in free-ranging animals. Four specific aims are being addressed:

1. Determine the hierarchy of blood distribution supporting thermoregulation and tissue oxygenation in divers during exercise and recovery.

2. Assess the physiological triggers for blood distribution that may compromise sequential dive performance.

3. Evaluate the role of vascularization in the neuro-protection of diving mammals.

4. Apply physiological theories of recovery to free-ranging cetaceans exposed to anthropogenic noise.

Methods

Using a comparative approach, we are conducting metabolic, cardiovascular and thermal tests on trained bottlenose dolphins (*Tursiops truncatus*) and beluga whales (*Delphinapterus leucas*), as well as deep-diving narwhals (*Monodon monoceros)* and elephant seals (*Mirounga angustirostris*) wearing custom-designed biologging tags. Emphasis is on determining the simultaneous timeline for recovery of thermal, metabolic, respiratory, cardiovascular, and tissue metabolite homeostasis following intense exercise as may occur in response to anthropogenic disturbance.

Results

Major results during this period include the development of physiological-based metrics to inform bioenergetic modeling (John *et al.*, 2024) and critical habitat designations (NAMMCO, 2022; Williams *et al.,* 2022) for deep-diving cetaceans threatened by anthropogenic activities. In addition, we have developed new physiological monitors for evaluating brain function (Kendall-bar *et al*., 2023) and the thermo-vascular responses of diving and exercising cetaceans. Studies monitoring skin temperature and heat flux homeostasis are ongoing (Fig. 1; Chambault, P. *et al.*, in prep.).

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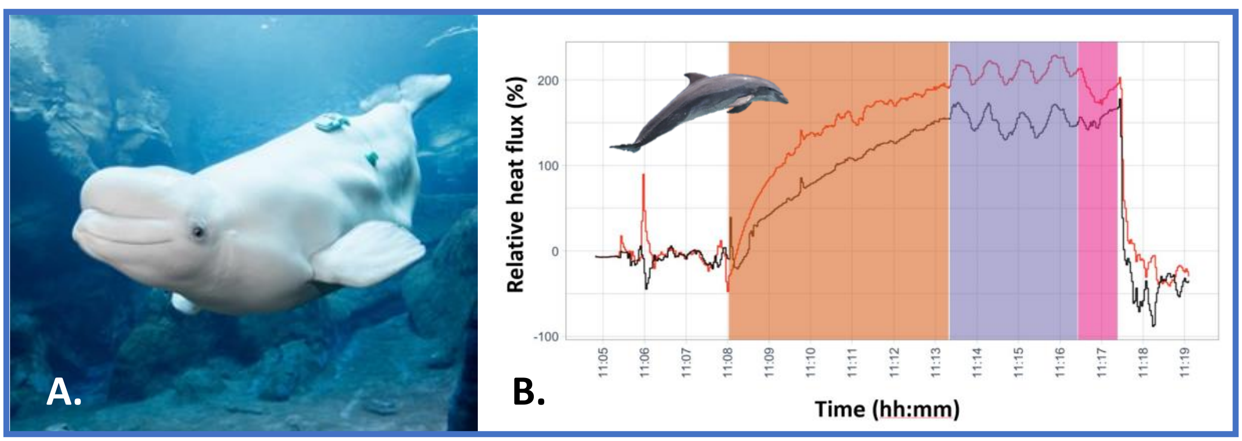

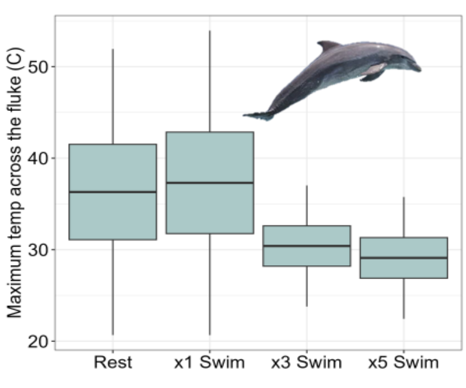

C.

Figure. 1. Instantaneous Changes in Thermal Balance in Active Cetaceans. Panel A shows the placement of a custom heat flux/skin temperature tag and sensors on a diving beluga whale. In B, instantaneous changes in heat flux from two sensors on the dorsal fin of a bottlenose dolphin during pre-exercise stabilization (orange), swimming (purple) and post-exercise recovery (pink) periods indicates the variability in vascularization. Interestingly, this variation led to differences in maximum skin temperatures of the fluke during recovery from single and sequential swims.

Internships Program for Diversity and Inclusion

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Background

There is a growing body of scholarship addressing demographic shifts in the U.S. population and the need for strategies to ensure a diverse and innovative future STEM workforce in the USA. Geosciences in particular have not improved over the last four decades (Bernard and Cooperdock 2018), where black, indigenous and people of color (BIPOC) faculty hold only 3.8% of faculty positions in the top 100 geoscience departments, despite much discussion of the barriers to inclusion and diversity. Similarly, in Ecology and Evolutionary Biology, white students earned 7 times more Ph.Ds. than BIPOC; even when correcting for total population, white students earned a higher proportion go Ph.Ds. (Massey et al. 2020). Marine mammal research is extreme in its paucity of diverse thought and representation. In a survey by the Society for Marine Mammalogy, less than 1% of marine mammal scientists (including students) in the United States (4 out of 464 U.S. respondents) identified as African-American, Black, or multi-racial (Archer and Cox, unpub. data). The lack of diversity in a field further reduces retention of those with diverse backgrounds, including BIPOC scholars – propagating a cycle in the field of marine mammal science; the very lack of diversity discourages minorities from participating in the community. Minority-Serving Institutions (MSIs) are substantially more effective in science education, due to supportive environments, high faculty-to-student ratios, and diverse faculty and student bodies.

Methods

The pilot project focused on three MSIs: Savannah State University, Hampton University, and San Diego State University, where three faculty were already working on marine mammal research. The faculty of these three MSIs partnered with several partner organizations: Cascadia Research Collective, International Fund for Animal Welfare, National Marine Mammal Foundation, Sarasota Dolphin Research Program, and Virginia Aquarium & Marine Science Center. Key components of the program included:

1) Academic year internships for early undergraduates (freshmen and sophomores) to incorporate them into active science labs and teach them scientific skills (e.g., acoustics, machine learning/computational analyses, genetics, photo-identification).
2) Weekly virtual cohort meetings in which there were "soft" skills modules (e.g., identifying research interests, conflict management; communication; creative thinking), scientific skills development, journal club, and career services.
3) Mentor training for the partner organization mentors.
4) Summer research internships at one of the partner organizations.
5) Travel to research conferences.

Objectives

The overarching goal of this program was to increase the number of under-represented minorities in U.S. marine mammal research. We aimed to establish the infrastructure necessary to support the growth of underrepresented groups in future generations of marine mammal scientists. Specifically, the objectives were to:

1. Establish a network of Minority-Serving Institutions and research organizations that can financially support internships at multiple stages of research, as well as provide the mentoring necessary to ensure a high level of retention of students who enter the field.
2. Build professional skills and confidence of early undergraduates in academic-year internships in which they are introduced to scientific process, technical skills, and professional development.
3. Increase accessibility of entry-level positions in marine mammal science through paid summer internships.
4. Prepare a cohort of researchers representing diverse backgrounds in marine mammal science, which can mentor later cohorts in a tiered mentoring scheme.
5. Increase undergraduate interns' proficiency in technical skills, oral and written communication, and the research process to ensure they are well positioned for a career in STEM.

Results

The internship program has run from Fall 2021 through Spring 2024. During that time, 16 interns from 3 institutions have participated (Table 1). Because the program focuses on early undergraduates, none of the interns have yet graduated, and all, with one exception, are still enrolled in STEM majors, yielding a 93% retention rate. For reference, SSU currently has a 24% graduation rate, Hampton 56%, and San Diego State 78%, indicating interns are exceeding average retention rates. Most are planning on graduating and then pursuing graduate school. This program has already made a significant impact on diversity in marine mammal science and in these students' lives. Specifically, two interns have stated they would have dropped out of school if they had not had this program. Four interns have moved from the ONR-funded internship into more lucrative scholarships and stipends (NOAA LMRSC, NSF, and Naval ROTC). In addition, one intern is currently applying for the Master's degree program at Savannah State University.

Notes:

This is a joint-funded program with the U.S. Marine Mammal Commission. The MMC will fund 2 summer interns in Summer 2024.

Table 1. Demographics of interns. One summer intern did not participate in the semester internships. A total of 16 interns participated in the program.

	Semester	**Summer**
African American	12	6
Hispanic	3	2
Female	9	5
Male	6	3
Total	**15**	**8**

Developing a bioenergetic model for right whales to assess population consequences of exposure to multiple stressors

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Background

Marine mammals are increasingly exposed to a multitude of anthropogenic stressors, which can have cumulative effects on targeted populations. Stressors can negatively affect the behavior and/or physiology of animals, resulting in energetic costs. Cumulative energetic effects can reduce the body condition (BC) of individuals. If the BC of individuals falls below a critical threshold, it can compromise individual survival and reproductive success. If a sufficiently high proportion of a population is exposed to stressors, it can lead to population level effects. The North Atlantic right whale (*Eubalaena glacialis*, NARW) is on a trajectory to extinction. While fishing gear entanglements and ship strikes are the largest direct anthropogenic threats to NARWs, reduced reproductive rate resulting from nutritional stress (poor BC) has been hypothesized as a factor further contributing to the population decline. While it is of paramount conservation value for the NARW to understand the population consequences of multiple stressors (PCoMS), informing the many necessary parameters of such models requires not only large amount of empirical data, but also data across the full range of possible values. With the NARW population being represented by individuals already under significant stress and in poor BC, baseline data from healthy southern right whale (*Eubalaena australis*, SRW) populations are needed to fully understand the relationships between different links in the PCoMS framework.

Objectives

The aim of this project is to develop a bioenergetic model for SRWs to predict PCoMS. The model will be developed in three work packages (WPs):

WP1: Quantify energetic costs of growth, maintenance and reproduction for SRWs (results presented at the 2022 ONR review).

WP2: Develop an individual based model (IBM) to estimate the optimal allocation of energy to growth, survival and reproduction to maximize lifetime reproductive success (this presentation).

WP3: Develop a population of IBMs to simulate PCoMS in SRWs (will be presented in the final project report).

Methods

The output from WP1 was used to build an IBM for SRWs based on the healthy (growing) population in Australia. First, the effect of maternal body length on the cost of gestation and lactation was estimated. Second, the lower BC threshold for survival was identified using both a tissue-mass model (developed in WP1) and measurements of an emaciated SRW calf that died in Australia in 2021. Using this threshold and the time required to migrate back to the feeding grounds (~30 days based on satellite tag data), the lower BC threshold at departure from the breeding grounds was estimated. For mother-calf pairs, the cost of lactation was added to the mothers own costs. Based on the mean body length of calves at departure from the breeding grounds (7.5m), the number of days and energy required for mothers to grow their calf to this desired length was estimated. The resulting energetic cost was then used to determine the lower BC threshold for pregnant/lactating females at the time of birth, as well as the BC at the time of departure from the feeding grounds. An IBM was developed, where each day in the life of a whale was simulated, from weaning until death (at 80 years). The age, length and BC of the simulated whale changed as the animal aged, grew and ingested/spent energy, respectively. A yearly migratory cycle was followed, consisting of summer feeding, autumn migration, winter breeding, and spring migration. On the feeding ground, the prey density determined the rate of energy intake, which in turn affected the BC (after subtracting the energy costs). An individual would reach sexual maturity when its age and body length exceeded 5 years and 12m, respectively. Mature animals would reproduce if their BC exceeded the minimum requirement to go through gestation and lactation. If the BC of pregnant/lactating females fell below the critical thresholds at any time during the reproductive cycle, gestation/lactation would be terminated, respectively. Similarly, if the BC of an individual fell below the lower BC threshold for survival, it died. The effect of different environmental conditions (prey density) and levels of anthropogenic disturbance (effect sizes and exposure) was simulated, and the effects on female lifetime reproductive success (number of calves produced) was investigated.

Results

The tissue mass model found that the point of emaciation for SRWs occurred at a BC of -42%. This matched the BC of the emaciated calf that died in 2021, which was -40% (equivalent to a 5% blubber mass). The lower BC threshold at departure from the breeding ground decreased from -12.6 to -30.4% for lactating females (12-16m in length). Birth size and growth rates of SRW calves was positively related to maternal body size at the time of birth, which meant that larger females were able to grow their calves faster and depart the breeding grounds earlier (after 60-95 days for 12-16m long females). To complete early-lactation, the BC of the mother at the time of birth needed to be 37.4 to -12.6% (for 12-16m long females). Based on a 30-day migration duration, pregnant females needed to build up a minimum BC of 52.0 to -1% (for 12-16m long females) at the time of departure from the feeding grounds, to complete both late-gestation and early-lactation. The IBM was able to mimic the seasonal loss and gain in BC of SRWs in Australia, within the observed BC range (-40% to 60%). Even though SRW females reached sexual maturity at an age of 5 years, most took 8-9 years to build up sufficient BC to reproduce, similar to published estimates from Australia. Younger/smaller females (<14m) had 4-year inter calving intervals on average, which then decreased to 3-years once they reached their asymptotic length (~14m). Lower prey density resulted in an older age at first parturition, and longer inter-calving intervals, which in turn resulted in lower lifetime reproductive success (fewer calves). Due to their higher mass-specific energy expenditure, smaller animals were more sensitive to anthropogenic disturbance, which prolonged the age at first parturition. For sexually mature females, a reduction in BC due to disturbance would lead to a termination of gestation on the feeding grounds. If the female had already migrated to the breeding grounds, it would reduce the residency time on the breeding grounds, and calf survival. The main threat to adult survival was if the disturbance took place on the breeding grounds, as the animal risked starving to death before reaching the feeding grounds.

Vital Rates of Cuvier's beaked whales: A multi-regional comparative assessment

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Background

For populations subject to chronic disturbance, long-term, recurrent exposure to stimuli may not measurably reduce adult survival but may impact individual fitness in ways that reduce population viability over time. In beaked whales on military training ranges, repeated foraging disruption associated with frequent exposure to mid-frequency active sonar may impact the rates at which females give birth and wean calves. These more subtle effects can be very difficult to measure in a slow reproducing, cryptic species, such as Cuvier's beaked whale (*Ziphius cavirostris*, hereafter "Zc"), but may indicate unsustainable levels of exposure are occurring before a measurable decrease in adult survival is observed.

A longitudinal study of Zc in the Ligurian Sea, off the northwest coast of Italy, has been developing methods to study population dynamics of Zc since 2003. Here, we adapt and apply these methods to Zc from four other regions, each with varying levels of exposure to military training, with the goal of improving our understanding of vital rates in this data-deficient species.

Objectives

This project has three primary objectives, which will allow us to leverage existing photo-ID collections toward the project goal: 1. Validate cross-regional applicability and expand published methods for Zc age-sex classification using identification photographs; 2. Develop a comprehensive data management system to standardize photo-ID data among study areas and facilitate consistent, systematic age-sex classification across collections; and 3. Derive and compare vital rates among study areas.

Methods

Following discussions with the Ligurian Sea team, a series of updates to their published methods of Zc age-sex classification were developed to make the process more broadly applicable to typical photo-ID collections from other regions. We applied the revised method to known age-sex whales from Southern California (SoCal), Isla Guadalupe (IG), and the Ligurian Sea, then compared whether the new methods successfully age-sex classified whales within and among the three regions.

Next, we developed a data management system consisting of an MS Access database and user interface that was interoperable with ImageJ, which included a set of custom macros to extract image metrics. This system facilitated the compilation of identification data, systematic review and scoring of images, and collection of scarring density measurements needed to age-sex classify each whale. A copy of the system was distributed to each project team along with a comprehensive set of written and video protocols. Each team also received at least one virtual training session and ongoing data processing support as needed.

Finally, the completed data and supporting images from each region were returned to MarEcoTel for validation and cross-regional compilation. The screened data were combined to create age-sex classified capture history data for the following analyses: basic descriptions of demographic processes in the observed data, multi-event mark-recapture estimation of age-sex specific vital rates (Tenan *et al.*, 2023), and a supplementary assessment of appearance and scarring density.

Results

The comparison of appearance and scarring density data from the Ligurian Sea, SoCal, and IG demonstrated limited regional variation in thresholds used to distinguish male and females, and also that the revised protocol was suitable for use in the project. The results of this assessment were published as a manuscript (Coomber *et al.*, 2022).

The project data management system thus incorporated these standards and was distributed to all regional teams. Completed datasets from each region have been combined for analysis.

The combined data currently includes 3,777 daily identifications of 1,208 individuals from two Pacific regions (the Southern California Tactical Training Range and Isla Guadalupe, MX), two Atlantic regions (Cape Hatteras, NC, USA and Terceira Island, the Azores), and two subareas in the Mediterranean Sea (the Ligurian and Ionian Seas). In most areas, effort included opportunistic and directed data collection, with the number of annual IDs increasing over time (Table 2).

Roughly two thirds of identified whales could be sexed via reproductive history, genetics, and/or appearance. Sex ratios from most regions were relatively even with the exception of the Mediterranean collections, which were biased towards males. Excluding the very small Ionian Sea sample, the number of IDs on sighted more than one day ranged from 25% (Hatteras) to 65% (Guadalupe), and the number sighted in more than one year ranged from 7% (Azores) to 60% (Guadalupe). The percentage of females observed with at least one calf ranged from 2-14% of each collection (Table 1).

The four largest collections provided sufficient numbers of annual captures of age-sex classified IDs to attempt mark-recapture estimation of survival rates, after effort type (dedicated), image quality, and individual distinctiveness filters were applied to meet assumptions of the method. Among these, the Hatteras sample was insufficient for the multi-event (age-specific) estimation of survival rates, and only single-state methods will be used. While multi-event modeling will proceed for the remaining three collections, data were limited for some age-sex classes, particularly younger whales, which often fail to meet distinctiveness criteria in this species. These analyses, the comprehensive summary of the observed data, and the analysis of scarring rates are presently underway.

Table 1. Summary of regional Zc photo-ID collections. IDs are unique individuals.

Region	Sub-area	First Year	Last Year	IDs	*Sex F*	*Sex M*	*Sex U*	>1 Day	% IDs >1 Day	> 1 Year	% IDs >1 Yr	Moms	% IDs Moms
Azores	Terceira	2012	2022	42	*13*	*16*	*13*	12	29%	3	7%	1	2%
Mediterranean	Ionian	2017	2019	16	*1*	*8*	*7*	1	6%	0	0%	0	0%
Mediterranean	Ligurian	2003	2022	315	*63*	*95*	*157*	193	61%	167	53%	35	11%
Mexico	Isla Guadalupe	2006	2023	102	*32*	*32*	*38*	68	67%	61	60%	14	14%
US East Coast	Hatteras	2007	2020	415	*144*	*141*	*130*	105	25%	58	14%	25	6%
US West Coast	Southern California	2006	2023	320	*125*	*114*	*81*	126	39%	97	30%	39	12%

Table 2. Total number of IDs per year in each regional collection.

Sub-area	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Terceira										1			7	2	11	8	9			7	
Ionian															13	2	1				
Ligurian	1	33	54	49	16	11	14	3	23	24	52	53	60	42	65	81	73	22	110	41	
Isla Guadalupe				2	6	3	14				1		1	26	40	44	49	37	45	53	43
Hatteras					6				5		7	35	30	20	65	101	112	108			
Southern California				2		43	19	18	28	17	27	27	39	17	35	37	47	43	5	71	45

Identifying predictive relationships for daily lactation costs in marine mammals

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Background

Lactation is an energetically demanding life history stage for marine mammals and one that has been identified as particularly vulnerable to adverse energetic effects associated with anthropogenic disturbance. As such, reproductive females are often a focus of models implementing the Population Consequences of Disturbance (PCoD) framework, which describes how disturbances at the individual level can have cascading effects on populations through behavioral and physiological linkages. Given concerns associated with disturbance often center around disruptions to foraging behavior, many PCoD models rely on bioenergetic models to characterize fluctuations in mass dynamics and body condition, often at relatively fine temporal scales. Quantifying energetic costs associated with lactation remain challenging for marine mammals, particularly cetaceans where empirical measurements of milk intake are largely infeasible due to their body size and fully aquatic nature.

Objectives

The objectives of this study are to 1) use published values of daily milk intake rates and milk energy density to understand how these relationships change throughout lactation across terrestrial and marine mammals, 2) explore predictions of cetacean lactation costs using the predictive relationships from Obj. 1, and 3) compare output with daily lactation costs from existing or ongoing efforts to assess feasibility of this approach for estimating daily lactation costs in future PCoD efforts.

Methods

Literature reviews were conducted using systematic searches as well as 'snowballing' to identify relevant studies reporting on either milk intake and/or milk composition of eutherian mammals. Studies were included if they met the following criteria: 1) conducted using labeled water (milk intake only), 2) species not bred with the purpose of promoting milk production genes, and 3) accessible data (in text, tabular format, or a digitizable figure) across a relatively short measurement interval (e.g., days rather than months or the entirety of lactation), 4) a minimum of at least three time points covering a minimum of 25% of lactation per species, from one or more studies. We limited milk energy density data to marine mammals and closely related terrestrial species.

Generalized additive models (GAMs) were used to develop predictive relationships between milk intake or milk energy density and the proportion of time into lactation, which was determined by the age of the offspring during the measurement interval and the average duration of lactation of a given species. Separate models were run at the species and family level. Analyses were run using the *mcgv* package in R.

Results

Data from 17 species were included in the milk intake analysis spanning four orders (Primates, Rodentia, Artiodactyla, and Carnivora). Lactation coverage ranged from 26.2 - 91.9% of the estimated lactation duration, with a mean of 60.5%. All species exhibited a decline in mass-specific milk intake across lactation, with inter-specific differences in the rate of decline (Fig. 1). Pinnipeds exhibited minimal change in mass-specific milk intake rates during lactation compared with other species. Mass-specific milk intake rates varied considerably among species, particularly carnivores. Otariids exhibited the lowest rates of mass-specific milk intake among carnivores (Fig. 1).

Data from 18 species were included in the milk energy density analysis spanning three orders (Artiodactyla, Carnivora, Proboscidea). Lactation coverage ranged from 35% - 100% with a mean of 83.8%. Almost all species exhibited an increase in milk energy density during lactation, but the magnitude and shape of the curve were species-specific (Fig. 2). Milk energy density trends of some species were asymptotic while others exhibited linear increases.

Next efforts will focus on addressing Objectives 2 and 3.

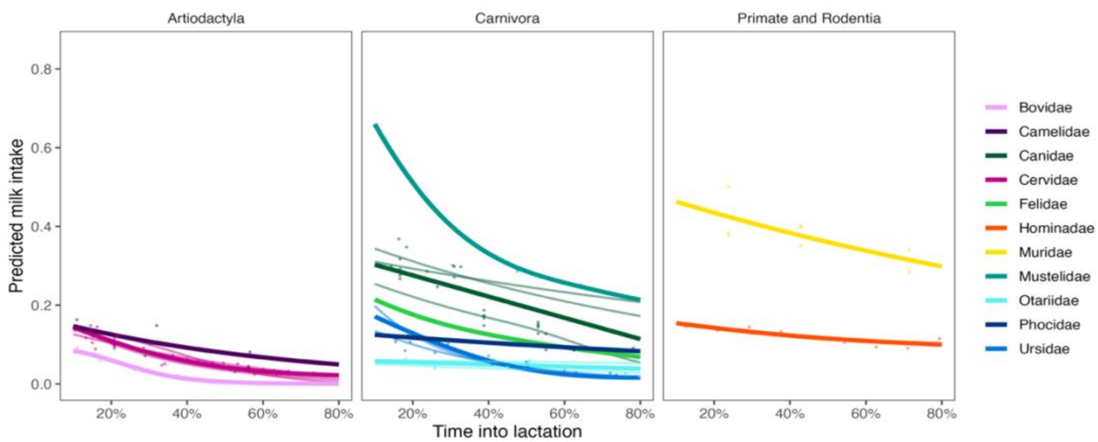

Figure 1. Predicted values of milk intake (g milk day^{-1} g^{-1} offspring mass) from species- (thin lines) or family-specific GAMs (thick lines). Points represent data from individual species.

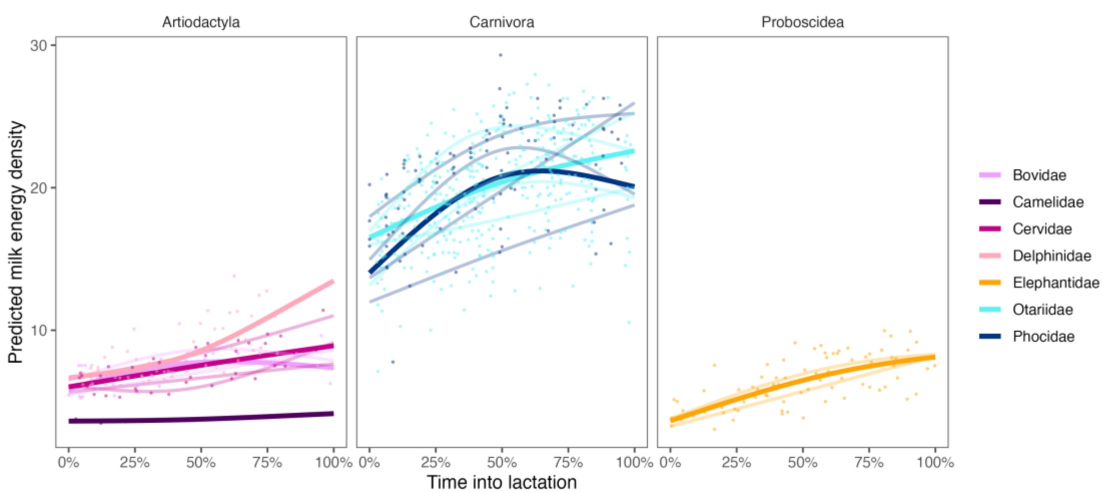

Figure 2. Predicted values of milk energy density (kJ g^{-1}) from species- (thin lines) or family-specific GAMs (thick lines). Points represent data from individual species.

Dolphin population photogrammetry (DoPoGram): Assessing feasibility of generalizable method to determine delphinid population health.

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Background

The importance of understanding the population status of long-lived species is critical for their effective management (Taylor & Gerrodette, 1993). Traditional monitoring methods often fail to quickly identify demographic shifts (Taylor et al., 2007). Variations in vital rates (e.g., fertility, survival) correlate closely with population growth (Pirotta et al., 2015; Senigaglia et al., 2016), necessitating innovative methods for early indicators of decline. Population dynamics, including age-structure, are crucial for assessing population stability and future trends (Pirotta et al., 2018; Jackson et al., 2020).

Stable populations usually exhibit a consistent proportion of calves, juveniles, and adults (Gamelon et al., 2016), whereas deviations from such may indicate population growth or decline (Coulson et al., 2005). Changes in age-structure influence vital rates offering insights into future population trends (Booth et al., 2020; Holmes & York, 2003).

Aerial photogrammetry, particularly using Unoccupied Aerial Systems (UASs), offers a non-invasive approach to monitoring cetaceans (Dawson et al. 2017). However, research on small-toothed whales is limited due to methodological challenges (Cheney et al., 2022; Currie et al., 2021; Fearnbach et al., 2018).

The aim of this research was to assess the feasibility of developing a method of using UAS-photogrammetry to determine delphinid population status.

Objectives

Three interconnected study objectives were conducted:

1) Calibrate and ground-truth the precision of UAS-photogrammetry using dolphins in facilities.
2) Age and develop a length-at-age growth curve for spinner dolphins (*Stenella longirostris subsp.)* in Hawai'i.
3) Estimate the length of a large proportion of individuals comprising a stock of spinner dolphins through UAS-photogrammetry to infer stock age-structure (Fig. 1).

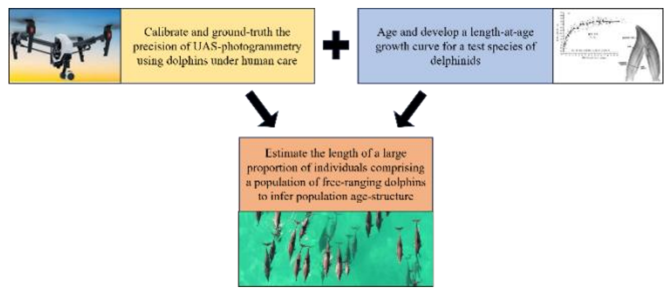

Figure 1: Schematic overview of the study objectives that form the basis for assessing the feasibility of using Unoccupied Aerial Systems to determine delphinid population age-structure.

Methods

1) First, the precision and accuracy of UAS-photogrammetry were tested to measure and estimate Total body Length (TL, a proxy for age) at five altitudes (16-50m) for five male bottlenose dolphins (*Tursiops truncatus*) at Dolphin Quest O'ahu, Hawai'i, USA. These measurements were compared to hands-on measurements of the individuals. TL was then estimated using the blowhole-to-dorsal-fin distance (BHDF, a proxy measure for TL) when individuals surfaced. Results from this study were then applied to a long-term dataset (>40 years) to assess the sensitivity of UAS-photogrammetry to estimate the age-structure of

a free-ranging community of bottlenose dolphins in Sarasota Bay, Florida.

2) Between 2007 and 2024, 34 stranded spinner dolphins were recovered by the University of Hawaii's Health and Stranding Lab. All 34 individuals were aged (obtained from counting of tooth growth layer groups) and measured (TL, BHDF), and a growth curve was developed.
3) UAS-photogrammetry was used to measure the TL and BHDF of free-living spinner dolphins off Kona Coast, Hawaii during September 2021 and 2022. UASs were flown from two research vessels simultaneously to sample a maximum of dolphin groups while limiting re-sampling.

Results

1) We documented a strong positive correlation between TL and BHDF measurements (R^2 = 0.76) in bottlenose dolphins. The average difference in TL between physical measurements and UAS-derived measurements for stationary dolphins was 0.1% ± 1.3 SE across all altitudes and 3.10% ± 1.21 SE when estimated based on BHDF measures. Optimal age-class assignment was best when using two age-class bins (0-10 years vs. 10+ years; ~87%) and three age-class bins (81% accuracy for 0–2, 2–10, and 10+ years, representing calves, juveniles, and adults).
2) The Gompertz function best fitted the growth pattern of stranded spinner dolphins, showing rapid calf growth in the first two years. This growth model will aid in estimating the ages of free-ranging spinner dolphins using UAS-length measurements, informing the assessment of the spinner dolphin stock's age structure. Additionally, a strong positive relationship between TL and BHDF was found in the stranded spinner dolphin specimens (R^2=0.92), confirming that TL can be estimated from BHDF for spinner dolphins.

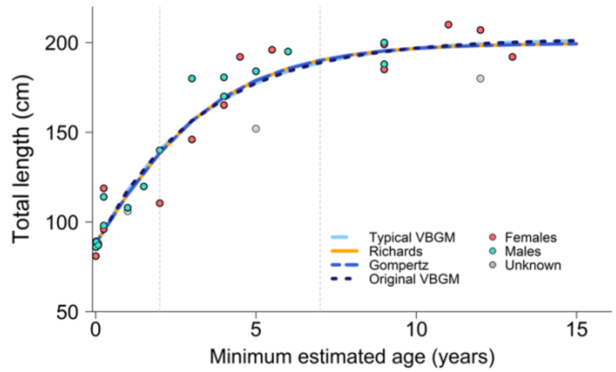

Figure 2: Visual representations of each of the four-candidate growth model fit (n = 34). The most parsimonious fit was obtained with the Gompertz growth model. VBGM: von Bertalanffy Growth Model. Red dots represent females (n = 16), blue dots represent males (n = 15), and grey dots display unknown sexes (n = 3). The dashed vertical grey lines represent the ages of 2 and 7 years.

3) In 2021 and 2022, we surveyed 860-900 km of coastline each week, encountering 211 ± 22 SE spinner dolphins daily in 2021. The TL of individual spinner dolphins was measured in 64 (124 flights) and 35 (48 flights) dolphin groups in 2021 and 2022, respectively. In 2021, we assessed the age-structure of the stock using the three days with the highest dolphin counts (295, 271, and 267 individuals, respectively). Age-structure estimates remained consistent across days (p>0.1), averaging 10.2% calves (0–2 years), 51.4% juveniles (2–7 years), and 38.4% adults (7+ years). Analyses for 2022 are currently ongoing. Before determining the stock's status, it is crucial to understand birth and mortality rates and to compare these age-structure estimates to calculated stable age-structure. We will use available information for other spinner dolphin populations for these comparisons.

Quantifying key vital rates in Blainville's beaked whale reproduction, including age at weaning, pregnancy rates and calf survival to inform population of consequences of disturbance models

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Background

Population Consequences of Disturbance (PCoD) models provide a conceptual framework tracing effects of disturbance through life functions to population status around which prediction models are constructed. Despite the potential utility of PCoD models as mitigation tools for the U.S. Navy, key information on vital rates is lacking for beaked whales, leading to a high degree of uncertainty in model predictions. This highlights the need to fill these data gaps to better parameterize PCoD models.

Here, we are quantifying key metrics to improve the efficacy of PCoD models for Blainville's beaked whale (*Mesoplodon densirostris, Md)*. These parameters include reproductive metrics (e.g., fecundity, calf survival, lactation duration, age and length of calves at weaning) and health metrics (body condition, growth rates and energetic cost of reproduction) from a reference (undisturbed) *Md* population in Abaco, Bahamas. These metrics are being compared to a population ~170 km away at the U.S. Navy's Atlantic Undersea Test and Evaluation Center (AUTEC) where disturbance from Navy sonar regularly occurs [1, 2] (see N000141812778).

Objectives

We are using aerial photogrammetry, capture-recapture (photo-identification), and remote biopsy sampling methods with the following objectives:

1. To document and monitor pregnancies to determine baseline reproductive rates and success, for comparison to *Md* at AUTEC.
2. To quantify the energetic cost of reproduction by relating changes in body condition in lactating females to calf growth.
3. To determine the age of calves at weaning and lactation duration by documenting suckling bouts.
4. To measure the calf's percentage of mother's length and length of calves at weaning.
5. To compare body condition of whales at AUTEC (N000141812778) of similar state/sex classes to Abaco.
6. To document growth rates and develop growth curves.
7. To document genetic relatedness of individuals in social units to investigate *Md* mating strategies.

Methods

Vertical images of *Md* were obtained using a small, remotely controlled hexacopter system (custom-built LEMhex44) fitted with a SF11 laser altimeter launched from a 6.8 m RHIB. Images were collected using a micro 4/3 camera (Sony Alpha5100 24-Megapixel CMOS mirrorless camera) with a 35 mm f1.8 lens, mounted on the hexacopter, pointing directly downwards. When beaked whales were sighted, the hexacopter was launched and positioned over individual whales when they surfaced, obtaining high-resolution images from an altitude of ~30 m.

For absolute length, size in pixels were converted using a measure of scale (scale = altitude/lens focal length). Precision was determined by photographing a 2.0 m pole alongside the RHIB. Measurements were linked to individuals and their life history data, based on the pattern of natural scars discernible from the air and boat platforms.

For each whale imaged, total body length (distance from the tip of the rostrum to the fluke notch) and width profiles (at 5% increments along the body axis, Figure 1) were measured using MorphoMetriX v2 [3]. We used a 'Bezier fit' which applies a smooth fitting curve to the points selected in the measurement, which is useful if the animal is curved (e.g., rolling as it surfaces). Widths were expressed as a proportion of length to control for individual differences in sizes, and width profiles used to derive metrics for evaluating body condition and identifying pregnancies that can be confirmed from blubber hormones, and follow-up photo-identifications. CollatriX [4] was used to collate MorphoMetriX measurements and calculate body condition or Body Area Index (BAI) using the parabola method to calculate a parabola for the sides of the whale using width measurements and the surface and then calculated the area under this curve [5]. This approach is being replicated at AUTEC (N000141812778).

Results

There were 50 vessel days (3780 km travelled, 344 hr search effort) and an additional 22 days (1350 km, 143 hr) in the Abaco study site when there was no access at AUTEC in 2019 and 2020 (N000141812778). There were 169 sightings of 12 cetacean species, including 49 *Md* groups with median group size of 3 (range 1 – 9). Fifty-five individuals were photo-identified, including 14 calves (5 neonates). Fifty-six percent of non-calf individuals matched to our existing catalogue (n = 23), four of which were adult females first seen in Abaco over 24 years ago. Biopsy samples were collected from 18 whales: six adult females, five adult males, and two subadult females and four subadults of unknown sex. One female was biopsied three times in differing reproductive states.

The UAS was launched for 45 flights during which 2,318 aerial photogrammetry images were obtained. High quality images were collected and measured for 25 whales, including eight adult females in different states of reproduction with mean total length (TL) 3.91 m (range 3.3 – 4.5, n = 64 images), three adult males mean TL 3.86 m (range 3.61 – 4.2, n = 18 images), five subadults mean TL 3.24 m (range of 3.05 m – 3.58, n = 17 images) and seven calves mean TL 2.34 m (range 1.57 – 2.85, n = 30 images). The mean distance between mature male's erupted teeth was 0.13 m (range 0.12 m – 0.17, n = 15 images).

Calf's proportion of the mother's length ranged from 48% of its mother's TL for a neonate, increasing to 69% for 11-month-old calves. Empirical growth rates calculated annually for a calf and a maturing male photographed repeatedly were 0.065 and 0.023, respectively.

Pregnancies were predicted for three females based on width profiles (Figure 2); all females were later resighted with calves. Body Area Indices (BAI) for lactating females declined with increasing calf age (Figure 3). Interestingly, the adult male with the lowest BAI exhibited the highest site fidelity suggesting a high energetic cost to female defence.

Two additional upcoming field seasons will increase small sample sizes and allow statistical modelling approaches to be applied to this unique and valuable dataset.

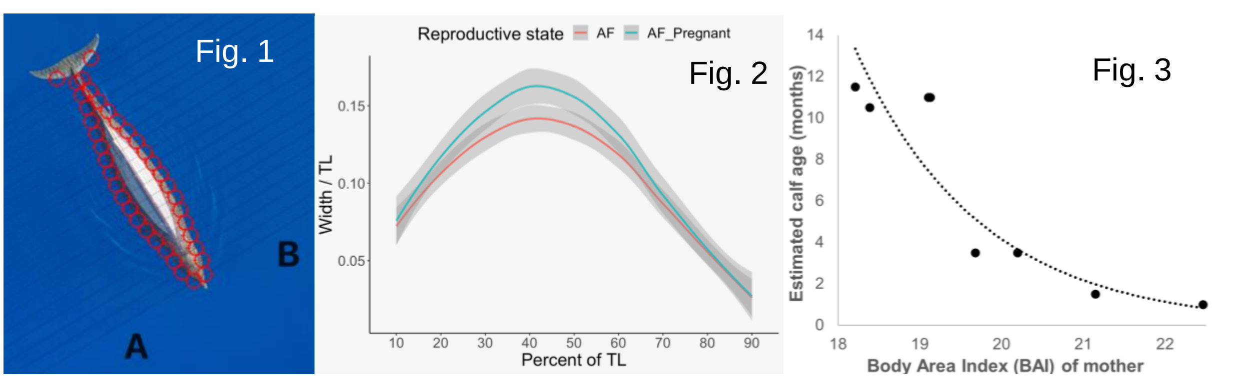

Fig. 1. Width profiles at 5% increments along the body axis measured using MorphoMetriX v2 [3]; Fig. 2. Width profiles for pregnant and non-pregnant females; Fig 3. Relationship between calf age and Body Area Index of its mother.

VESOP II: Developing broadly applicable models to predict vital rates from remotely sampled health measures including epigenetics

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Background

To better understand how stressors affect marine mammal individuals and populations, we must find ways to translate health indicators from animals in the field into estimates of survival rates and reproductive success. As part of our previous ONR project, we collected data from hands-on bottlenose dolphin (*Tursiops spp.*) health assessments and longitudinal photo-identification studies, and with guidance from veterinary/physiology experts we developed the Veterinary Expert System for Outcome Prediction (VESOP) model. The model uses a Bayesian framework to recognize patterns among a small number of health indices (e.g., blood markers of inflammation and lung disease scores). These patterns were predictive of increased 1- and 2-year mortality risk for individual dolphins and, when averaged across cohorts, correlated well with capture-mark-recapture analysis estimates of population annual survival rates (Schwacke et al., 2023).

In parallel, a joint ONR/SERDP project is investigating epigenetic analyses for Population Consequences of Multiple Stressors (PCoMS) models. In humans, epigenetics can predict not only chronological age (actual time since birth) but also biological age (physiological age based on organ/system health). The SERDP project has established an epigenetic clock using blood samples from managed dolphins of known age to predict chronological age (Barratclough et al., 2021). Human studies show evidence for an association between environmental stressor exposure and changes in DNA methylation (DNAm) that indicate age acceleration (when biological age is greater than their chronological age). Importantly, epigenetic analyses can be conducted on skin tissue from remote biopsies, supporting application of a VESOP modeling approach to species for which hands-on health assessments are not currently feasible.

Objectives

To develop a tool for evaluating how stressors impact wild marine mammals of interest to the U.S. Navy, we are extending the VESOP model to: 1) integrate dolphin epigenetic biological age as a new health metric into the existing model for predicting survival and reproductive success; 2) develop a model for Blainville's beaked whales (*Mesoplodon densirostris*) focusing on triiodothyronine (T3) and cholesterol levels in the blubber, as well as epigenetic biological age in skin tissue; & 3) develop a model for northern elephant seals (*Mirounga angustirostris*) as a case study for capital breeders. These tasks will advance our ability to assess and monitor the health of diverse marine mammal species through case studies that identify quantitative links between health indicators and vital rates critical for the development of PCoMS models.

Methods

To develop a dolphin biological age metric, we first built a model to estimate chronological ages using wild dolphin skin samples (Barratclough et al., *in review*). We are now exploring a variety of techniques for establishing biological age/age acceleration metrics, e.g., identifying and understanding epigenetic signatures that predict VESOP health score and/or specific health outcomes. We will use archived beaked whale skin samples (n= 48) to evaluate chronological age and biological age from DNAm patterns, and we will supplement the dataset with ongoing remote biopsy field collection efforts. We are also performing laboratory assay development to understand how hormone concentrations in blubber and blood samples compare. We will then combine these two sets of health data with survival estimates from a capture-mark-recapture model in a VESOP framework. For elephant seals, we are using DNAm data to develop a chronological age clock, combining samples from wild adults and pups before and after rehabilitation. We will evaluate approaches for biological age estimates and combine them with blood diagnostics and survival outcomes to develop a VESOP model for capital breeders.

Results

We evaluated elastic net regression and random forest regression (individually and in combination) to develop an epigenetic clock and predict chronological age using DNAm data from 476 dolphin skin samples. The hybrid model predicted ages most accurately, with a median absolute error (MAE) for calves (n=28) of 0.149 years, subadults (n=273) of 1.46 years, and adults (n=110) of 3.25 years. Age predictions were less accurate in the oldest cohort (n=65), with an MAE of 5.90 yrs. Overall MAE across all ages was 1.91 yrs. Samples from dolphins exposed to *Deepwater Horizon* oil had high epigenetic age predictions relative to observed age (age residual), perhaps due to markers associated with chronic lung disease and/or inflammation. This was supported by a negative correlation between DNAm age residual and VESOP predicted survival probability in a beta GAM model while controlling for sampling location.

To identify cellular processes that contribute the most to these predictions, we used the Genomic Regions Enrichment of Annotations Tool (GREAT) to find overrepresented genes within the 338 DNAm sites given the most weight by random forest regression model. Genes involved in meiosis and gamete production, neuroplastin and its function in synapse formation/memory, skeletal muscle development and maintenance, disassembly of cellular machinery, organic cation transport, and myoblast proliferation are significantly enriched in the dolphin age model.

The case studies with beaked whales and elephant seals are in progress and integrated into other ONR-funded projects. Our results suggest that DNAm patterns can inform wildlife population health assessments and identify marine mammal populations impacted by specific stressors. We will continue to investigate the gene regions and ontologies of CpG sites that underlie biological age via patterns associated with specific health conditions or environmental stressors.

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Assessing Nutritional Stress & Pregnancy in Blainville's Beaked Whales at the Atlantic Undersea Test & Evaluation Center (AUTEC)

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Background

Studies at the U.S. Navy's Atlantic Test and Evaluation Center (AUTEC) in The Bahamas indicate that Blainville's beaked whales (*Mesoplodon densirostris*, *Md*) move away from Navy sonar sources and are displaced from their regular feeding habitat [1,2]. Bioenergetics models suggest that beaked whales require relatively high-quality habitat to meet their energy requirements, and that regular displacement from preferred feeding habitats could impact survival and reproduction through compromised body condition [3]. This is of particular concern for pregnant and lactating females that have increased energetic demands and findings of low reproductive success of *Md* at AUTEC have led to a hypothesized population consequence of repeated disturbances [4]. However, empirical data on *Md* female body condition is lacking, constraining a direct assessment of whether nutritional stress from disturbance is a realistic mechanism for explaining the apparent population responses.

Objectives

We are combining photogrammetry, photo-identification and blubber hormone measures in *Md* at AUTEC:

1. To measure width profiles to assess individual body condition and detect pregnancy, and ground-truth inference about nutritional stress from hormone measures (cortisol and thyroid hormones, T3 and rT3) comparing females with (lactating) and without dependent calves.
2. To ground-truth photogrammetric inference based on body shape using hormone-derived identification of pregnancy (progesterone and testosterone).
3. To compare state-specific size (total length) and body condition (body area) of whales at AUTEC to the reference population to investigate size differences as a potential indication of longer-term nutritional stress.

Methods

Real-time localizations of *Md* were relayed by NUWC's Marine Mammal Monitoring (M3R) to the field team on a 6.8 m rigid-hulled inflatable (RHIB) used for photogrammetry operations, photo-identification, and biopsy sampling.

Vertical images of *Md* were obtained using a small, remotely controlled hexacopter system (custom-built LEMhex44) fitted with a SF11 laser altimeter. Images were collected using a micro 4/3 camera (Sony Alpha5100 24-Megapixel CMOS mirrorless camera) with a 35 mm f1.8 lens, mounted on the hexacopter to be downward-pointing. When beaked whales were sighted, the hexacopter was launched and positioned over individual whales when they surfaced, obtaining high-resolution images from an altitude of ~30 m.

For absolute length, size in pixels was converted using a measure of scale (scale = altitude/lens focal length). Precision in these measurements is determined by photographing a 2.0 m pole alongside the RHIB. Measurements were linked to individuals and their life history data, based on the pattern of natural scars discernible from the air and boat platforms.

For each whale imaged, total body length (distance from the tip of the rostrum to the fluke notch) and width profiles (at 5% increments along the body axis) were measured using MorphoMetriX v2 [5]. This approach allows whales to be measured using a 'Bezier fit' which is especially useful if the animal is curved (e.g., rolling as it surfaces) as it applies a smooth fitting curve to the points selected in the measurement. Widths are expressed as a proportion of length to control for individual differences in sizes, and width profiles are used to derive metrics for evaluating body condition and identifying pregnancies that can be confirmed from blubber hormones, and follow-up photo-identifications. CollatriX [6] was used to collate MorphoMetriX measurements and calculate body condition or Body Area Index (BAI) using the parabola method to calculate a parabola for the sides of the whale using width measurements and surface and then calculated the area under this curve [7].

This approach is being replicated at a reference, "undisturbed" site off Abaco Island (N000142012756).

Results

AUTEC is a challenging place to conduct field work due to frequent inclement weather and limited range access. These challenges increased during this project by unpredicted, additional restrictions including new Federal regulations preventing UAS use on navy ranges (yr 1), COVID (yr 2), and a new Bahamian permitting agency which prohibited research at AUTEC (yrs 3-4). As a result, time in the field at AUTEC was very limited.

There were 47 vessel days (4,600 km travelled, 340 hr search effort) but only 19 days at AUTEC (2018, 2019, 2023). The remaining field days took place at the Abaco site and are reported in N000142012756. At AUTEC, there were 25 sightings of six cetacean species. Eighteen *Md* groups were sighted, and 27 individuals were photo-identified, including five calves. Fifty percent of non-calves matched to our existing catalogue (*n* = 11); including two adult females first seen in 2005. In addition, five "new" whales were identified during a transit between Abaco and AUTEC. Biopsy samples were collected from six adult females and one adult male.

During field work at AUTEC, the UAS was launched for six flights during three encounters and over 180 aerial photogrammetry images were obtained. High quality images were collected for an adult female, an adult male, and a dependent calf (est. 6 months old). Mean total length (TL) and ranges were adult female TL 4.19 m (3.84 – 4.5 m, *n* = 6 images), adult male TL 3.61 m (*n* = 1) calf TL 2.38 m (2.3 – 2.46 m, *n* = 4). The distance between the male's teeth was measured at 0.2 m. TL for the AUTEC whales compared to TL for the same stage/sex classes from Abaco (Figure 1) showed no clear differences.

Based on measurements of the width profile of the adult female when compared to females known to be pregnant at the Abaco site, we predict the AUTEC female is pregnant (Figure 2). This female was also biopsy sampled so we will be able to validate the photogrammetry results. The female's mean BAI (21.5, sd 0.90) when compared to Abaco pregnant females further supports pregnancy (Figure 3). The AUTEC calf's mean TL and BAI (22.21, sd 0.55) fall between the averages for 3.5 month-olds to 11 month olds from Abaco.

Small sample sizes for AUTEC inhibit valid comparison of these metrics to the Abaco reference population. A final field effort is planned this year which will increase sample sizes enabling inferences to be made about potential population level effects of disturbance.

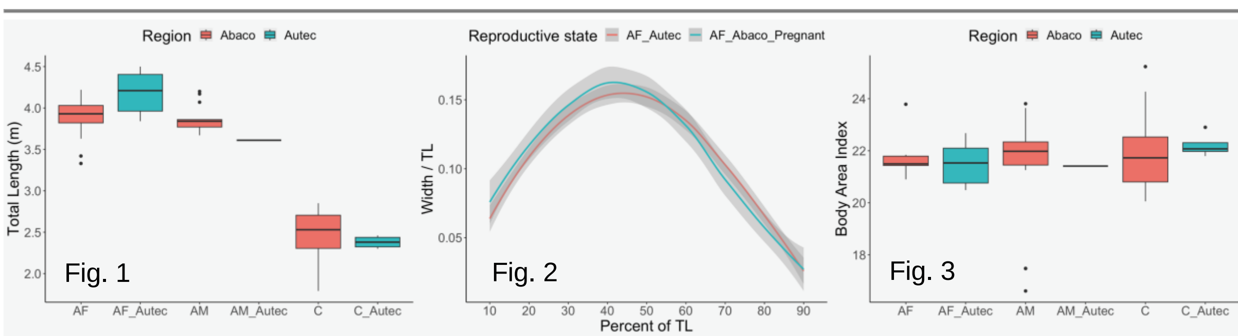

Fig. 1. Total length for same state/sex stages at Abaco and AUTEC [C = Abaco calves, ages 3.5 – 11 mths]. Fig. 2. Width profiles for an AUTEC adult female and pregnant Abaco females. Fig. 3. Comparison of Body Area Index (BAI) [AF = Abaco pregnant females, C = Abaco calves, ages 3.5 – 11 mths].

Engineering and design to enhance heart rate detection in cetacean-borne tags

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Background

Cetaceans are increasingly exposed to a wide range of direct and indirect anthropogenic disturbances including fisheries, ship traffic, and acoustic disturbances of varying frequencies. Reliable technologies are needed to quantify the impact of stressors on physiological function, particularly for cetaceans, which remain some of the most vulnerable and poorly understood marine mammals. Despite decades of tag studies on large cetaceans, the diving physiology of these animals in both nominal and stressed states remains poorly understood.

In recent work, we completed a major advancement in our ability to bring the physiological laboratory to the open ocean using whale-borne tags equipped with surface electrodes (Goldbogen et al., 2019). The original prototype was basic in design with a time-depth recorder coupled to a dECG recorder. This tag successfully measured a blue whale's ECG via the custom-built dECG recorder/ housing (UFI/Meer Instruments) incorporated into a custom suction cup-attached tag (CATS Diary WiFi; Customized Animal Tracking Solutions). Surface ECG electrodes (E-244 electrode; In Vivo Metrics) were embedded in the bases of 2 suction cups. The tag was attached using a 6-m carbon fiber pole and a 6.3-m inflatable boat in Monterey Bay, California. Analysis of dECG data to determine heart rate followed previously established protocols (Bickett et al., 2019). Despite the initial success of obtaining the first ever digital heart rate profile from a wild cetacean without prior restraint, it has been challenging to obtain consistently high signal-to-noise ratios with multi-sensor tags. The noise appears to be secondary to movement artifacts and the contact quality of the electrodes with the skin. Signal artifacts due to body movement, such as flipper movement or fluke strokes, is largely tag placement-dependent and results in intermittent noise. In contrast, consistent noise is likely a function of the contact quality at the electrode-skin interface, which may be reduced due to high drag and turbulence around the tag (i.e. due to poor streamlining of the tag). Therefore, tag design improvements are needed to enhance heart rate detection in wild cetaceans.

Objectives

The project aims to design a suction-cup attached tag that records a digital electrocardiogram (dECG) signal of baleen whales. The goals of the initial project phase were to 1) construct a platform to conduct controlled bench tests of different dECG recorders, electrodes, and configurations, 2) evaluate the dECG recorder (UFI recorder) with different ECG surface electrodes (silver/gold), and 3) iterate on a recorder housing and electrode configuration that improved electrode contact with the skin and minimized motion artifacts in the signal.

Methods

To support bench testing the ECG tag, we constructed a plexiglass platform with embedded electrodes that could be connected to the UFI Square Wave Simulator. Thus, when a tag is positioned over these stimulating electrodes, it should measure the signal being produced by the signal generator. The stimulating electrodes were positioned in the platform such that they would align with the previous CAT dECG Tag suction cup-embedded electrodes when a tag was suctioned to the platform. Early tests with this platform suggested good functionality of the testing setup with the previous CATS dECG housing when the suction cup-embedded electrodes covered in conductive paste were fully depressed onto the platform. Intermittently, when contact was lost (which could be observed through the clear plexiglass), the signal observed in the UUB3GPa software was completely lost. When contact was ensured, signal magnitudes as small as 50 uV were clear.

Results

Observations of electrode contact on the CATS dECG tag showed close contact to the plexiglass surface but not at the center of the cup where the electrodes are typically placed. Tests of a single ECG suction cup (with embedded electrode) showed that even when fully depressed, the electrode is not contacting the attachment surface and if the cup is sitting any higher, the electrode is further from the surface. On a whale, the skin may be pliable enough that the negative pressure pulls the skin surface into the cup and enhances contact. However, these bench tests suggest that the previous CATS dECG housing and electrode configuration can record clean signals, but only when electrode-surface contact is ensured. Tests of three types of electrodes (Ag-AgCl Ag-AgCl in elevated configuration, and gold 3 types of electrodes tested in D-tag suction cup electrodes all recorded similar magnitude signals suggesting electrode material type and relative position in suction cup does not influence signal quality.

Bench tests indicated that removing the heart rate sensing electrodes from the attachment suction cups may be helpful to allow for 1) proper suction without leaks due to the electrode-cup interface, 2) improved electrode contact that is independent of the depression of the attachment suction cups, and 3) a possible added damping effect that reduces motion artifact in the ECG signal due to tag movement/jiggle. The electrode suction cups were removed from one CATS dECG tag housing and replaced with normal cups. Custom-designed wing housings were designed and 3D printed to attach to existing CATS housing through the suction cup set screws. This design made use of existing CATS housings, which contain pressure and kinematic sensors, and connectors allowing future iteration without major irreversible changes being made to the non-ECG components of the tag. The wing allowed two major changes to be made: 1) the electrodes to be moved out of the attachment suction cup and into lower-profile suction cups that are closer to the body and 2) construction of a spring-based assembly within the wing that can damp motion noise due to tag jiggle and provide a compression force to maintain electrode contact on the animal regardless of attachment suction cup depression.

Initial designs of the wing focused on the spring assembly which consists of a suction cup cap with a vertical stem, a plunger with a flat, circular top, and a cup with an internal false floor (Figure 6). By connecting the plunger to the stem of the suction cup cap through a circular opening the false floor of the cup, we allow constrained movement of the suction cup (and ultimately the embedded electrode) in the vertical direction. Mounting this assembly in the underside of the wing with a spring placed above the plunger creates a spring-damped system that allows the suction cup and electrode to move vertically (and to a lesser degree horizontally) while the tag body moves around it. An FDM (fusion deposition modeling) printer was used to print wing designs to allow for tests of fit with suction cups (VacMotion) and electrodes (BioMed) and initial movement damping testing.

The second iteration of the wing improved the stability of the ECG wires in the channels of the wing. Initially a tunnel was created over the channel through which the ECG wire embedded electrode tubing was thread. Because the fit of the tunnel was intended to be tight, this design made it challenging to successfully navigate the wire/tubing through the entire length of the tunnel/channel. The current wing design makes use of several shorter tunnel sections so that the ECG wire and tubing can be threaded through this area in sections.

Developing a robust satellite-linked smart tag for long-term monitoring of large cetacean behavior

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Background

Long-term tracking devices have been key to assist the Navy with the implementation of monitoring programs to assess impacts and minimize risks of their activities to large whales in training and testing areas. Recently, Oregon State University (OSU) has worked with the tag manufacturer Telonics to develop a new consolidated-style tag (the "RDW" tag) which incorporates a pressure sensor, accelerometer, and onboard processing capacity to monitor critical behavioral events like whale feeding behavior over several months without requiring recovery (Palacios et al. 2022). This tag is still under development as there is a need for additional in-situ validation of its onboard event detection algorithm and the tag uses a multi-part physical design (e.g. separate penetrating tip, tag housing and end cap). Tags attached to large whales can experience strong kinetic forces during the deployment process, or interactions with conspecifics. A design which integrates multiple components into a continuous unit can increase its resistance to external forces and minimize the chance of breakage (Zerbini et al. 2018). Until this tag has been demonstrated as robust and well-validated, there is a limited ability to directly measure basic behavioral characteristics of large whales over long-time scales.

Objectives

The overall goal is to redesign and adapt existing components of a large cetacean behavior monitoring tag (the RDW tag) to produce a more robust design, with defined performance characteristics for monitoring the dive and feeding behavior of large whales. The primary objectives are:

1. Develop a robust tag housing design that integrates multiple components (housing, penetrating tip, attachment elements, and endcap) into one continuous unit and conduct destructive laboratory testing to establish its structural integrity characteristics.
2. Conduct field deployments and follow-up monitoring of the robust tag to demonstrate its functionality, identify any tag breakages and confirm minimal effects of the tags while attached to the whales.
3. Conduct in-situ validation of the robust tag's event detection algorithm

Methods

In this first year of the project, we worked closely with the OSU CEOAS Machine and Technology Development facility to develop a robust tag housing design that integrates multiple tag components into one continuous unit. We used computer-assisted design (CAD) software to consider competing designs and fabrication methods including machining from a single piece of cold-rolled stainless steel, fabrication as a continuous piece of metal using Metal Additive Manufacturing (MAM; 3D printing with metal), or a combination of these methods to reinforce and reduce the number of separate parts. We concurrently regularly consulted with the tag manufacturer Telonics to facilitate integration of the existing RDW electronics package into the new housing design.

Laboratory tests simulating stresses that may be experienced by the tag during deployment and while attached to a whale are being conducted in OSU facilities dedicated to measuring the response of materials to applied forces. These tests are also being conducted on a similar consolidated-style tag with demonstrated robustness characteristics (Zerbini et al. 2018) for comparison.

Results

The selected design included a penetrating tip, manufactured using MAM to improve the cutting surface, which is press-fitted and welded onto a machined tube/pressure chamber, while also capturing the tag retention elements (Figure 1). The tag endcap was extensively re-designed to fit entirely within and seal the distal end of the pressure chamber for greater protection from impacts. Pressure tests of the endcap seal with antenna and pressure sensor pass-throughs were successful down to 3000 m. Laboratory tests quantifying the response of the housing to applied forces are ongoing.

Design elements to integrate the internal electronics components of the tag with the re-designed endcap have been developed concurrent with other work and focused on a modular design to facilitate adaptation of new features in the future.

Figure 1: Visualization of the robust tag housing (middle) with re-designed endcap assembly (left) and internal electronic components (right)

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Advancement of Attachment of Biologging Tags

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Background

The current average tag attachment time with suction cups remains on the order of ~24 hours, several days short of the recording capabilities of the current generation of sound and movement tags themselves. The project team has investigated use of adhesives to augment suction cup attachments and improve the reliability and duration of tag deployments. Preliminary field testing of a cyanoacrylate (CA) adhesive applied to suction cups in 2021 demonstrated that attachment via liquid adhesives is practical in the field. The deployment with CA resulted in a 35 h attachment to the animal, but, based on field-based observations, the flexible material used in the suction cups is not ideal for long-term adhesion. The repetitive hydrodynamic loading of the flexible cups, and the relatively small contact area between the cup lip and the whale skin, limit adhesive performance. Additionally, the quality of the tag placement during attachment will affect longevity of the tag on the animal during deployment. Tag bodies are designed to reduce hydrodynamic loading when correctly aligned with the flow. However, tag placement and orientation on the animal during pole tagging is often variable and highly dependent on animal behavior. Tag attachment longevity would be improved by consistently placing tags in preferred locations (e.g., on the midline of the animal between the blow hole and the dorsal fin, aligned with the axis of the body) with a consistent attachment force.

Objectives

Building on these preliminary results we will address these gaps by: ***A) Designing, fabricating and testing a new DTAG attachment mechanism that combines suction cups and liquid adhesives for improved attachment longevity; and B) developing a DoD-approved drone-based tagging system to deliver the hybrid adhesive tags to the animals in a consistent and repeatable manner.***

Objective 1: An adhesive attachment mechanism for multi-day tagging

The design of a hybrid attachment system requires knowledge and expertise from mechanical engineering, materials science and biology. Our preliminary results indicate that common cyanoacrylate adhesives (CAs) can be used to adhere silicone to whale skin, but there are still open questions that need to be investigated to realize a reliable adhesive-based attachment method for tags. An interdisciplinary approach will be used to address the following research questions:

Are there liquid adhesives in addition to CA, that can be used to secure engineered materials to biological tissue? How do material properties (such as impedance) of the engineered materials influence the adhesive bond between the material and skin? Can the surface roughness of the engineered materials be modified to improve adhesive performance? How do external environmental factors (hydrodynamic loading, the marine environment, temperature) affect adhesive performance?

Objective 2: Deployment and field testing the adhesive attachment mechanisms

UAV based tag deployment: Our primary objective is to increase the average duration of tag attachment using adhesives to yield reliable multi-day recording of sound and movement data from cetaceans using a minimally invasive attachment method. This will facilitate collection of the necessary data (both duration for baseline vs. exposure comparisons and sample sizes) for behavioral response studies (BRS) and generate new knowledge about the behavior of marine mammals over longer time scales to better understand natural variability in behavior in individuals. Reliable and repeatable attachment force and tag location will enhance the performance of the adhesive attachment system. The PI's have demonstrated the feasibility of UAV tag deployment, and the proposed work will result in a redesigned system capable of generating consistent attachment forces from low drop heights. The drop heights required by the current system range between 6 – 8 *m*. The corresponding drop times are long enough for the animals to change direction, resulting in missed attachments and variability in the placement location.

Results

During the start of the project the team has been working on developing the experimental designs and instrumentation for the controlled force vs displacement testing that will be used to evaluate the performance of the adhesives with the engineered materials for the attachment system. At Michigan we have fabricated the fixturing and the sensing for the testing work, Figure 1. At Syracuse, the components for the testing system that will be used to evaluate adhesive performance with dolphin cadavers have been selected, purchased, and ordered. Once these components arrive on sight they will be assembled into the portable testing system. Controlled loading for the pull-off adhesion testing will be assembled into the portable testing system. Controlled loading for the pull-off adhesion testing will be conducted with an Instron Universal Testing Machine. The team is currently evaluating materials and geometries for the test samples that will be used to evaluate performance of the adhesive bond in the lab first on compliant engineered surfaces, and then with the dolphin cadavers.

Plans for the Next Period

In the coming year we plan to focus on the following for the two primary project objectives:

Objective 1: An adhesive attachment mechanism for multi-day tagging: Using the experimental instrumentation built specifically for this project, we will evaluate the performance of the adhesives first on compliant engineered materials, and then with biological tissue. In the next phase of the project we will focus on the evaluation of adhesive performance on cadavers before moving to tests with animals in managed settings.

Objective 2: Deployment and field testing the adhesive attachment mechanisms: UAV based tag deployment: We will with the program management team to identify and purchase compatible UAV systems for the work with funds from co-funded projects. Once these systems have been purchased we will integrate the deployment system for the adhesive tags, and test/evaluate those designs in controlled experimental environments.

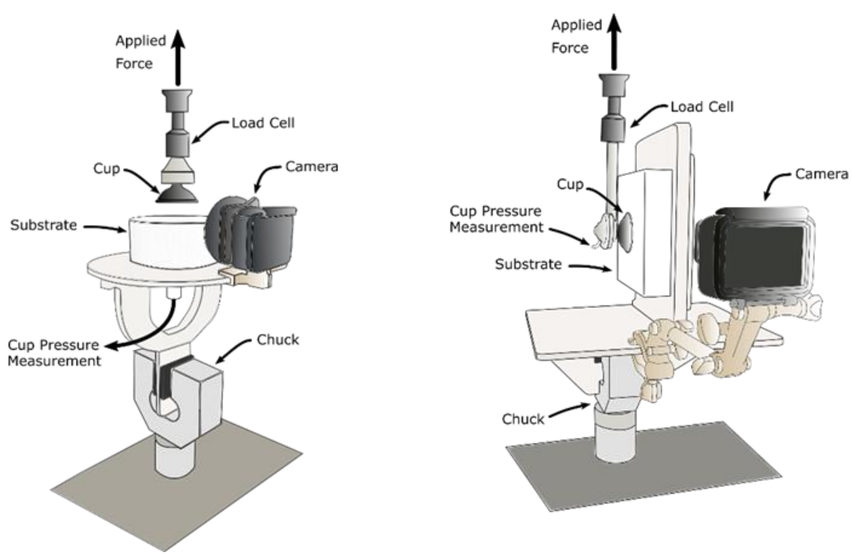

Figure 1 – An image of the experimental test setup that will be adapted to test the performance of adhesive bonds between engineered materials at the University of Michigan. Failure during normal and shear loading at variable rates will be tested. Materials with a range of compliance will be tested with the adhesives to investigate how an impedance mismatch affects the strength of the bond.

Targeted management approaches for minimizing Navy activity impacts on long-lived vertebrates

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Background

Significant progress has been made linking behavior with health and vital rates to predict the potential for a Population Consequence of Disturbance (PCoD). PCoD models rely on input parameters such as survival and reproductive rates that have generally been thought to adequately represent the population. However, these demographic measures are typically derived from population averages, assuming that the likelihoods of survival and reproduction are equivalent across individuals. Recent research in long-lived vertebrates has discovered that the likelihoods of reproduction and survival are highly variable across individuals. Therefore, the population consequence of a disturbance is not likely to impact individuals across all demographic groups equally. For example, some females might be particularly resilient to disturbance whereas others might be more sensitive. Data on northern elephant seals (*Mirounga angustirostris*) and bottlenose dolphins (*Tursiops truncatus*) has shown that a few individuals appear to be more resilient to interannual variations in resource limitation or other stressors and thus disproportionately contribute to population viability; however, traits that contribute to this resilience are not known.

Objectives.

To quantify the importance of individual variability for management efforts informed by PCoD models, we are integrating multiple sources of data from northern elephant seals. In this robust model system, critical data on the phenological, ecological, physiological, and genetic drivers of lifetime fitness can be consistently obtained. Our research will leverage existing long-term data and produce new data to answer the following questions: (1) Which phenotypic and/or genotypic traits mediate lifetime reproductive success (LRS)? (2) How does the relationship between chronological age (i.e., time since birth) and biological age (i.e., markers for DNA methylation in the blood) relate to LRS? (3) What are the implications of variation in LRS for population dynamics and management?

Methods

First, we will evaluate whether phenotypic traits (at-sea behavior, diet, life-history phenology) and/or genotype modulate individual heterogeneity in LRS. As an alternative explanation, we will explore whether variation in LRS simply occurs due to random chance. Next, we will leverage existing samples to quantify similarities between chronological and biological age and determine the degree to which LRS impacts these epigenetic health metrics. Finally, we will synthesize the findings above by modeling the impact of variation in female LRS on population dynamics under targeted versus random management approaches.

Results

Our major accomplishment for this reporting period was publishing two peer reviewed papers related to Aim 1 [Beltran, Hernandez et al. 2023 Ecology Letters. Physiological tipping points in the relationship between foraging success and lifetime fitness of a long-lived mammal.; Hoelzel et al. 2024 Nature Ecology and Evolution. Genomics of post-bottleneck recovery in the northern elephant seal]. These publications show that substantial among-individual variation can be explained by behavioral strategies, foraging success, and underlying genetic architecture.

Postdoc Geno DeRango also made great progress on his manuscript that relates to Aim 1. The working title of the manuscript is "Examining individual niche decisions for the northern elephant seal: insights into phenology and fitness of a colonial-breeding marine mammal". He examined potential mechanisms behind breeding site selection [female age, post-foraging arrival time and mass gain at-sea] and how these decisions may result in fitness consequences for these long-lived mammals, from robustness of stress responses [cortisol and ACTH hormones] to lifetime reproductive output [pup production and mass at weaning]. He found that older females put on more mass and had longer foraging durations, which was associated with arrival to denser breeding sites. Denser sites were also correlated with higher cortisol and ACTH levels, likely as an adaptive physiological response after exposure to increased numbers of conspecific interactions and associated stressors. When exploring fitness effects, maternal mass gain, and not habitat density, was a strong predictor of reproductive success [numbers of pups born and weanling mass]. This further highlights the importance of successful foraging and resource acquisition in a capital breeder and displays how females may offset fitness costs of dense breeding sites.

We also organized and obtained additional samples for Aim 2 [epigenetics analysis], and continued to collect mark-recapture data so that fitness outcomes could be measured. This involved hundreds of days and thousands of hours of fieldwork for lab members, including undergraduate students at a minority-serving institution. To facilitate the research aims, a DURIP equipment grant was used to upgrade the fieldwork infrastructure including purchasing a truck and boat/engine and therefore increase the capabilities of our unique elephant seal research program. We performed biochemistry and hematology analysis on the blood samples we collected and submitted epigenetic samples [archived and fresh buffy coat samples] to the Clock Foundation for DNA methylation analysis. We recently received the DNA methylation and provisional analysis has demonstrated that accurate chronological ages can be estimated from DNA extracted from blood in elephant seals. To our knowledge this is the first time epigenetics has been performed in this species.

Understanding the timing and location of key life history events of the most demographically valuable individuals can help to strategically plan Navy activities in space and time to minimize the impacts of disturbance. These findings will help quantify the population consequences of a Naval exercise, which can be applied to other threatened, endangered, and at-risk species such as beaked whales that are of high concern to the ONR Marine Mammals and Biology Program. Our work will also set the stage for elucidating the interactions between Navy exercises and climate change, as marine mammal core habitats become displaced and potentially overlap with active Navy sonar training areas.

Identification of Navy-relevant oceanographic hotspots guided by ethical practices and experiential learning

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Background

The United States Navy's position as one of the largest global maritime fleets and concurrent investment in the development and testing of sophisticated sonar technologies means negative interactions with marine wildlife are likely to occur. Identifying where marine organisms are most concentrated can reduce the frequency of negative Navy-wildlife interactions. Biological hotspots (areas of intense biological activity that promote feeding activity in higher trophic level species) have become an important monitoring tool for describing locations where certain species and environments are at greater risk. However, the research community lacks detailed information about the location of biological hotspots in the marine environment, and a clear foundation on what constitutes a marine hotspot. Large marine vertebrates such as whales, seals, seabirds and sea turtles have been identified as ecosystem sentinels, or indicators of overall ecosystem processes and climate change impacts. Northern elephant seals *Mirounga angustirostris* are an ideal vessel for detecting hotspots of biological activity in the ocean because they are fast, quiet, far-ranging, and deep diving. Our research group co-leads a long-term elephant seal monitoring program which contains the tools to review what is known about hotspots and to understand how hotspots vary across scales using drones, stable isotope analysis, and instruments attached to animals. The research program can facilitate valuable quantitative skills development for undergraduate students, highlight career opportunities in the DoD workforce, and train graduate students to facilitate a safe field environment for future scientists. The proposed work cannot take place without considering the ethics of animal handling and compensating young field researchers.

Objectives

Thus, we propose a holistic research program for facilitating Navy-relevant research on biological hotspots grounded in ethical considerations and experiential learning. The characterization and subsequent avoidance of known biological hotspots pertaining to foraging, reproduction, migration, and multi-species aggregations of marine animals would be advantageous when planning Naval exercises. Avoiding these areas would not only reduce the direct impacts on the organisms, but also potential negative cascading effects to coastal communities dependent on ocean ecosystem services such as ecotourism and fisheries.

Results

We were able to provide important field experience to 24 undergraduate students, and all 7 graduate students and postdocs in the Beltran lab. Each trainee was taught and mentored to handle animals, collect data, participate in lab work and undertake self-directed research projects to continue the research efforts. All of the field assistants were financially compensated for their time.

PhD student Danial Palance has collected, read, and synthesized over 300 peer-reviewed research publications into a database detailing publication metadata, taxa examined, hotspot type, and location of each study to create a comprehensive conceptual framework for marine hotspots. The review is now being drafted into a manuscript for publication.

An Assistant Project Scientist, Taiki Adachi, drafted a manuscript on the effect of body condition on daily time-activity budget in wild elephant seals. He used cutting-edge biologging tools to show that northern elephant seals in poorer body condition have higher energetic costs and less efficient foraging and sacrifice sleep to spend more time foraging more when they are at higher risk of predation.

PhD student Allison Payne been able to facilitate dozens of workshops that focus on preventing sexual harassment and sexual assault in field settings. These workshops have given her the opportunity to hone her skills in climate setting, conflict resolution, and field safety. She also submitted a manuscript to Proceedings of the Royal Society B titled Summarizing the impacts of biologgers on walkers, swimmers, and flyers. In writing the paper, she mentored a recent post-undergraduate student through the process of compiling a review paper.

Developing Hawaii-based capacity to analyze accelerometry tag data to evaluate responses of marine mammals to disturbance

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Background

The Hawai‘i region is the U.S. Navy's largest and most strategic island base in the Pacific and is part of the U.S. Pacific Fleet which covers 100 million square miles. The Navy Hawai‘i region overlaps with the habitat of >30 species of protected whales (e.g. the endangered false killer whale, *Pseudorca crassidens*), dolphins and the endangered Hawaiian monk seal (*Neomonachus schauinslandi*). It is important to ensure a strong and locally-based science team to monitor the potential impacts of military activities on these species.

Building upon ongoing research conducted by the Marine Mammal Research Program (MMRP) at the Hawaii Institute of Marine Biology (HIMB; University of Hawaii at Manoa; UHM), we have proposed to develop analytical tools and build local Hawaiian capacity to measure both baseline metrics and behavioral responses of marine mammals to stressors using archival tags. Because of its ongoing efforts, the MMRP can ground-truth, expedite and deploy tri-axial accelerometer tags on marine mammal species of particular interest to the U.S Navy.

Objectives

Over the last ~18 months, MMRP at UHM has sought to implement a robust accelerometry tag research program through the hiring of a post-doctoral researcher (Dr. William Gough) with extensive CATS (Customized Animal Tracking Solutions) camera tag experience. Dr. Gough’s objectives have included:

1. Establishing infrastructural elements and broadly-applicable protocols to enhance data storage and management at the MMRP.
2. Collecting and processing new tag data from species of interest such as the humpback whale (*Megaptera novaeanligae*), short-finned pilot whale (*Globicephala macrorhynchus*), false killer-whale, and Hawaiian monk seal.
3. Mentoring graduate and undergraduate students from both UHM and nearby academic institutions.
4. Leading tag data training exercises for various audiences within the Hawaiian community.

Methods

In the lab, Dr. Gough has spent time implementing infrastructure to enhance the capacity of the MMRP to store and access data. Dr. Gough has also assisted in multiple field efforts to collect novel CATS tag data on species of interest to the Navy. Finally, Dr. Gough has performed various types of CATS tag data training, from in-person one-on-one mentoring of students to small group intensive workshops and classroom seminars.

Results

Dr. Gough has established a section of the MMRP lab devoted to tag setup, calibration, diagnostics, and data processing. He has also contacted representatives from the Animal Telemetry Network (ATN) and developed a project structure that can be used to store raw tag data (now) and processed tag data (in future) to be made available to the wider research community. In the field, Dr. Gough has implemented protocols to maintain data integrity and completeness for future processing and analysis.

Dr. Gough has assisted on six data-collection expeditions including: 1) a 10-day field trip to Lanai in November 2022, 2) a 12-day field trip to Maui in February 2023, 3) individual days on Oahu throughout June-July 2023, 4) another 10-

day field trip to Lanai in November 2023, 5) a 15-day field trip to the island of Hawaii (Big Island) in February 2024, and 6) an 8-day visit to the Okinawa Churaumi Aquarium (Okinawa, Japan). Work on Lanai helped produce 6 deployments on short-finned pilot whales (~56 data hours). Work on Maui and Big Island produced 58 tag deployments on humpback whales, including non-neonate calves, juveniles, mothers, and male escorts. Efforts on Lanai and Maui also resulted in two tag deployments on false killer whales, the first time that these data have been collected for this species. Work on Oahu was in support of a NOAA PIFSC team as they successfully deployed and recovered a CATS tag on a Hawaiian monk seal. Work at the Okinawa Churaumi Aquarium resulted in morphometric scans of 8 false killer whales in addition to preliminary time-synced CATS and ECG heart-rate tag data for breath-hold dives in a single false killer whale.

Dr. Gough has been working closely with multiple MMRP PhD graduate students (Jens Currie, Brijonnay Madrigal, Kirby Parnell, Augusta Hollers) to process and analyze tag data, with a focus on quantifying behavioral signatures that can be used as biological baselines and related to anthropogenic environmental changes or disturbances. In addition to each graduate student’s individual research goals, Dr. Gough has helped to foster a collaborative environment between the graduate students and worked to integrate applicable metrics and knowledge across projects.

Dr. Gough has also trained five MMRP undergraduate interns to work with CATS tag data in a variety of ways. Two of these interns, Trevor Thompson and Cameron Nemeth, have been mentored by Dr. Gough for university credits at UHM. With Dr. Gough's guidance, Cameron is leading a project on humpback whale turning capacity, with the goal of producing a peer-reviewed publication within the next ~6 months.

Beyond the MMRP, Dr. Gough has spent a portion of his time training members of the Hawaiian community on the use of tags and accelerometry data. This includes high school students taking part in the Summer Marine Mammal Intensive Learning Experience (SMMILE) run annually by members of the MMRP, undergraduate students taking a course led by Dr. Bejder, and members of the local research community through a four week tag data processing workshop similar to one previously co-led by Dr. Gough in 2020. Dr. Gough is also on the graduate committee for University of Alaska Fairbanks PhD graduate student Jessie Hoffman as an expert in accelerometry tag data. Dr. Gough has also assisted members of the NOAA Hawaiian Islands Humpback Whale National Marine Sanctuary group as they process and analyze CATS tag data collected independently on humpback whales.

Dr. Gough has presented his research as a department seminar at UHM and as an oral talk at the Society for Integrative and Comparative Biology’s annual meeting in January 2024. He is also working on three first-author manuscripts, with ~8-10 co-authored manuscripts in progress.

Sponsor: ONR
Status: ONGOING

Determining the energetic cost of locomotion in pilot whales and killer whales

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Background

Little is known about the basal metabolic rate or energetic cost of locomotion in larger cetaceans. Understanding the basal metabolic rate of larger cetaceans is necessary because measuring total daily energy expenditure is difficult and estimates are often made by scaling basal metabolic rate. To understand movement, kinematic bio-logging tags have been deployed on many cetacean species, allowing the fine-scale measurements of activity during swimming and diving. However, correlating activity measured by the tags with measurements of energy use is necessary to better predict the energy expenditure of different behaviors. Because these measurements require trained animals, we focused on two of the larger species in professional care: the short-finned pilot whale (*Globicephala macrorhynchus*) and the killer whale (*Orcinus orca*). These results will allow researchers to estimate energy utilization from kinematic tag deployments in order to improve Population Consequences of Disturbance (PCoD) models, which are used to understand human impacts on marine mammal populations (Pirotta et al., 2018).

Methods

This project involves combining respirometry (the measure of exhaled gases) and kinematic tags during submerged swim trials. Respirometry allows us to measure oxygen consumption, and thus estimate metabolic rate. Measuring the metabolic rate before and after swimming enables us to determine both basal metabolic rate and the locomotion costs of different activity levels (measured via the tag). We are using kinematic tags designed at the University of Michigan that measure speed via a pin-wheel and contain tri-axial accelerometers, magnetometers, and gyroscopes, in addition to pressure and temperature sensors (Gabaldon et al., 2019). Each swim trial takes place following an overnight fast. The whale breaths into the flow meter for 10-15 minutes to measure basal metabolic rate, followed by 3-3.5 minutes submerged swimming, followed by 12-15 minutes of breathing into the flow meter to measure oxygen consumption during recovery. The difference between the basal and recovery oxygen consumption is attributed to the locomotion cost of swimming. We are working with killer whales at Marineland (Antibes, France) and Loro Parque (Canary Islands, Spain), and pilot whales at SeaWorld (San Diego, USA).

Objectives

The first main objective was to design and calibrate a flow meter capable of measuring metabolic rates in larger cetaceans. Building on the design for smaller cetaceans (Fahlman et al., 2015), we worked with engineers at adm+ engineering to build a larger version capable of handling the subsantial flow rates produced by larger cetaceans—in excess of 250 liters per second. We calibrated it using a custom built flow chamber, using pitot tube and vane anemometers to determine flow rate. The second main objective was to conduct experimental swim trials. This allows both the measurement of basal metabolic rate and the correlation of locomotion cost with activity level. This builds off of our work with common bottlenose dolphins (*Tursiops truncatus*; Allen et al., 2022). This activity-energetics correlation can then be applied to tags deployed on cetaceans in the wild.

Results

We have designed, built, and calibrated the larger flow meter. Across the two killer whale facilities, we have conducted 35 useable trials among 5 killer whales (1800-4000 kg body mass). Results are preliminary, but average basal metabolic rates are 1.08 x Kleiber (the "mouse to elephant" equation generated from terrestrial mammals; Kleiber, 1975). This suggests that the basal metabolic rate of killer whales is close to values predicted for similarly sized terrestrial mammals. Analyses relating activity level to locomotion costs are ongoing. Due to constraints on animal access, we have been unable to collect data from pilot whales. We plan to collect basal metabolic rate measurements from pilot whales at some point in 2024.

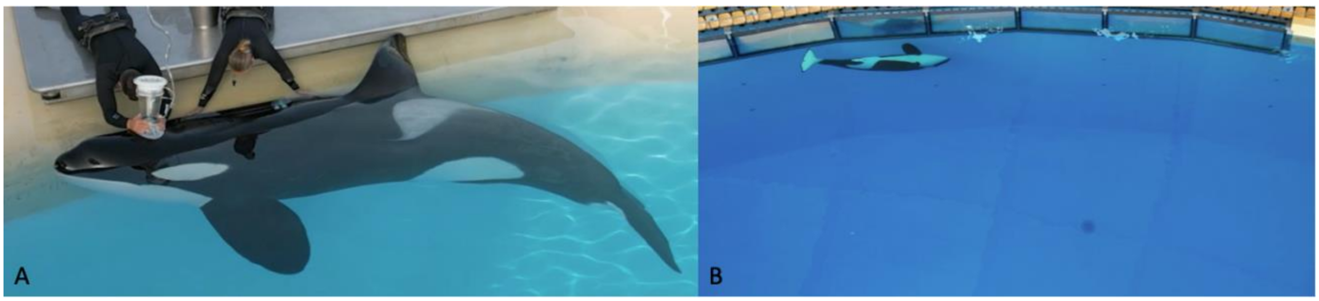

Figure 1. A) A killer whale breathing into the flow meter while at rest. The whale is wearing a kinematic tag on its dorsal side. B) Killer whale swimming a 3-minute submerged swim trial, before returning to breath into the flow meter.

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Towards an understanding of the cumulative effects of multiple stressors on marine mammals: Interdisciplinary working group with case studies.

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Background

Marine mammals are exposed to multiple stressors, including underwater sound, chemical pollution and interaction with fisheries, and assessment of their cumulative impacts is a top priority in marine ecology. Assessment of cumulative effects has been required by U.S. regulations since the 1970s. Recognizing its importance, the US National Academies formed a panel to report on approaches to studying cumulative effects in marine mammals (NRC 2017). In their comprehensive review of the state of the science, NRC (2017) concluded that current approaches have little predictive value. The problem is hard because many individuals are exposed to different stressors at varying levels over long time periods; these stressors may interact to exacerbate or even inhibit each other's effects. The NRC (2017) panel developed a framework to analyze the Population Consequences of Multiple Stressors (PCoMS) and provided a working definition of interaction between stressors. It concluded that interactions are hard to predict but are more likely if stressors share a common pathway for their adverse effect.

Objectives

The overall objective of the project is to advance understanding of the cumulative effects of multiple stressors on marine mammals through case studies and new technology in collaboration with an expert working group (WG).

Methods

Our approach to advance this topic relies on an interdisciplinary expert WG supported by dedicated researchers and several sub-teams, with each sub-team focused on either carrying out a specific case study or evaluating a new technology. The WG and WG researchers focus on developing general methods to quantify interactions between stressors and assess their cumulative, population-level effects.

The case studies funded under this project are: (1) Atlantic bottlenose dolphins exposed to chemical stressors – we have conducted an experiment to test whether the response of dolphins to vessel approaches depends on how much the chemicals have impacted their health; and (2) North Atlantic right whales – our study investigates how prey availability interacts with sublethal health effects of entanglement, vessel strike and noise. We are also collaborating with a separately funded case study on northern elephant seals. Each case study has excellent data on exposure to and/or effects of stressors. Our key innovation is the effort to estimate the effects of interactions among multiple stressors. We are also applying new technology using epigenetic biomarkers to examine evidence for age acceleration in relation to exposure to multiple stressors and to identify biomarkers for health conditions.

Results

As well as guiding the work of the case studies, the WG has produced two major outputs which significantly advance this topic. The first (Tyack et al. 2022) is policy-focused and advocates a cumulative risk approach to managing multiple stressors on populations, suggesting steps for integrating assessment of cumulative risk from multiple stressors into ecosystem-based management. The other (Pirotta et al. 2022) focuses on the combined effects of multiple stressors, proposing an "assumption spectrum" for their analysis, from data-driven through to process-driven analytical approaches, highlighting the role of these approaches in a framework to assess and manage the combined effects of multiple stressors.

Bottlenose dolphins

Forty-eight controlled vessel approaches were conducted on 43 bottlenose dolphin individuals in Barataria Bay. Each of these individuals had its health assessed either prior to this study or in 2023 when 24 dolphins were encircled in net corrals for health assessment. The surface observation data collected during the vessel approaches were scored by a panel of eight experts who assessed whether the animals changed their behavior coincident with the vessel approach. An analytical framework has been developed to relate health and responsiveness as assessed by the experts and is being applied to the complete dataset. Nine of these dolphins were carrying Dtags at the time of the vessel approach, and these data will be examined for subtle, short-term responses that may be missed in the analysis of the surfacing data.

North Atlantic right whales

Model development for right whales is based upon the extensive historical data available for this species and associated stressors. A Bayesian state-space PCoMS model for survival and reproduction (Pirotta et al. 2023) is parameterized to include the effects of entanglement, vessel strikes and an index of annual prey abundance as stressors. Model outputs indicated that blunt and deep vessel strike injuries and severe entanglement injuries had the largest effect on health. Prey abundance had a smaller but protracted effect on health across individuals. The model has been, and continues to be, further developed; for example, whale length photogrammetry measurements have now been integrated into the model (Pirotta et al. 2024). Specifically, the asymptote of calving probability is modelled as a function of the cube of length, as an indicator of body volume. This relationship helps explain the decline in calving over the last few decades (Pirotta et al. 2024). In parallel, work on a spatially explicit version of the model is progressing.

Northern elephant seals

A conceptual framework for an elephant seal PCoMS model based on an extensive tracking and sightings database has been developed. The model operates on an annual time scale using cross-sectional data from two time points in the annual life cycle of a female elephant seal and is designed to capture changes that happen during the two foraging trips that take place over the annual cycle. An initial model combines morphometric measurements, physiological measurements relating to hormonal changes, and demographic data. We are now planning how to include stressors into this model.

Epigenetics

Epigenetics, specifically DNA methylation, allows animal age to be estimated from blood or remotely sampled skin. Epigenetic markers have been identified for chronological aging using blood and skin of known-age Navy dolphins (Barratclough et al. 2021), and a chronological clock based on wild skin samples has now been established for wild dolphins from different locations (Barratclough et al. submitted). These data also form a case study within a review of analysis of DNA methylation data for wildlife. We are also quantifying the relationship between methylation and health metrics, which would allow us to use methylation data to infer the health status of sampled individuals.

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Finding patterns within the noise: Modelling baleen whale response to multiple stressors through replicate physiological sampling of gray whales

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Background

The overall goal of this project is to disentangle many of the intrinsic and extrinsic stressors that impact the physiology and ecology of baleen whales in order to better identify and evaluate the effects of individual anthropogenic sources of disturbance. Additionally, we aim to investigate how impacts of stressors on individual whales may scale-up to population-level consequences.

Objectives

(1) Distinguish between relevant and non-relevant stressors to a baleen whale.
(2) Quantify temporal thresholds and recovery rates of whales to stressors.
(3) Improve predictions of how multiple types of stressors will impact different demographic groups.
(4) Describe the population consequences of individual exposure to different stressors.
(5) Describe how relevant stressors interact to cause different physiological responses in individual
baleen whales.
6) Inform the development of the population consequences of multiple stressors (PCoMS) framework through novel data collection.

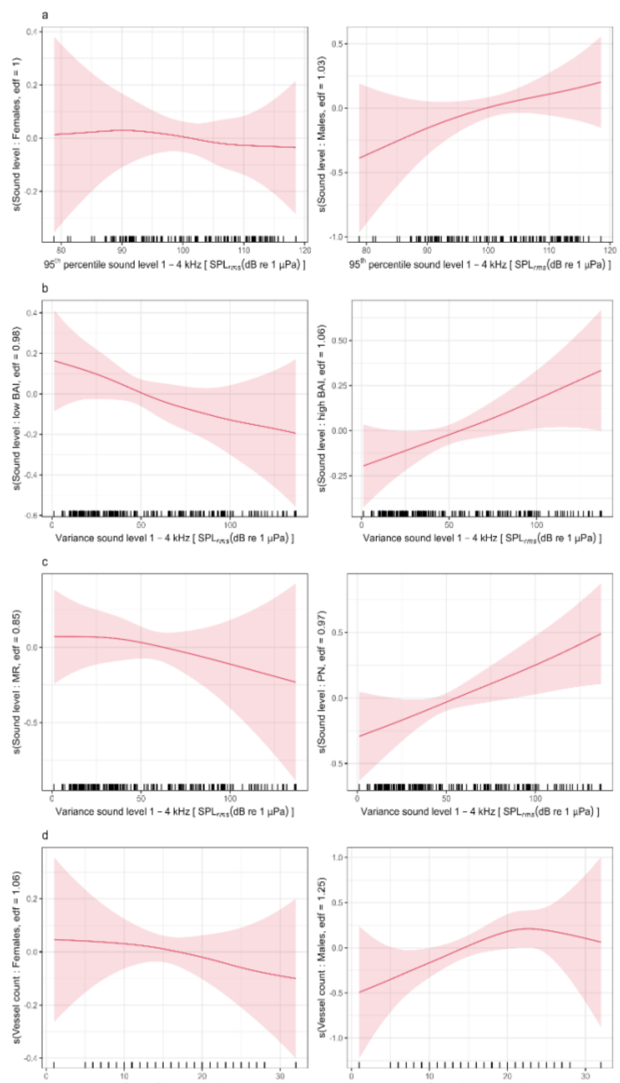
Methods

Since 2016, we have studied the ecology and physiology of Pacific Coast Feeding Group (PCFG) gray whales that forage during summer and fall months off the coast of Oregon, USA. Between 1 June and 15 October each year, when weather conditions allow, we survey for PCFG gray whales in a 5.4m RHIB; when whales are encountered, we collect photo-identification data, conduct drone overflights, and collect fecal samples opportunistically. Individual whales are identified through photo-ID analysis and compared to existing catalogs to obtain sex and age information. Photogrammetry analysis is performed on individual whales filmed using drones to measure total length and body condition. Fecal samples are analyzed for apparent concentrations of hormone metabolites of the glucocorticoid cortisol (GC), progesterone, testosterone, and tri-iodothyronine (T3, the active form of thyroid hormone). From 2017 to 2022, two hydrophones were deployed in the study region to sample 10–13,000 Hz underwater sound. One hydrophone was deployed near the Port of Newport in a high vessel activity area, and another 17 km north within a protected Marine Reserve. We assessed the association between GC content and metrics of exposure to sound levels and vessel traffic at different temporal scales, while controlling for contextual variables such as sex, reproductive status, age, body condition, year, time of year and location. We developed a Bayesian Generalized Additive Modelling approach that accommodates the many complexities of these data, including non-linear variation in hormone concentrations, missing covariate values, repeated samples, sampling variability, and some hormone concentrations below the limit of detection. To assess how stressors may impact the vital rates of this population, we are developing a Bayesian state-space model to link individual health and length (informed by photogrammetry data) with calving probability within the Population Consequences of Disturbance (PCoD) framework.

Results

Our dataset of PCFG gray whales includes 1271 sightings of 206 individual whales, 359 fecal samples of 96 individuals, and 832 drone flights of 150 individuals. Soundscape analysis revealed that median sound levels (50 – 4,000 Hz) were related to noise from various vessel types and sizes, which were up to 6 dB higher at the Port of Newport, with greater diel variability compared to the Marine Reserve. Sound levels were highest near the Port of Newport between 5am and 6pm, likely driven by nearby vessel traffic (not wind speed or swell height). Models estimating relationships between GC concentrations and exposure to stressors (vessel counts and soundscape metrics of different frequency bands and temporal periods) showed large variability, but emerging patterns indicate a strong context-dependency of physiological variation, i.e., depending on sex, body condition and proximity to a port (Fig. 1). Our results highlight the need to control for baseline hormone variation related to these contextual variables, which otherwise can obscure the functional relationship between GCs and stressor exposure. Preliminary results of the PCoD model indicate that a female's probability of successfully calving is positively associated with her length and health states in the previous year. The next step of these analyses will involve the compilation of these data to inform an underlying stress state, which could then be related to stressor exposure and, in turn, affect health and/or calving probability.

Figure 1. From Pirotta et al. 2023 (Fig. 4). Estimated relationships between gray whale fecal glucocorticoid (GC) metabolite concentrations and a subset of stressor variable combinations. In (a), interaction between sex and the 95th percentile of sound levels in the high-frequency band (1 – 4 kHz) between 5 AM and 6 PM in the day prior to sampling; GC concentrations increased with sound level in males (right panel), but not in females (left panel). In (b), interaction between Body Area Index (BAI) and the variance of sound levels in the high-frequency band (1 – 4 kHz) in the 24 hr prior to sampling; GC concentrations decreased with sound level for individuals with low BAI (left panel), and increased with sound level for individuals with high BAI (right panel). In (c), interaction between closest hydrophone location (Marine Reserve, MR, and Port of Newport, PN) and the variance of sound levels in the high-frequency band (1 – 4 kHz) in the 24 hr prior to sampling; GC concentrations increased with sound level at PN (right panel), but not at MR (left panel). In (d), interaction between sex and the count of charter vessels in the day prior to sampling; GC concentrations showed an increasing trend with the count of charter vessels in males (right panel), but not in females (left panel). Each panel reports the posterior effect, with the shaded areas representing the 95% credible intervals. The effective degrees of freedom (edf) of the spline are also reported on each panel.



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Validation of the bone conduction hypothesis for baleen whale hearing

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Background

The mechanism by which baleen whales receive low-frequency sounds is not adequately understood.

Previously, we hypothesized that sound waves propagate through the water toward the animal and set the skull into motion. We envisioned resonant vibrations of the bulla and ossicles with respect to the base of the skull.

Mechanical models of the bulla, pedicles, and the ossicles, point to a view of the skull as an "antenna" that captures sound energy and feeds it into the motion of the bulla relative to the periotic bone and skull. Since the periotic bone houses the inner ear, it is coincident with the ossicular chain at the stapes footplate, while the malleus is fused to the tympanic bulla.

Objectives

Test the validity of our bone-conduction hypothesis as the primary mechanism for low-frequency auditory sensitivity in mysticetes.

Gather vibroacoustic measurements from an instrumented gray whale skull underwater and compare them to our finite element modeling simulations.

Methods

A juvenile gray whale specimen (LACM 97758, total length=540 cm, weight=869 kg) was obtained from the Natural History Museum of Los Angeles County. After removing most of the soft tissue superficial to the natural skull, the specimen was CT-scanned and segmented to create a volumetric reconstruction of all bony components. That volume was then used to build and execute finite element models of vibration, and also to 3D print a plastic, life-size replica of the skull.

We tested our hypothesis by conducting vibroacoustic experiments performed on specimens underwater and in air. Specimens tested include: (1) a natural juvenile gray whale skull (LACM 97758); (2) a plastic replica of that same gray whale skull; and (3) an adult gray whale skull (LACM 84202; in air only).

To test the validity of our bone conduction hypothesis, the natural gray whale skull was instrumented with 7 accelerometers distributed across the base of the skull and bullae. The apparatus was then submerged in water and exposed to low-frequency sounds projected from a transducer 1 m anterior to the skull's center of gravity. The frequency range was determined by the characteristics of the transducer and by the peculiarities of the pool, between 170 Hz and 1000 Hz.

Results

In this study, we tested the bone conduction hypothesis using vibrational analysis performed on the skulls of gray whales.

Sounds were projected underwater toward skulls instrumented with accelerometers on the base of the skull and the tympanic bullae (Figure 1).

Figure 2 shows the frequency response functions of the velocity amplitude for the mediolateral axis and the anteroposterior axis. They demonstrate that skull vibrations, due to interactions with the sound waves in the water, result in the differential motion of the bullae that is significantly amplified, relative to the skull, and therefore the periotic.

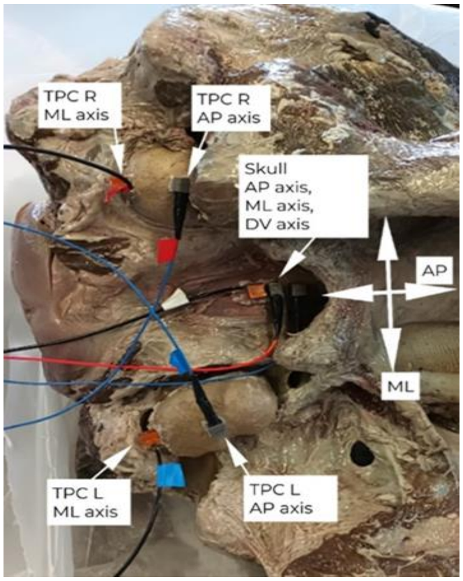

Figure 1. Gray whale skull and TPCs instrumented with seven accelerometers. Each accelerometer is oriented to measure along a specific single axis: AP = anteroposterior (rostrum-tail axis), ML = mediolateral (left-right), DV = dorsoventral (back-belly axis). Some visible evidence of decomposition is indicated by discoloration in the soft tissues.

The long wavelength low-frequency sound waves in the water set the skull and embedded periotic bones into motion, and these vibrations are in turn transmitted to the pedicles, the bullae, and then the ossicular chains. The suspension of the bullae from flexible pedicles appears to be the key feature.

Motions of the bullae relative to the skull base were detected by recordings from multiple accelerometers (Figure 2). This relative motion drives the ossicular chain to activate the stapes footplate, showing that the bone conduction hypothesis is consistent with these results.

In addition, we compared the anatomic geometry of the skull and auditory structures in multiple mysticetes. Comparative morphology provides an opportunity to understand how structure relates to function across species.

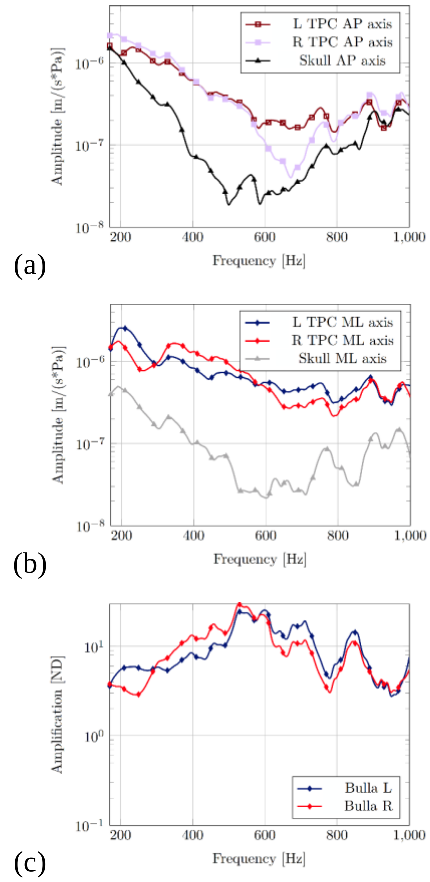

Figure 2 – Frequency Response Functions. (a) velocity of each bulla relative to the base of the skull for mediolateral direction. (b) velocity of each bulla relative to the base of the skull for anteroposterior direction. (c) Relative amplification of mediolateral velocity. Experiment on 9/13/2023 on the natural skull under water, ensonified head-on.

Our finite element analysis tools simulate biomechanical and vibroacoustic functions, indicating that the skull functions as the "external ear" of the auditory apparatus in all three species of mysticetes we have examined (fin, minke, & gray).

Based on all this evidence, we conclude that the bone conduction hypothesis for mysticete sound reception is consistent with the experimental data, and therefore can be considered validated.

Auditory brainstem response and evoked response measures of the upper limit of hearing in the minke whale (*Balaenoptera acutorostrata*)

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Background

Frequency-specific sensitivity to sound, typically characterized as an audiogram, is the fundamental piece of information necessary to evaluate the potential for anthropogenic sound to affect marine mammals. Of the roughly 130 species of extant marine mammals, only one major phylogenetic group has no representative audiogram – the mysticete whales. Because of practical limitations on capturing and holding mysticetes for behavioral audiometry, evoked potential audiometry likely offers the only means for directly measuring hearing sensitivity in mysticetes. Evoked potential audiometry estimates hearing thresholds by measuring the relationship between acoustic stimulus levels and the amplitude of auditory evoked potentials (AEPs), which are electrical signals produced by the brain in response to sound. The approach remains hampered by animal access and animal size; AEPs become increasingly difficult to record as the distance between skin-surface recording locations and the site of the AEP source increases. Therefore, small mysticetes that predictably utilize coastal waters provide the best chance for catch-and-release opportunities, and developing and successfully implementing AEP hearing measurements.

Objectives

The objective of this proposed effort is to catch and release adolescent minke whales (*Balaenoptera acutorostrata*) and obtain AEP hearing thresholds in order to produce a mysticete audiogram.

Methods

An oceanic catch-and-release site (CARS) to temporarily hold adolescent minke whales was constructed off the coast of Lofoten, Norway (Figure 1). Adolescent minke whales were targeted because their small size (3-5 m; ~240-1050 kg) makes them amenable to AEP hearing tests. Adolescent minke whales also have a predictable northward migration along the coast of Norway where rocky barrier islands make a CARS feasible.

Barrier and guide nets ranging from 100 m to 600 m length with depths of 40 to 60 m were deployed to guide migrating whales between two small islands. A catch basin was created utilizing the channel between the islands; a barrier net was stretched across the west end of the channel to block exit from the basin, and additional nets were used to create a partial barrier with a 40-m opening to the basin on the east end. When a whale entered the basin, the opening was closed by pulling a net across it and securing it to another stationary barrier net.

An aquaculture pen (10,000 m^3 volume; 90-m circumference) consisting of a reinforced PVC ring with inner and outer nets was co-located with the catch basin. The outer net was modified with a 10 m (w) x 15 m (h) rectangular net door. Whales caught in the basin were corralled into the aquaculture pen with two small boats and a 100-m corralling net. During corralling, the aquaculture pen door was opened and the inner net was placed on the sea floor. Once a whale was corralled through the door and into the aquaculture pen, the net door was closed and the inner net pulled to the surface and secured to the aquaculture pen frame. A roller system was subsequently used to slowly pull the inner net to the surface and constrain the whale in a net hammock for the hearing test (Figure 2).

Sounds for the hearing tests were projected from an underwater transducer placed near the whale at 0.5 m depth. Acoustic stimuli consisted of clicks, chirps (short-duration frequency upsweeps, 2.8-32 kHz), or repetitive tone-bursts. Evoked potentials were recorded using a three-electrode montage with signals amplified (94 dB) and filtered (either 0.3-3 kHz or 0.03-3 kHz) prior to being digitized (16-bit resolution) at 250 kHz. Stimulus projection and AEP recordings were controlled via the Evoked Response Study Tool (Finneran, 2009).

Results

The project is entering its fourth year of a four-year effort. In the first year, the CARS was demonstrated to be effective at catching and temporarily holding minke whales. In the second year, a minke whale was caught in the basin and corralled into an aquaculture pen for a hearing test. The whale was released prior to testing because of an unexpected tonic immobility and tachypnea. Modifications to the handling procedures were made to address the stress response during future captures, and in the third year, two minke whales were caught and subjected to hearing tests. Parameters for recording the auditory brainstem response (ABR) were determined in the first whale, and the first-ever recording of a mysticete ABR was completed. A comparable ABR was also recorded in the second whale, along with information on the frequency range of hearing obtained with repetitive tone bursts. The minke whale ABR was characterized by a dominant wave at a latency of ~9 ms and amplitudes ranging from 450-575 nV. The second whale was unexpectedly found to be sensitive to sound frequencies of at least 45 kHz and potentially as high as 90 kHz. The whale was also sensitive to sound at 4 kHz, the lowest frequency tested.

Figure 1. Aerial view of the CARS off Lofoten, Norway.

Figure 2. Minke whale constrained in the net hammock for the AEP hearing test.

References

Finneran JJ (2009) Evoked Response Study Tool: a portable, rugged system for single and multiple auditory evoked potential measurements. Journal of the Acoustical Society of America 126 (1):491-500. https://doi.org/10.1121/1.3148214

NEUROBIOLOGICAL AND PHYSIOLOGICAL MEASUREMENTS FROM FREE SWIMMING MARINE MAMMALS

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Background

Diving mammals must function effectively during extended periods without oxygen. To achieve this, they exhibit dramatic cardiovascular responses to diving, including intense bradycardia and major re-distribution of blood flow. Despite these circulatory adjustments, marine mammals can routinely experience extreme hypoxemia even during normal foraging dives. How tissues tolerate regular hypoxia and how diving mammals avoid acute deleterious effects of low oxygen is currently unknown but may be managed by hemodynamic adjustments to conserve the available O_2. As management of available O_2 determines both dive duration and activity levels that can be maintained, improved understanding of the physiological consequences during diving will improve our understanding of observed behavior.

Objectives

Non-invasive sensors, such as near-infrared spectroscopy (NIRS), developed for measuring metabolic activity of organs in humans, provide new opportunities for understanding how mammals manage O_2 during diving. In this study we aimed to develop an animal-borne NIRS system that can function on free-ranging small cetaceans. Enabling studies to investigate the physiological adaptations involved with diving and to assess how stress, e.g. exposure to sound, alters physiology and diving capacity. We divided this project into phases:

1) Characterization of light propagation and transmission in the near IR spectrum for bottlenose dolphin tissues at multiple sites on the body.

2) Upon completion of 1 and 2 we built a wearable NIRS bio-logging tag and tested this in the bottlenose dolphin during spontaneous breathing, static dives and while swimming to investigate hemodynamics.

Methods

1) Tissue light propagation and transmission

We conducted experiments to characterize parameters of light propagation (scattering and absorption coefficients and scattering phase function) from brain, muscle, and chest in the bottlenose dolphin at two managed care facilities (OV; Mirage, Las Vegas). We used both frequency-domain NIRS and time-domain NIRS to calculate scattering and absorption coefficients.

2) Design, fabrication and testing of **a near-infrared spectroscopy bio-logging tag**

The electronics from single LED/receiver Artinis system (PortaLite mini) were integrated with custom movement and sound sensors (IMU, speed, depth, and a single hydrophone) into a biologging tag that was secured to the animal with suction cups. We conducted experiments with dolphins housed in managed care (OV and Dolphin Quest-Oahu (DQ-O)) to verify the tag performance and to investigate hemodynamics and blood oxygenation dynamics in the dolphins. We also tested the NIRS tag with collaborators at University of Santa Cruz to study elephant seals. In both species, we investigated changes in oxygenation and blood flow with changes in respiration, heart rate, behavior and activity at the surface and while diving. This enabled the investigation and validation of the physiological drivers of fluctuations in the NIRS signals at the level of individual tissues.

Results

1) Tissue light propagation and transmission

Our results show that with the current hardware (4 cm distance between light and detector), the tissue properties absorb and scatter too much light to enable cerebral NIRS in the bottlenose dolphin. On the white portion on the chest, on the other hand, absorbs less light. As such, the sensor configuration of the PortaLite mini (detector distance and light frequency/intensity) can be used to measure hemodynamics at this site (Fig. 1). Figure 2 presents simultaneous measurements of ECG and NIRS measurements to demonstrate the viability of this approach.

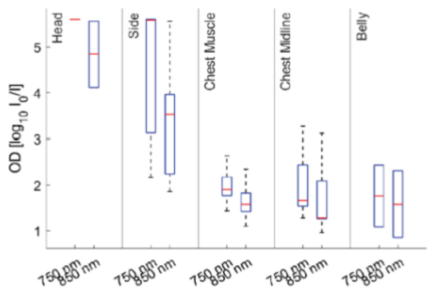

Fig. 1. Optical density at different body locations on a bottlenose dolphin.

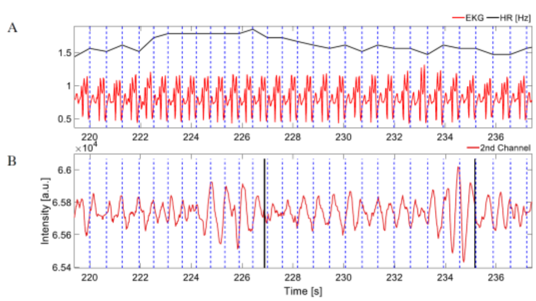

Fig. 2. Simultaneous A) ECG and B) NIRS data from a dolphin. A) red lines: ECG; black line instantaneous heart rate. B) red line: NIRS showing total blood volume changes;
black lines a respiration event. In both A and B: dotted blue line represent onset of systole.

2) NIRS Tag

In the elephant seal, data in translocated animals and during sleep apneas on land provided reliable data on cerebral NIRS (Fig. 3).

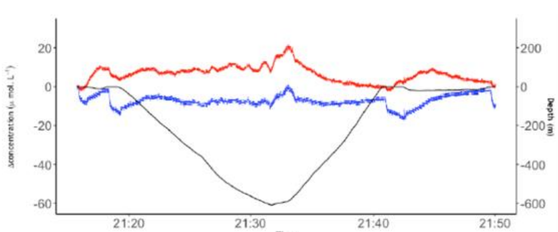

Fig. 4. Data showing dive depth (black) and oxy (red) and deoxy (blue) hemoglobin during a 600 m dive

The biologging tag with the integrated Portalite NIRS system (light/detector distance of 4 cm) was tested with bottlenose dolphins in managed care (OV and DQ-O) during prescribed tasks: quiet breathing, static breath-holds, and while swimming (Fig. 4).

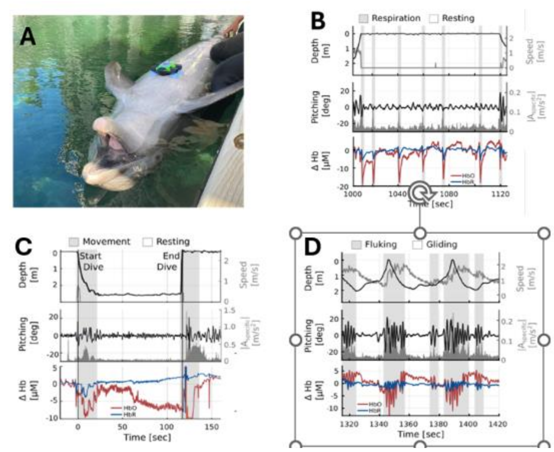

Fig. 3. A) NIRS tag placed on the chest of a bottle nose dolphin, and data during B) spontaneous breaths (quiet breathing) at the surface, C) during static breath-hold, and D) while swimming freely. In panels B-D) top panel: depth (black), and speed (gray); middle panel: pitch (black), and acceleration (gray); bottom panel: oxy (red) and deoxy hemoglobin (blue).

Discussion

This project has resulted in first of their kind non-invasive physio-logging devices that can be used to investigate the biomechanics, biology, and physiology (cardiovascular and cardiorespiratory function) of free-swimming cetaceans, and elephant seals. In the dolphin brain fNIRS measurements in adult bottlenose dolphins may be possible in specific locations at source-detector distances > 100 mm, and at wavelength from 700-900 nm or 1100-1150 nm. Data collected from the chest in the dolphin, showed that continuous measurements of heart rate and oxygenation, were possible, but motion artifacts made the heart signal difficult to detect. In the elephant seal, provided high resolution data and suggest that NIRS can provide the physiological measurement tool.

Central Nervous Lymphatic Structures In Marine Mammals: A Morphological Comparison Of Shallow And Deep Divers

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Background

The peripheral lymphatic system provides a route for fluid transport throughout the body, supporting vital homeostatic functions including removal of metabolic waste and excess fluid, and immune surveillance. The brain and spinal cord, that comprise the central nervous system (CNS), are unique in that they lack classic lymphatic vessels. However, it has been shown that the highly metabolically active brain clears waste products through an alternative mechanism. The recently discovered brain lymphatic system (i.e., glymphatic system) is the analog of the lymphatic system in the brain and derives its name from the network of star shaped Astro-glial cells that help to provide an avenue for cerebrospinal fluid flow into and out of the brain. The glymphatic system plays a key role in CNS health by driving clearance of wastes and excess fluid using cerebrospinal fluid that bathes the tissues of the brain. The glymphatic system also modulates the brain immune response and distributes metabolites and gases within the brain.

Glial cells are a major anatomical component of the blood brain barrier. Vasculature in the brain differs from the periphery in that it is entirely ensheathed by polarized glial end feet, creating a unique compartment called the perivascular space. The donut-shaped perivascular space is a tunnel surrounding all vasculature penetrating the brain that creates a low resistance pathway for the fast transport of CSF deep into the parenchyma and serves as the primary neuroimmune interface between the blood and neural tissues. The glymphatic system is primarily active during sleep cycles, where physiological drivers like the cardiac cycle, respiration, and vasomotion work together to move cerebrospinal fluid through the brain. Glymphatic flow is directly affected by changes in cerebral blood flow, intracranial pressure, and noradrenergic tone, highlighting the interplay between the vasculature and perivascular space in glymphatic function. Dysfunction of glymphatic flow can result in altered neurovascular coupling and brain function, progression of diseases such as Alzheimer’s, and can contribute to the pathogenesis associated with traumatic and ischemic brain injuries. Further, glymphatic clearance can be altered during apnea, with changes in pressure, and during states of low oxygen. Therefore, the brain lymphatic system of air-breathing divers, who routinely experience changes in pressure and hypoxia, could be susceptible to alterations in glymphatic function.

Marine mammals have evolved to be exceptional divers and are ideal models to study the structure and potential range of biological functions of the glymphatic system. A comparative understanding of the glymphatic system morphology among deep and shallow diving marine mammals may provide insights into the pathophysiology of specific diving-related CNS injuries and elucidate novel approaches to enhance CNS clearance of pathological proteins and improve neurorecovery. The characterization of this system may also help to improve CNS health and inform risk mitigation procedures for Navy human divers and marine mammals that perform operations for the US Navy.

Objectives

Our specific goals were to determine if glymphatic system structures, including perivascular spaces and meningeal lymphatic vessels, were present in two different cetaceans (bottlenose dolphins and common dolphins) and to characterize their location relative to draining dural venous sinuses using a combination of immunofluorescence and histochemical staining techniques, and CT angiography, respectively. Our results will demonstrate that multiple species possess all the structures required for a functioning glymphatic system.

Methods

Species-specific collection protocols were created utilizing computer aided design software based upon detailed dissections of frozen cetacean and pinniped specimens collected by the UNCW Marine Mammal Stranding Program. Standard histologic and histochemical techniques were employed to demonstrate the microscopic structure of the glymphatic system across several species. Meningeal lymphatic vessels were identified using highly conserved immunofluorescent antibodies and confirmed using gastrointestinal lymphatic structures as a control. Cetaceans are known to possess highly elaborate vascular structures, and CT angiography and digital reconstruction of intracranial venous circulation will be used to identify additional target areas of interest for future histologic comparisons among deep and shallow divers that may not be captured by standard sampling based upon human and murine models alone.

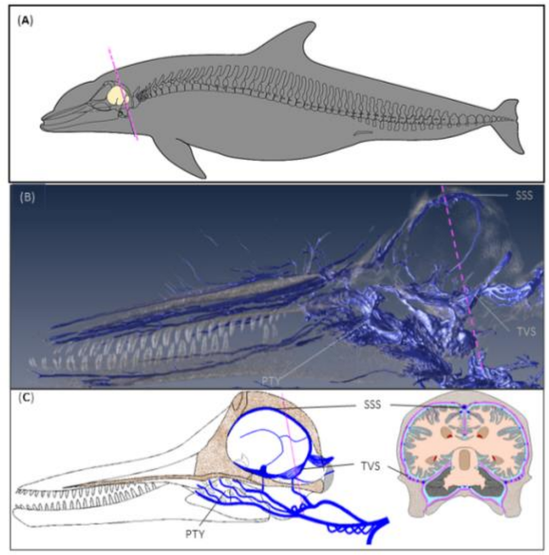

Fig.1. Figure 1. CT angiography digital reconstruction & schematic of the bottlenose dolphin displaying key structural features of the dural venous sinuses that drain CSF and glymphatic system waste.

Results

Together, our results demonstrate for the first time that two cetacean species possess all the structures that are required for a functional glymphatic waste clearance system including a network of perivascular spaces (PVS) surrounding CNS microvasculature, confirmation of meningeal lymphatic vessels, and presence of dural venous sinuses. Further, this functional arrangement highlights the dynamic interplay between the brain's vasculature and perivascular space that plays a vital role in glymphatic clearance within the brain.

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Graduate students: Olivia Jackson, Nathan Nelson-Manley (University of North Carolina Wilmington)

Defining the molecular physiologic impacts of stress on beaked whale hypoxia tolerance implications

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Background

Marine mammals are chronically and acutely exposed to a variety of human-induced disturbance, with the effects from anthropogenic noise a particular concern (Tyack 2009). In particular, vulnerability to the effects of military sonar systems has driven much of the last two decades of Behavioral Response Studies (BRS) across marine mammal taxa (Harris et al. 2018; Southall et al. 2016). Despite a large array of successful BRS (e.g Henderson et al. 2014; Houser et al. 2012; McCarthy et al. 2011; Miller et al. 2012; Sivle et al. 2015; Southall et al. 2016; Tyack et al. 2011) many gaps still remain in our ability to quantify the complex nature of responses to both acute and chronic military sonar events and how these stressors may impact tolerance to hypoxia during diving.

Management decisions are often based on evidence of behavioral response, but the absence of a behavioral response does not rule out other types of response that may impact individual fitness. For example, changes in the levels of stress hormones have been identified as important components for assessing individual health and are a known physiological response during disturbance (Keen et al. 2021). Measuring stress levels is extremely challenging for wild marine mammal species, but understanding disturbance-related changes in physiology that compromise individual breath hold tolerance and overall health is a critical data gap to parametrize population-level models for disturbance (Keen et al. 2021, Pirotta et al. 2018). This presents an **urgent need** to: 1) understand how stress impacts the ability of marine mammals to respond to noise; and 2) define the molecular underpinnings that connect stress and cellular pathways vital to the dive response.

Methods

Cell cultures were established from cetacean biopsies using a modified explant system. To induce stress signaling pathways, cells were treated with hydrocortisone (1 nM to 1 μM). Hypoxia signaling was induced by exposure to 1% oxygen in a humidified tissue culture incubator (37°C). RNA-Seq and western blotting were performed following manufacturer's recommended protocols.

Objectives

Our **overall goal** is to define the relationships between the molecular mechanisms that connect stress and diving. To accomplish this goal, we have combined the powerful tools of genomics and systems biology with novel cetacean cell culture systems to determine the gene expression and cell signaling pathways that connect stress and hypoxia in whale tissues. We hope these experiments will add i) an important layer of understanding to the mechanisms by which cetaceans are able to dive and ii) determine the impacts of stress on the dive response.

Results

We have established a system to create novel cetacean cell cultures from freshly-collected skin biopsies (**Fig. 1A**). This system enables long-term cultures of dermal fibroblasts beyond ~6 month and long-term storage in liquid nitrogen, with a 100% establishment rate for >20 samples across 7 taxa (*Tursiops truncatus*, *Stenella frontalis*, *Globicephala macrorhynchus*, *Kogia breviceps*, *Megaptera novaeangliae*, *Physeter* *macrocephalus*, and *Ziphius cavirostris*). Using this system, we performed RNA-Sequencing on cultures exposed to ambient oxygen or 1% oxygen for 24 hours (**Fig. 1B**). These studies revealed a diminished shift toward anaerobic glycolysis during hypoxia as compared to human fibroblasts (**Fig. 1C**). This was consistent with a sustained mitochondrial volume in the presence of hypoxia, along with sustained oxygen consumption despite low (1%) oxygen levels. In a parallel series of experiments, we established a western blotting protocol to identify activation of glucocorticoid receptor activation in Goosebeaked whale (*Z. cavirostris)* cells (**Fig. 1D**). These systems will be used in our future studies to analyze the relationships between hypoxia and stress (glucocorticoid receptor) signaling in cetaceans.

Conclusions

Overall, our approach will have the positive benefit of contributing to a predictive framework of physiological consequences of acute and chronic stress hormone exposure - particularly as it relates to anthropogenic noise - and how these consequences may contribute to behavioral responses and disease susceptibility in marine mammals by impacting cellular responses to oxygen deprivation and immune activation.

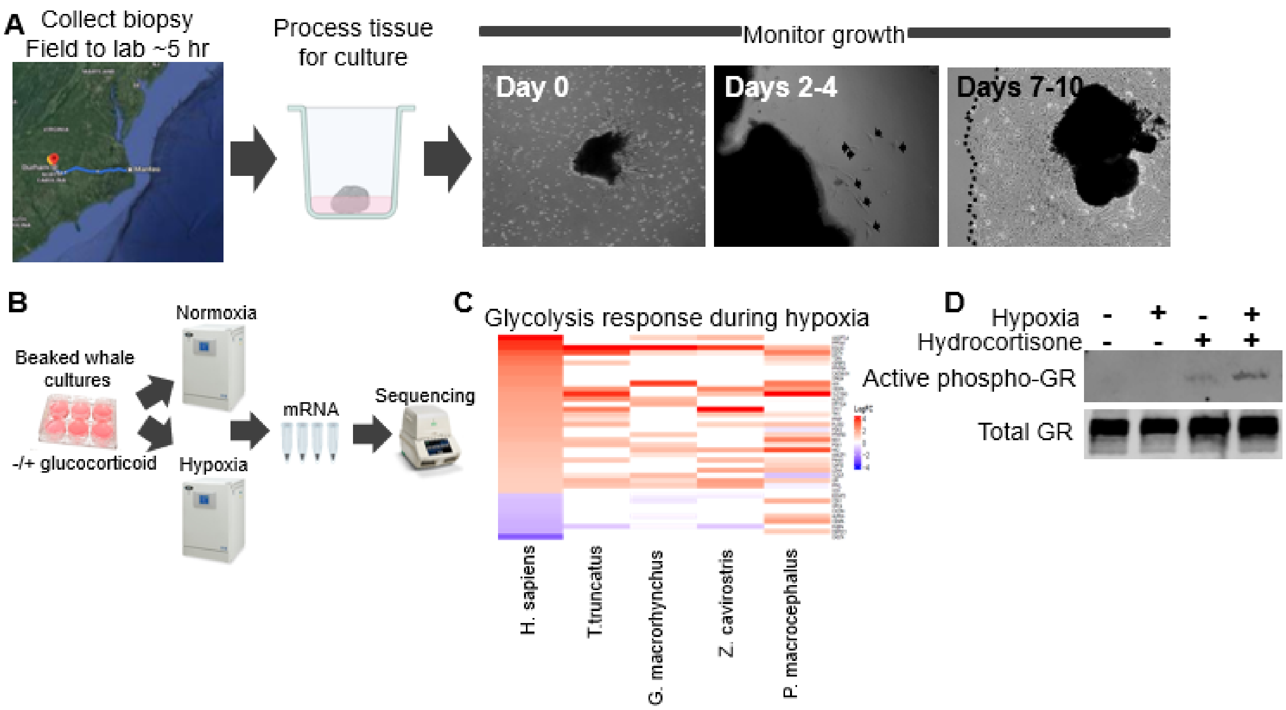

Figure 1. Cell culture systems to study the interplay of stress and hypoxia tolerance in cetaceans. **A.** Schematic of cell line establishment. **B.** Methods workflow for hypoxia studies. **C.** RNA-Seq analysis of glycolysis shift in cetaceans. Human cells are used as a well-annotated reference/comparator. **D.** Western blots showing activation of glucocorticoid signaling in response to hydrocortisone in *Z. cavirostris* fibroblasts.

Modeling Gas Dynamics In Shallow And Deep Diving Cetaceans: Accounting For Changes In Metabolic Rate And Blood Flow Changes

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Background

An increasing number of studies indicate that marine vertebrates are susceptible to gas bubble formation when exposed to stressful situations that alter their dive behavior and/or physiology. For example, the use of naval mid-frequency sonar has been implicated in mass stranding of beaked whales, where the stranded whales exhibited symptoms that were similar to those caused by inert gas bubbles in human divers. Thus, anthropogenic sound may cause behavioral or physiological changes that may harm marine animals, particularly whales and dolphins. Modeling efforts looking at the potential effect of physiological and behavioral changes following sonar exposure on tissue and blood N_2 tension (PN_2) in beaked whales have suggested that behavioral and physiological alterations may increase end-dive blood and tissue PN_2, and thereby increase the risk of gas bubble formation. However, these modeling efforts suffer several limitations including limited knowledge of physiology and anatomy, and the duration of the data sets used to estimate blood and tissue gas tensions. Thus, longer high-resolution data, and revised information about the physiology and anatomy may provide improved estimates of risk.

Objectives

In this project we (Aim 1) revised a previously published model that estimates blood and tissue N_2, O_2, and CO_2 levels. In the revised model, we allowed the diving metabolic rate, and blood flow to vary with changes in changes in activity (acceleration). We used the revised model to: Aim 2) to assess how changes in metabolic rate and blood flow alters tissue and blood gas dynamics in deep and shallow diving bottlenose dolphins, and (Aim 3) from high-resolution long term (5-19 days) dive records from Cuvier's beaked whales. Our goal was to compare model estimates of tissue and blood PO_2, PCO_2, and PN_2 from these different types of tags, and investigate how updated parameters, which alters the relationship between ventilation and perfusion, affect the end-dive blood and tissue gas tensions (Aim 2 and 3).

Methods

Aim 1: Revising the gas dynamics model

The gas dynamics model was revised to include estimates of diving metabolic rates from activity measurements collected using 3-axis acceleration sensors. The Overall Dynamic Body Acceleration (ODBA) was calculated, and the locomotion metabolic rate (LMR) calculated from the calibration equation published in the bottlenose dolphin. The total metabolic cost was then calculated as the sum of the basal metabolic rate (BMR) and LMR. In addition, the metabolic cost due to digestion was added while the animals were at the surface (dolphin and beaked whale) and for dives < 40 min (beaked whale).

Aim 2: Estimating diving metabolic rate and gas dynamics in the bottlenose dolphin and beaked whale

The developed in *Aim 1* was used to estimate diving metabolic rate from Dtag (acceleration and dive depth) recordings in 6 bottlenose dolphins (3 nearshore and 3 offshore). Data for dive depth allowed comparisons between activity/energetic cost and dive behavior and beaked whale. The model assumed 4 different tissues (compartments) with varying metabolic rate and gas solubility (central circulation, muscle, brain and fat). The metabolic rate for central circulation, and brain were adjusted so that all dives remained aerobic. The O_2 stores were made up of lung, blood and muscle (myoglobin) and the cardiac output and blood flow distribution adjusted to assure that most dives were within the calculated aerobic dive limit (cADL). We used the estimated diving metabolic rate for each dive and the available O_2 before each dive to calculate a varying index of aerobic capacity for each dive. As both the LMR and O_2 store varied dynamically, we called this index the dynamic ADL (dADL).

Results

Results from deep diving bottlenose dolphin showed that the dolphins spent more energy (higher ODBA) during ascent and at the surface as compared with descent and the bottom phase (Fig. 1). The cADL ranged from 10.3-38.5 min in near- and off-shore dolphins, and the dADL was 5.2 ± 1.1 min (range: 3.9-6.1 min) and 15.1 ± min (range: 10.8-22.5 min). In the offshore dolphins, the dADL decreased with dive depth as the metabolic effort increased with depth, from 18.7 ± 3.4 min, to 11.1 ± 0.4 min for shallow (< 30 m) to deep dives (> 100 m).

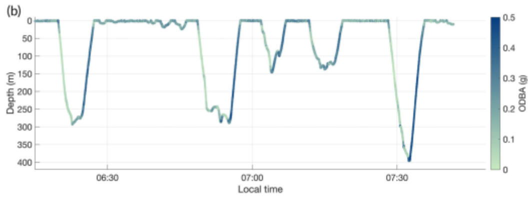

Fig.1. Partial dive record in a bottlenose dolphin show dive depth and ODBA (an index of metabolic effort).

In the beaked whales, 8 dive records ranging from 4.1-19.2 days were used. The deepest and longest dive recorded were 1952 m and 162.9 min, respectively. Dives were separated into short (S < 60 sec [1 min]), intermediate (I ≤ 1050 sec [17.5 min] and I > 60 sec), long (L ≤ 2000 sec [33.3 min] and L > 1050 sec), and extreme (E > 2000 sec) dive durations based on dive behavior. Foraging dives were mainly < 40 min (Fig 2), and it was assumed that animals digested food during the non-foraging dives.

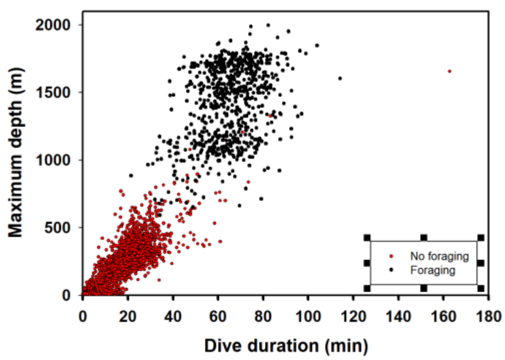

Fig.2. All dives recorded from the 8 beaked whales, separated into foraging and non-foraging dives.

The dADL increased for dives up to 2000 sec (33.3 min) and then decreased, as the model assumed a lower LMR for longer dives, Fig. 4). The model showed that even dives shorter than the bADL, cADL, or dADL, the muscle ran out of O_2.

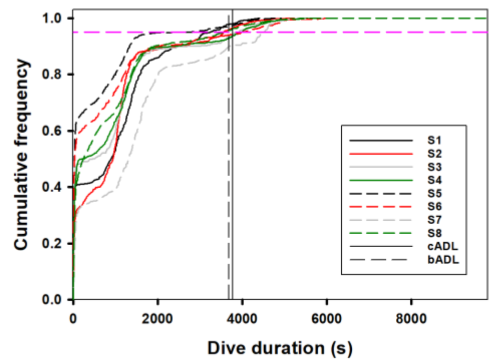

Fig.3. Cumulative frequency against dive duration for each for each individual animal Estimated average behavioral (bADL, solid vertical line) and calculated ADL (cADL, broken vertical line) are shown and also the 95% cumulative frequency (broken pink line).

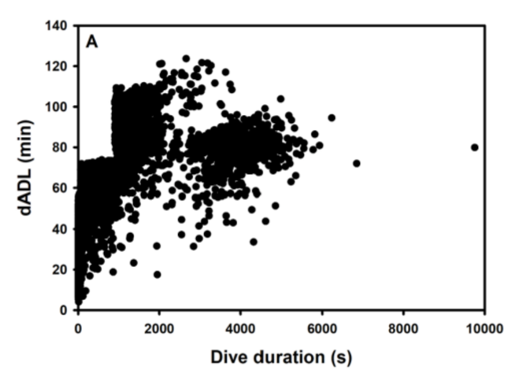

Fig.4. Dive duration against dynamic aerobic dive limit (dADL) in beaked whales.

Discussion

The revised model allowed us to estimate the metabolic requirements in the bottlenose during shallow (near-shote) and (off-shore) deep dives, and also in beaked whales. In the dolphin, all dives remained aerobic. In the beaked whale, on the other hand, the muscle compartment ran out of O_2 for many dives, even those well within the differently estimated aerobic dive limits. Despite an extremely low diving metabolic rate (1.23 ml O_2 min^{-1}), beaked whales may perform a large number of dives anaerobically, which may result in accumulation of CO_2 and lactate. As elevated CO_2 has been shown to enhance gas bubble formation, this may explain their susceptibility to formation of gas emboli and strandings when exposed to man-made stress.

BALEEN: Balaenopterids – A Layered Exposures and Effects Nexus

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Background

The physiological stress response and population impact when two or more stressors interact has not previously been described for marine mammals. The Smithsonian Institution baleen collection provides an unparalleled opportunity to investigate the interaction between two stressors in blue and fin whales. The Smithsonian collection contains baleen plates from over 1,400 individual whales, with accompanying biological data, collected by Japanese commercial whaling hunts in the Ross Sea and northward during the 1946-47 and 1947-48 seasons. Numerous studies measuring hormones and stable isotopes have demonstrated that whale baleen, an accretionary keratin structure, provides a historical record that reflects biological and environmental conditions at the time of growth. We are assessing foraging and movement by measuring bulk stable isotopes retrospectively in baleen, and inferring food chain length from Compound Specific Isotope Analyses of Amino Acids (CSIA-AA). We are also simultaneously assessing health by investigating energy storage and stress levels through measuring thyroid hormones and the glucocorticoids cortisol, corticosterone and aldosterone retrospectively in baleen. Vital rates are assessed by determining fecundity from retrospective baleen analyses of the reproductive hormones progesterone, estradiol and testosterone. The length of baleen plates from individual Antarctic blue and fin whales captured in 1946-48 represents the historical period of ~1940-48. This coincides with an extreme El Nino event and an Antarctic Oscillation negative phase shift, which produced significant warming in West Antarctica (~1942-1946). This major warming event (1st Stressor) likely impacted Antarctic krill recruitment and density as well as whale reproductive output during the 1940s. A second stressor was added in late 1945 when large-scale commercial whaling quickly ramped up at the end of World War II (WWII) (2nd Stressor). We anticipate that constructing historical trajectories from the baleen of known blue and fin whales will signify the most detailed investigation of the interaction between two stressors (warming event + whaling) on any marine mammal to date, while providing two case studies for application of the Population Consequences of Multiple Stressors (PCoMS) framework to actual marine mammal populations.

We also measured hormones and stable isotopes in baleen from humpback whales collected from animals stranded in Hawaii. Humpback baleen records environmental and physiological conditions from ~2013 to 2021, a time period that coincided with a 34% decline in the humpback whale population [1] and a 76.5% drop in mother-calf encounter rates in whales wintering in Hawaii [2]. Specifically, we examined stress and reproduction in the strongest marine heat wave recorded globally.

Objectives

Our overarching goal is to determine the physiological stress response and population impacts on baleen whales when two stressors interact. Our specific objects are to:

1) Utilize historical whaling data to determine body condition thresholds for pregnancy and lactation in blue and fin whales.
2) Characterize the relationships between behavior change, health and vital rates components of the PCoMS framework according to species, sex, age class and known reproductive status at time of death in the baleen of blue whales.
3) Evaluate behavior change, health and vital rates in the retrospective baleen of blue whales to determine the physiological response to a single historical stressor that is compounded by a second historical stressor in baleen whales.

Methods

- Analyze historical whaling data from blue and fin whales using general linear models (GLM’s) to examine variation in body condition of mature females according to reproductive state and pregnancy stage.
- Analyze baleen at Time of Death in blue whales for stress and reproductive hormones and for stable isotopes to characterize PCoMS relationships.
- Analyze baleen retrospectively for stress and reproductive hormones and stable isotopes from humpback whales impacted by the Pacific Marine Heat Wave event.

- Analyze baleen retrospectively for stress and reproductive hormones from Antarctic blue whale whales impacted by multiple stressors in the 1940's.

Results

We report on progress towards our first and third study objectives. We have expanded our third study objective to conduct retrospective baleen analyses of both stranded North Pacific humpback whales impacted by the 2014-2014 Pacific Marine Heatwave event as well as blue whales captured in Antarctica in the 1940's.

Whaling Data Examination:

We examined historical whaling data from Antarctic blue and fin whales obtained from the 1946-1947 and the 1947-1948 whaling seasons to investigate body condition thresholds needed to maintain pregnancy and lactation while on the feeding grounds. We focused on biological data from mature females taken from the Ross Sea and included 522 blue and 472 fin whales. Using GLMs and non-parametric multiple comparisons, we examined variation in body condition index (BCI) that was derived from blubber thickness and total body length. For females captured on the feeding grounds we examined female reproductive status (resting, pregnant or lactating) and early, mid and late pregnancy. In comparing the reproductive groups, pregnant females exhibited the highest BCI values, followed by resting and then lactating females in both species. GLMs demonstrated increased BCI over time at the feeding grounds in pregnant and resting blue and fin whale females. The BCI decreased over time in the blue and fin whale lactating groups, emphasizing the high cost of lactation and the importance of building adequate energy stores prior to parturition. Significant differences in blue whale BCI values were observed among pregnancy stages with significantly higher BCI values in the late pregnancy group than mid pregnancy followed by early pregnancy. Fin whale BCI values were not significantly different among pregnancy stages. In blue whales, a 40% increase was observed in the minimum blubber thickness of late pregnancy individuals compared to blubber thickness during early and mid-pregnancy, and may suggest that blue whale are especially susceptible to pregnancy loss in the later stages if blubber storage is insufficient.

Retrospective Baleen Analyses of Humpback Whales:

Retrospective $\delta^{15}N$ values in humpback baleen covering ~2013-2021 all show seasonal variations related to migration. However, $\delta^{15}N$ values coinciding with baleen growth in Hawaiian breeding grounds were always higher (~1‰) than values from feeding grounds in the northeast Pacific. In contrast, the $\delta^{15}N$ values of zooplankton from Station ALOHA north of Oahu are significantly *lower* (2.2‰, $p<0.001$) than similar zooplankton from Station PAPA in the Gulf of Alaska. Baleen $\delta^{15}N$ values were independent of pregnancy as inferred from progesterone levels and suggests protein catabolism related to fasting while on the breeding grounds. The average baleen $\delta^{15}N$ values in three plates analyzed were significantly different ($p<0.001$), suggesting while these whales migrate to the northeast Pacific, their specific feeding grounds are different.

Retrospective Baleen Analyses of Antarctic Blue Whales:

We retrospectively sampled baleen plates from nine adult females and four adult males at one-centimeter intervals (n=1,093) for bulk stable isotope analysis and to determine progesterone, testosterone and corticosterone concentrations. Seasonal oscillations in $\delta^{15}N$ values were used to recognize migration and delineate yearly intervals in retrospective baleen growth. In the whales examined, baleen growth represented between 7 and 10 years. Testosterone concentrations in the four males ranged between 1.8 ng/g and 59.0 ng/g. Testosterone concentrations were variable among the males but no clear pattern emerged. Progesterone concentrations in the four males ranged between 10.1 ng/g and 352.4 ng/g. Progesterone concentrations in the nine females ranged between 3.9 ng/g and 2,790.8 ng/g. We assumed a baseline progesterone of 470 ng/g in females based on progesterone data generated from the males and published literature. Progesterone concentrations were compared to known reproductive status at time of death, with elevated concentrations in early and mid-pregnancy females and baseline progesterone in a mature, resting female. Prolonged progesterone peaks consistent with the gestation period were evident in retrospective baleen profiles of all females. In six of the females four presumed pregnancies were identified. One, two, and three pregnancies were identified in each of the remaining three females. Corticosterone ranged between 0.2 ng/g and 19.9 ng/g among the males and females. Corticosterone was strongly correlated with progesterone concentrations ($p<0.0001$) and generally mirrored retrospective progesterone profiles in the adult females. These findings indicate a strong relationship between corticosterone and progesterone release and provide foundational data to further our understanding of stress and reproduction in blue whales.

References

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Development of blow hormone quantification for Blainville's beaked whales to provide physiologic data to better inform population consequences of disturbance (PCoD) models

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Background

Behavioral changes have been documented in beaked whales (family *Ziphiidae*) in response to naval sonar exposure, raising serious concern for the conservation of beaked whale species inhabiting U.S. Navy testing ranges or regularly exposed to sonar exercises. To better forecast the potential impacts of non-lethal repeated disturbance to exposed populations, models investigating the Population Consequences of Disturbance (PCoD) have been developed to predict effects of naval sonar and other stressors. However, for beaked whale species, these models often lack data on physiological responses and vital reproductive parameters (such as the duration of gestation), which can hinder model assumptions and outcomes. Hormone data can provide significant insight into biological processes that are of conservation concern – namely, reproduction and stress responses. Given the challenges of studying an elusive, deep-diving species and the importance of obtaining physiological data, there is a need to advance techniques that will help address significant knowledge gaps on beaked whales. Here, we present our work to develop a noninvasive method for collecting and analyzing hormones in exhaled respiratory vapor (blow) from Blainville's beaked whales (*Mesoplodon densirostris; Md*) during surfacing intervals. The long-term goal of this work is to advance an innovative and noninvasive technique that can be used to generate physiological data to improve our understanding of the impacts of disturbance on beaked whales and help support management decisions.

Objectives

This project aims to develop a blow hormone analysis, as a novel technique for studying hormones associated with reproduction and stress-related responses in *Md* whales, by achieving the following three objectives: (1) Assess collection methods (practical use of handheld pole) and sample quality of *Md* whale exhaled respiratory vapor for hormone analysis; (2) Validate enzyme immunoassay protocols for measuring progesterone, testosterone, and cortisol, and a colorimetric assay for urea in *Md* whale blow using standard parallelism and accuracy tests; then process and analyze all samples for each analyte; and (3) Characterize baseline levels and variation in progesterone, testosterone (as reproductive hormones), and cortisol (as a stress-related hormone) with sex, age class, and reproductive state of known *Md* whales.

Methods

Beaked whales were sampled during daylight hours in weather conditions that are optimal for sampled exhaled blow from beaked whales (Beaufort Sea state < 3 and wind < 8 kt). Field surveys were based out of Sandy Point, Abaco and will primarily be concentrated near the 1000 m isobath along the southwest side of Abaco Island (~15 miles offshore). Exhaled respiratory vapor (blow) samples from *Md* whales were collected using a sampling device fastened to the end of a carbon fiber pole (~7 m long), which was handheld at the bow of a research vessel (7 m zodiac RHIB). The sampling device was a sterile polystyrene dish (25 cm ´ 25 cm;

Corning® bioassay dish CLS431111, Sigma-Aldrich).

During sample collection, the vessel slowly approached an individual whale at idling speed on a gradually converging course to minimize disturbance to the whale. On anticipating surfacing behavior of the whale, the pole was extended and lowered to position the sampling device above the exhaling blowholes (0.1–0.3 m) to catch a portion of the aerosol droplets (see sample collection method depicted in **Figure 1**). To evaluate the efficiency of blow sample collection, we recorded the sampling outcome and sample quality score for every whale that was approached for a blow sample. Sampled whales were photographed to enable individual identification based on unique markings and to obtain known life history data using a long-term sightings catalogue. This field protocol involving animal research was assessed and approved by the Institutional Animal Care and Use Committee (IACUC) at the New England Aquarium (2021-01) and carried out under The Bahamas Department of Environmental and Planning research permits (BS-2022-332438, BS-2023-177568, and BS-2023-189542).

Results

A total of 40 blow samples have been collected from *Md* whales over 15 days on the water, spanning field surveys held in September 2022, May 2023, and October 2023. All sampled whales were individuals of known sex and reproductive maturity, involving both males (n = 6) and females (n = 5). Most samples were subjectively scored as *excellent* quality (52%; n = 21), with the remaining samples graded as *fair* (23%) and *poor* quality (29%). Enzyme immunoassays were modified to increase sensitivity and biochemically validated for reliably measuring progesterone, testosterone, and cortisol in *Md* blow samples; with all assay systems achieving parallelism (all $P > 0.05$) and accuracy tests (slope near 1.0)]. Nearly all samples had detectable cortisol (92%; <0.01–23 ng mL^{-1} unadjusted for sample volume) and progesterone (97%; <0.01–0.6 ng mL^{-1}), but there was lower detectability for testosterone across samples (60%; <0.03–0.1 ng mL^{-1}). Urea, a compound for potentially adjusting for unknown total volume and water content of blow samples was measurable in most samples (70%; 0.02–0.1 mg dL^{-1}). Final steps will be examining results among individual *Md* whales across sex and reproductive states to determine biologically meaningful variation in blow hormones. This work extends the application of blow hormone analysis to free-swimming deep-diving cetacean; and presents an important contribution towards to advancement of methods to assess the physiology of vulnerable beaked whale populations.

Figure 1. Noninvasively collecting exhaled respiratory vapor from a Blainville's beaked whale, using a polystyrene dish fastened to the end of a long pole and positioned above the exhaling blowholes; collected under The Bahamas Department of Environmental and Planning research permit BS-2023-177568.

Further Investigation of Blow or Exhaled Condensate as a Non-Invasive Tool to Monitor the Physiological Response to Stressors in Cetaceans

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Background

Breath or blow collection is feasible from free-ranging cetaceans and offers promise for monitoring and detecting physiologic responses to stressors, (such as sound), and to aid in determining "biologically significant" disturbance to inform the Navy and its operations. Moreover, blow collection is a useful tool that is also valuable in Navy relevant controlled acoustic and hearing research studies on managed care cetaceans, offering the ability for immediate physiological assessment after sound exposure.

Objectives

The overall purpose of this project is to further investigate and validate the use of blow as a non-invasive tool for monitoring the physiological response to stressors, in a cetacean species that can be accessed under both controlled conditions i.e., aquarium belugas, and whales in the wild. Objectives include: (1) Determination and validation of a suitable marker of dilution which can be measured in beluga blow samples and used to standardize hormone measurements (2) Validation of the thyroid hormones T3 an T4 in blow (3) Determination of feasibility and utilization of genetic and transcriptomic techniques to characterize molecular markers of health in blow samples. (4) Initiation of the transition of blow sampling techniques from aquarium whales to free swimming whales.

Methods

Three belugas under professional care at Mystic Aquarium have been trained through positive reinforcement to exhale into a collecting device at rest or after surfacing during swimming. Samples were also taken before and after opportunistic stressor events, at various times during the day paired with blood, as well as at different heights to determine limits of signal. Moreover, blow and blood cortisol were measured in two belugas each month between 2020 and 2023. Immediately after collection, samples were processed and/or archived for hormonal, dilution factor, and molecular studies.

Validation of extraction methods for cortisol, thyroid hormones (T3, T4), urea (potential dilution marker) were carried out in addition to assay validation of commercially available enzyme linked immunoassay (EIA) kits for measurement in blow. A dilution series using a pooled blow sample was used to investigate urea as a dilution marker. Raw hormone values, as well as urea corrected values, were evaluated to determine biological relevance.

RNA and DNA samples were extracted and processed as described in a prior ONR effort (N00012-18-1-2779) (Unal et al., 2018; Unal and Romano, 2021) to determine targeted gene expression, sex, and genetics in blow.

Results

A commercial EIA (Bioassay Systems) for urea, utilizing minimal volume (10ul) was validated for use with blow as a dilution factor. There was no correlation between urea in blow vs. blood ($p>0.05$), however, urea correlates with cortisol in blow ($r=0.433$, $p<0.001$). Application of urea as a correction factor to cortisol measured in different volumes of a blow sample, or different dilutions, results in good agreement between subsamples (i.e. between volumes, and dilutions). While cortisol follows a similar pattern in blow and blood this relationship was not significant ($p>0.05$). Correction of blow cortisol with urea, however, was significantly positively correlated with blood cortisol ($r=0.295$, $p=0.036$).

Despite multiple attempts with different extraction protocols and different commercially available kits, T3 and T4 were not detected in blow. Samples were sent to The Scripps Research Institute for measurement of T3 and T4 utilizing Liquid Chromatography Mass Spectroscopy for comparison.

The total RNA yield for preserved blow and blood samples was highly variable among individual whales and sampling dates with an average of 1,390ng (42ng-8,625ng) for blow and 6,928ng (2,264ng to 13,909ng) for blood. Gene expression for two target genes (IL8, TNFα) and one reference gene (RPL8) varied across months showing up to a 5-fold change in target gene expression (in log scale) but did not show a statistically significant difference among seasons ($p>0.05$). Target gene expression profiles followed different patterns for the two whales. For Whale-1, IL8 and TNFα gene expression was significantly correlated ($r = 0.513$, p=004). There was no statistically significant correlation between blood and blow fold change in expression for either of the genes ($p>0.05$).

Cortisol was detected in approximately 60% of samples collected within 12 in of the blowhole and 50% of samples collected at 1m above the blowhole. Cortisol and Cortisol/Urea were similar between free swim samples collected at 12 in and monthly (stationary) collections. Extraction was necessary to detect cortisol in free swim samples.

Both DNA and RNA were successfully amplified in blow collected up to 1.5 m above the blowhole yielding 344 ng of DNA and 644 ng of RNA on average. Free swim samples had DNA and RNA yields of 142 ng and 177 ng on average. The sex-linked genes were amplified successfully for each free swim blow sample and sequencing confirmed the presence of single base pair differences for mtDNA.

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Molecular Indicators of Physiological Stress and Its Health Effects in Cetaceans

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Background

The cellular and biochemical mechanisms that directly link stress responses and health in marine mammals are poorly understood at present. Downstream effectors and targets of stress hormones may provide substantial information about allostatic load and health and the link between them. Hormones that are altered in response to stress elicit physiological responses by binding to intracellular receptors in target tissues (e.g., adipose tissue) and altering gene expression. Our previous work in pinnipeds has shown that expression of stress-responsive genes in blubber, including metabolic enzymes and markers of adiposity, is predictive of stress hormone levels, survival, and reproductive success (Deyarmin et al., 2019; Pujade Busqueta et al., 2020). However, information on such gene markers in cetaceans is currently lacking.

Objectives

The main objective of this proposal is to identify biologically relevant markers of stress and its consequences in blubber of cetaceans potentially exposed to anthropogenic disturbance. We plan to leverage a bank of existing samples collected from free-ranging bottlenose dolphins (*Tursiops truncatus*) to identify potential stress markers using transcriptomics (Aim 1), validate markers in bottlenose and common dolphins (*Delphinus* spp) with acute and chronic levels of exposure to anthropogenic noise (Aim 2A), and integrate gene expression data with blubber cortisol measurements and available body condition, behavioral, and reproductive success data (Aim 2B). A critical component of Aim 1 is optimizing extraction of sequencing-quality RNA from cetacean blubber samples, which has been a significant challenge in the field.

Methods

RNA was extracted from previously collected *Tursiops* and *Delphinus* blubber samples, as well as pinniped (*Mirounga angustirostris*) skin as a control. Blubber was either flash-frozen or preserved in *RNAlater*® reagent upon collection. Some flash-frozen samples were incubated in *RNAlater ICE*® reagent prior to RNA extraction. Tissue was homogenized by either bead-beating (NextAdvance Bullet Blender®) or cryogenic milling (SPEX 6875 Freezer/Mill®). RNA was extracted using one of the following: TRIzol® and Qiagen RNeasy® Mini Kit, Qiagen RNeasy® Lipid Mini Kit, Qiagen RNeasy® Fibrous Tissue Mini Kit, Bio-Rad Aurum™ Total RNA Fatty and Fibrous Tissue Kit, and Omega Bio-Tek E.Z.N.A.® Total RNA Kit II. The effect of two sequential phase extractions on RNA quality was also assessed.

Results

We found that cetacean blubber samples that were preserved in *RNAlater*® reagent upon collection yielded higher-quality RNA than those that had been flash-frozen (Fig. 1). Treatment of flash-frozen tissue with *RNAlater ICE*® reagent did not improve RNA quality. Cryomilling produced over threefold higher RNA yields and higher-quality RNA than bead beating (Fig. 1). Efficiency of cryomilling small (< 100 mg) samples was improved by freezing tissue in a 5-mm² mold with lysis buffer prior to processing. Isolation of RNA from powdered tissue using TRIzol® reagent with two phase extractions, followed by RNA cleanup using Qiagen® RNeasy Mini Kit, yielded the highest-quality RNA (max RNA Integrity Number, RIN: 6.8), which was of sufficient quality for transcriptome sequencing.

References

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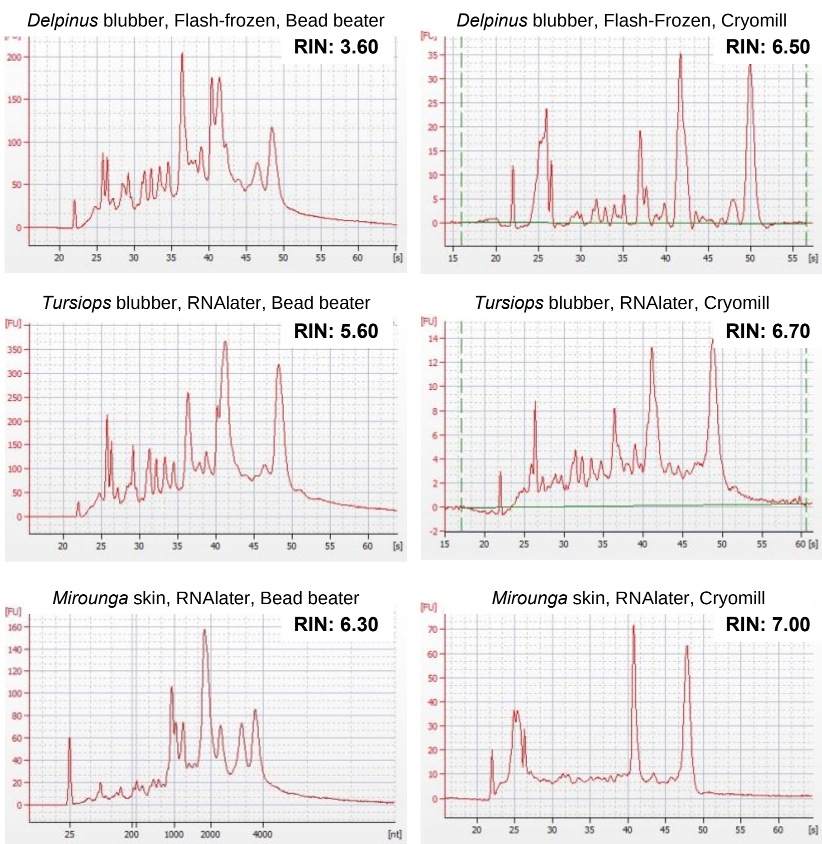

Figure 1. Microcapillary gel electrophoresis of total RNA isolated from blubber of free-ranging common dolphins (*Delphinus* spp.) and bottlenose dolphins (*Tursiops truncatus*) and skin of elephant seals (*Mirounga angustirostris*). RNA Integrity Numbers, or RINs, were calculated using electropherograms. Tissue samples were preserved by flash-freezing or treatment with *RNAlater*® reagent upon collection and homogenized either by bead beating or cryomilling prior to RNA extraction using TRIzol® reagent and Qiagen® RNeasy Mini Kit. RNA samples with RIN values > 6.0 are suitable for transcriptome sequencing.

Bioimpedance And Electrical Impedance Tomography For Mammals In Water

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Background

Our aim is to develop a monitoring system based on Bioimpedance (BioZ) and Electrical Impedance Tomography (EIT). This non-invasive method allows measuring regional gas filling, and blood distribution within the lungs (regional ventilation and perfusion) to study regional gas distribution. The method also allows measurement of heart rate, pulmonary perfusion, breathing frequency and impedance changes, which provides a proxy for tidal volume.

So far, we have: 1) Developed a dolphin-suitable electrode belt, 2) Evaluated BioZ in Dolphins, and 3) validated feasibility of EIT in Dolphins, both in beached animals and in the water. Our results show the ability to image regional airflow during breathing in dolphins, and we also can monitor cardiac activity. Some issues were identified, including a requirement for a neoprene insulation and improved EIT belt. A new design was developed, and the improvements validated.

Objectives

The overall aim is to improve understanding of lung function and mechanics in breath-hold diving dolphins while breathing and breathing-holding underwater. Specific aims are to (1) validate instrumentation for BioZ/EIT in dolphins in water, (2A) design and evaluate a waterproof wireless BioZ data logger, (2B) conduct and analyze EIT measurements of physiological activity.

Methods

-Development of dolphin electrode belt

We have developed an electrode belt to be both high-performance in terms of electrode contact quality, and comfort, and easy to assemble and put on and remove. In previous work, electrodes based on suction cups have been used, but these are uncomfortable for the animal and cumbersome to place. We focused on conductive polymers, which are safe for dolphins and the aquatic environment. Ten different electrode and conductive sheets were tested to characterize the contact impedance. Based on this selection of electrode material, a proof-of-concept belt was built using a neoprene material. While this system worked well, early tests showed the conductivity of the water to be an issue, since electricity will preferentially flow through the seawater, rather than the body. This issue was solved with the design of a belt/vest system which covers and partially insulates the electrodes (Fig.1).

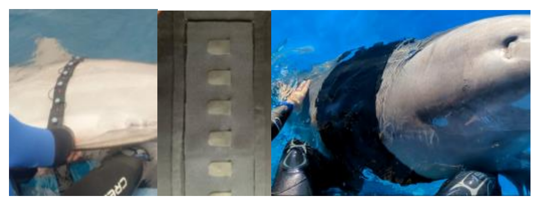

Fig.1. Dolphin electrode-belt designs. (Left) original design with cables and electrode clips. (Mid) modified belt/vest design uses a neoprene layer to cover electrodes (Right) Dolphin wearing new design

Results

Using our EIT belt, two scenarios were tested: 1) Beached dolphins on land (wearing only the electrode belt) and 2) Dolphins submerged while breathing at the surface. Our goal was to evaluate the feasibility of our test configuration and the equipment developed. These two scenarios were chosen to permit testing with a commercial EIT system (Sentec AG, Switzerland) connected to a 4-meter custom cable, which connects to the electrode belt clips placed under the neoprene vest.

We show illustrative results for two different dolphin postures. In the first results (Fig.2), EIT measurements were performed in a dolphin holding its position vertically in a pool. In the left-hand images, we analyze a breath of the dolphin, and show an expiratory and then an inspiratory phase over approximately 2 seconds. After the breath, the dolphin maintains the vertical posture without breathing, and sequential segments of these data are shown as images. In this configuration, the dolphin is making muscular efforts to maintain itself in position in the water, and a 140 cycles/minute signal is shown in the dorsal region of the image.

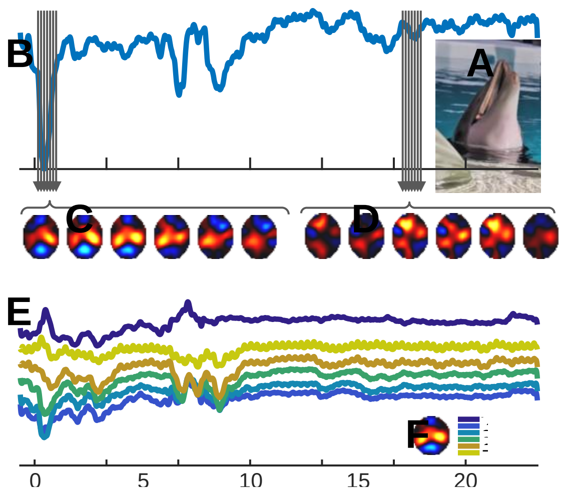

Fig.2. Sample EIT images and waveforms from (A) dolphin oriented vertically, B: Global EIT signal (proxy for lung volume): C & D: EIT images during breath (C) and apnea (D). Conductivity changes (+=red, –=blue). E: EIT waveforms in ROIs shown in (F)

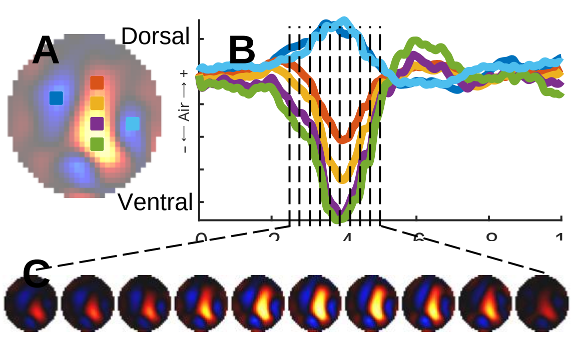

Fig.3. Sample EIT images and waveforms from a dolphin oriented horizontally, (A) color-coded points corresponding to waveforms. (B) EIT signals vs time (s) during a breath. The red indicates decreased air in the central region, with some blue (air) lateral spaces. (C) EIT images corresponding to vertical lines in (B). Note the phases during expiration, where dorsal regions respond 0.5-1.5s later than ventral regions. Much less phase difference occurs during inspiration.

In Fig.3 we show a detail of a dolphin breath while positioned horizontally. In this figure waveforms at regions of interest show the different time course. In the four central ROIs, there is a progressive expiratory delay. Expiration starts in the ventral lung and moves dorsally. Interestingly, inspiration shows little delay. This effect is like expiratory flow limitation in humans, especially at high flows and in lung disease (asthma). The lateral regions of the image show an increase in air content. This could be due to a pendelluft-type effect, or due to abdominal gas pushed into the image plane by the diaphragm.

Summary results

Results of initial tests demonstrate the feasibility of the monitoring technique. EIT provides a novel tool which helps gain new physiological knowledge previously unavailable in a breath-hold diver. In the very short and forceful breathing of dolphins, there is considerable heterogeneity between lung regions. In addition to our results, several challenges have been identified. One is that the movement of dolphins does interfere with the data quality. It will therefore be important to develop protocols in which we can identify and segment data corresponding to quiet activity in the animals.

Discussion

While the tool developed under this proposal is specifically for the dolphin, slight modifications can also make this technology available for human breath-hold and SCUBA divers. Thus, EIT may be a valuable diagnostic tool to enhance diver safety. EIT may also provide novel data to assess how respiratory limitations in divers using re-breathers are affecting lung physiology.

Metabolic costs and immune system impacts from chronic cortisol elevation and decreased energy acquisition

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Background

Models predicting the impact of disturbance or stress on marine mammals try to link the effector to behavioral and physiological responses that affect life history functions. This requires relating behavioral or physiological responses of an individual to a "cost," such as missed reproductive or foraging opportunities, immune suppression, or increased metabolic expenditure. Here, we attempt to assess the costs and mode of action for two potentially common responses to disturbance – chronic elevations in cortisol (the "stress" response), and reductions in energy acquisition.

Chronic cortisol elevations or reduced energy acquisition resulting from anthropogenic disturbance potentially impact immune function, metabolic hormones, and metabolic expenditure. These costs can be qualified and quantified, yet there is little information on them in cetaceans. Their determination in a representative odontocete will inform efforts to model the life history consequences of disturbance, and would benefit the US Navy in developing more accurate models of anthropogenic impacts to cetaceans.

Objectives

The objectives of this project are to: 1) determine body composition through stable isotope dilution and validate morphometric body composition estimates in the bottlenose dolphin (*Tursiops truncatus*); 2) determine the costs of chronic stress and reduced energy acquisition by comparing metabolic rates, body composition, metabolic hormone levels, and immune function in individuals under baseline and either chronic cortisol elevation or reduced energy acquisition conditions.

Methods

Body composition (year 1- complete)

Ten fasted dolphins were orally administered a known quantity of deuterated water. Prior to and four hours following administration (the equilibration period), a voluntary blood sample was collected to determine deuterium enrichment of the body water. Using the known dose of deuterated water and the equilibration enrichment, the total body water (TBW) was calculated. Assuming water content was contained in only the fat free mass, lean and fat mass compartments were calculated according to standard equations.

Dolphins undergoing the deuterated water procedure were simultaneously subject to length and girth measurements at multiple locations along the body, as well as ultrasound measures of blubber depth. Using a truncated cones method and assumptions about the water content of blubber, the measurements were used to estimate body composition and then validated against results from the TBW method.

Effect of chronic cortisol elevation (year 2)

Five dolphins will be trained to station and rest under a metabolic dome for post-absorptive measurements of oxygen consumption. Three baseline measurements will be obtained over a week and during the morning to characterize each individual's resting metabolic rate (RMR). Immediately following the last baseline measurement, each dolphin will be fed 60 mg of hydrocortisone at six-hour intervals to chronically elevate circulating cortisol. The hydrocortisone administration will continue over a week and RMR measurements will be repeated at day three, five and seven of the treatment. The body composition of each animal will be measured on the final day of the baseline and treatment weeks using the deuterium dilution method. Voluntary blood samples will be collected following each RMR measurement and analyzed for metabolic hormones (cortisol, free T3, DHEA, adiponectin) and immune markers (IL-6, TNFα, CRP, IgG and IgM). Statistical analyses will be used to assess impacts of the cortisol elevation.

<u>Effect of reduced energy acquisition (year 3)</u>

The procedure for determining the effect of chronic cortisol elevation will be replicated, except that a week-long 25% reduction in daily energy intake will be used as the treatment instead of oral hydrocortisone administration.

Results

The first year of the study is complete and the second year is in progress at the time of this report. Fat mass, as determined from deuterium dilution and truncated cones methods, ranged from 33-72 kg and 35-51 kg, respectively. Body composition (%lipid) using the respective methods ranged from 15.6-32.0% and 16.2-23.1%. Relationships between the two methods are shown in Figure 1; the truncated cones model appeared suitable for body composition predictions as the mean residual error when compared to isotopic dilution was <4% of body mass. The best univariate predictor of %lipid was the curve length of the dolphin (p=0.04, r^2=0.41). However, the amount of variance explained improved when curve length and mass were used in a multiple regression with an interaction term (p=0.11, r^2=0.61).

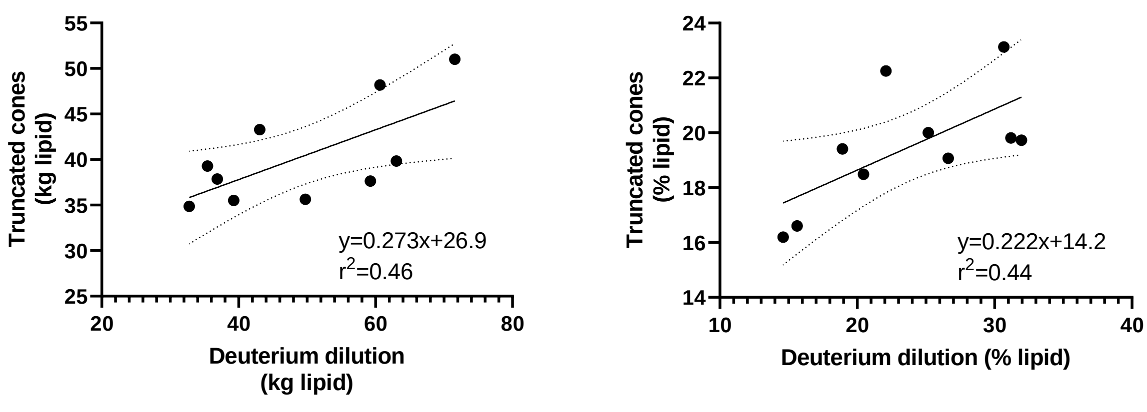

Figure 1. Relationship between (left) total lipid content predicted with the deuterium dilution and truncated cones methods, and (right) %lipid content obtained with the same techniques.

A framework of best practices for drone-based photogrammetry of cetaceans

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Background

Over the past decade, the advancement of unoccupied aircraft systems (UAS, or drones) has revolutionized researchers' ability to obtain morphological data on marine megafauna, particularly cetaceans. Studies on various cetacean species have used drones to distinguish morphological differences between populations and identify shrinking body sizes over generations due to habitat changes. While using drones has clearly brought major insights into cetacean ecology, several different drone systems and protocols have been used in these efforts, and no centralized framework has been established for quantifying and incorporating measurement uncertainty across different drone systems. This lack of standardization and assessment of uncertainty creates confusion over using one analytical method over another and difficulty in comparing results across studies, ultimately hindering collaboration and interoperability and limiting ecological insight.

Objectives

1) Develop a framework of best practices for collecting and processing drone-based imagery to produce morphological measurements with associated photogrammetric uncertainty.
2) Develop a suite of user-friendly hardware and software tools that are integrated within this framework and accessible to other researchers.

Methods

We draw from many years of trials, errors, headaches, and effort working in a variety of habitats and conditions on many different cetacean species. We started the Marine Mammal Institute's Center of Drone Excellence (CODEX) and developed several open-source, user-friendly hardware and software tools including a) "LidarBoX", a 3D printed LiDAR altimeter system that can be easily installed and swapped between several commercial drones (e.g., DJI Inspire, Mavic, Phantom), b) "DeteX", a graphical user interface (GUI) that uses a deep learning model for automated detection of cetaceans in drone-based videos, c) "MorphoMetriX v2", photogrammetry software that provides flexibility in obtaining custom morphological measurements of megafauna, d) "XtraX", a GUI that uses a deep learning model for automatically extracting body size (length and condition) measurements of cetaceans from still frames, e) "CollatriX v2", software for linking important metadata to photogrammetric measurements and automatically calculating different body condition metrics, and f) "Xcertainty", an R package for quantifying and incorporating photogrammetric uncertainty associated with different drones. We integrate all these lessons learned, open-source tools, and analytical approaches into a single framework to help researchers enhance the quality, speed, and accuracy of obtaining important morphological measurements to manage vulnerable populations.

Results

We developed a framework that integrates our several published open-source hardware and software tools, including automated tools, providing a centralized reference to help researchers enhance the quality, speed, and accuracy of obtaining important morphological measurements to manage vulnerable populations. Our framework consists of best practices for 1) Data collection (before takeoff and during flight), 2) Post-processing of imagery (image selection, measuring, linking metadata with measurements, and incorporating uncertainty), and 3) Morphometric analysis (incorporating measurement uncertainty from different drones into analyses). This framework produces morphometric measurements with associated uncertainty so that measurements are comparable across drones. As such, this framework is accommodating to research projects on various budgets, facilitating to multi-lab comparisons, and adaptable to long-term datasets where different drones are inevitably used over time. While we focus on measuring the morphology of cetaceans from drones, this framework can equally be applied across other taxa. We aim for this framework to be utilized by researchers around the world as a step-by-step guide on how to conduct drone-based photogrammetry in a cost-effective and accurate manner.

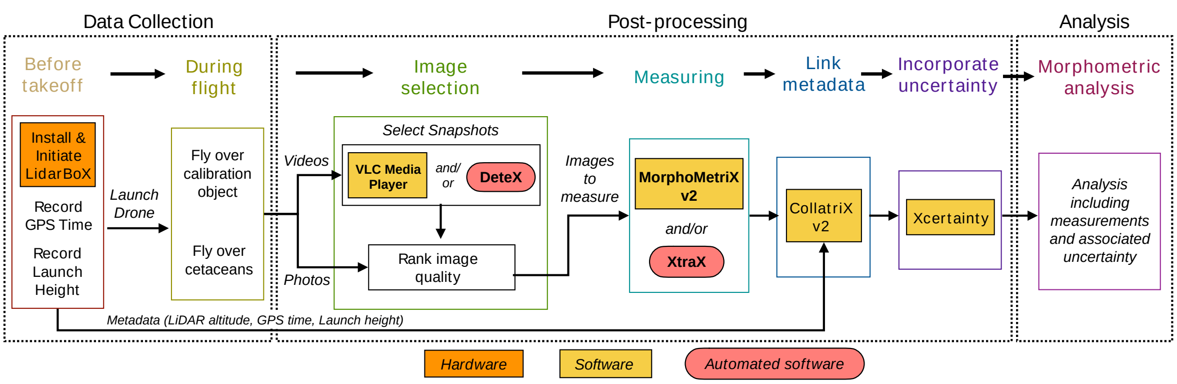

Figure 1. A framework of best practices for drone-based photogrammetry. Note the several open-source hardware and software tools developed by the Marine Mammal Institute's Center of Drone Excellence (CODEX), including LidarBoX, DeteX, MorphoMetriX v2, CollatriX v2, and Xcertainty.

POSTER

ABSTRACTS

Defining the molecular physiologic impacts of stress on beaked whale hypoxia tolerance implications

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Background

For deep-diving marine mammals like beaked whales, long duration dives are critical to survival. However, during these dives, their tissues likely experience extreme fluctuations in oxygen availability. The ability to study these fluctuations *in vivo* is difficult, but new cell culture models afford an unprecedented opportunity to study molecular and cellular adaptations to diving *in vitro*.

Beaked whales' deep-diving behavior is thought to contribute to their susceptibility to sonar disturbance (Cox et al., 2006), but the physiological and cellular mechanisms behind this vulnerability are not well-understood. Studying these mechanisms can elucidate diving adaptations and help formulate testable hypotheses for sonar response studies. Expanding what we know about baseline dive behavior and physiological limitations can be used to inform Population Consequences of Disturbance models and make effective management decisions.

Methods

Using small skin samples from free-swimming cetaceans, we are able to establish fibroblast cell cultures for *in vitro* experiments. Many of these skin samples are collected in partnership with the Atlantic Behavioral Response Study, especially those from goose-beaked whales (*Ziphius cavirostris*).

Cells are established and maintained in tissue culture incubators (kept at 37°C and 5% CO_2) and hypoxia experiments use 1% O_2. Analysis of gene expression is conducted using RNA-sequencing and RT-qPCR. Protein expression is assessed using western blotting, immunofluorescence, and proteomics. We use metabolic assays (Agilent Seahorse) and live-cell imaging (with a Sartorius IncuCyte) to assess cell growth.

Objectives

Our objectives are to better understand the molecular underpinnings of dive behavior and to use that knowledge to inform studies of anthropogenic disturbance.

By approaching these questions at the levels of genes, gene expression, proteomics, organelle organization, and cellular performance, we aim to elucidate multi-level responses and their implications for whole-organism response.

Results

Our results suggest that marine mammals have adapted multiple responses to tolerate low oxygen levels that converge on oxygen carrying and mitochondrial energetics.

Metabolic assays revealed an elevated respiratory capacity in cells from cetaceans, especially in the deep-divers. This is reflected in our gene expression data in which cetacean cells, as compared to human cells, exhibit a diminished shift in expression of glycolysis pathway genes.

Consistent with enhanced spare respiratory capacity and a reduced shift toward glycolysis, we observed sustained mitochondrial volume when cetacean cells were exposed to hypoxia, whereas human cell lines show a reduction in volume in response to prolonged hypoxia (**Fig. 1A**).

Protein-level analysis also pinpointed unique features of beaked whale cells that relate to oxygen transport. Using western blots, we identified downregulation of bisphosphoglycerate mutase (BPGM) in cetacean cells (**Fig. 1B**). BPGM is an enzyme responsible for producing 2,3-diphosphoglycerate (2,3-DPG), which binds to and decreases oxygen affinity of hemoglobin in red blood cells. Deficiency in this enzyme would increase hemoglobin's oxygen affinity. Conversely, these cells also showed a shift from adult hemoglobin to fetal hemoglobin, which is associated with a decrease in oxygen affinity. Based on these observations, we hypothesize that cetacean cells reduce BPGM levels under normoxic conditions, which increases their oxygen affinity, but under hypoxic conditions switch to fetal hemoglobin as a mechanism to improve release of oxygen at the blood/lung interface. Future studies are aimed at better understanding the impact of these changes on oxygen transport.

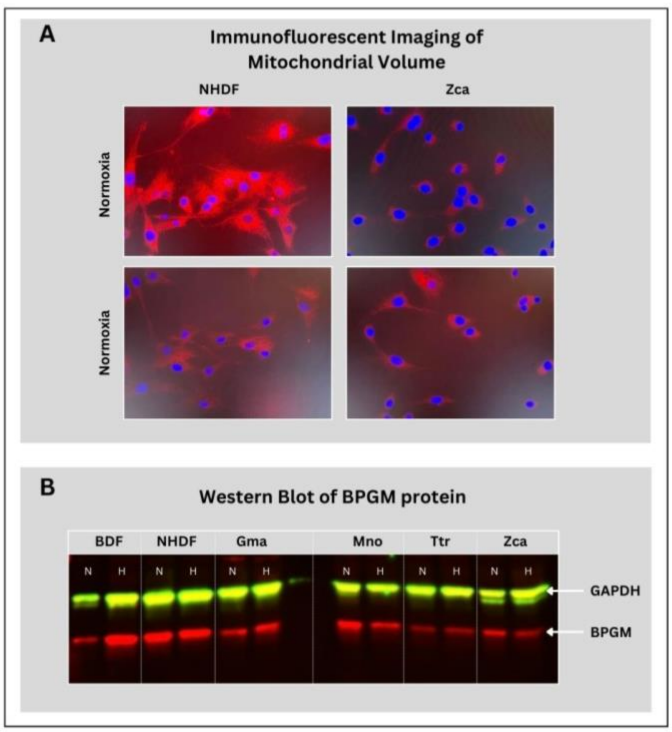

Figure 1. Hypoxia responses in cetacean cells. **A**. Immunofluorescence of fibroblasts from humans (NHDF) and goose-beaked whales (Zca). Nuclei are stained blue, and the Tom20 protein, which indicates mitochondrial volume, is stained red. The top row shows normoxic conditions and the bottom row is after one day in hypoxia. **B.** BPGM protein expression under normoxic ('N') and hypoxic ('H') conditions. Western Blot with staining for proteins including BGPM, red indicated by arrow, and GAPDH (green), which is used to control for protein volumes across samples. From left to right, the cell lines include fibroblasts from cow (BDF), human (NHDF), short-finned pilot whale (Gma), humpback whale (Mno), Atlantic bottlenose dolphin (Tta), and goose-beaked whale (Zca).

Learning stage-wise GANs for whistle extraction in time-frequency spectrograms

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Background and Objectives

Whistle contour extraction aims to derive animal whistles from time-frequency spectrograms as polylines. For toothed whales, whistle extraction results can serve as the basis for analyzing animal abundance, species identity, and social activities. During the last few decades, as long-term recording systems have become affordable, automated whistle extraction algorithms were proposed to process large volumes of recording data. Recently, a deep learning-based method demonstrated superior performance in extracting whistles under varying noise conditions. However, training such networks requires a large amount of labor-intensive annotation, which is not available for many species. To overcome this limitation, we present a framework of stage-wise generative adversarial networks (GANs), which compile new whistle data suitable for deep model training via three stages: generation of background noise in the spectrogram, generation of whistle contours, and generation of whistle signals. By separating the generation of different components in the samples, our framework composes visually promising whistle data and labels even when few expert annotated data are available.

Results

Regardless of the amount of human-annotated data, the proposed data augmentation framework leads to a consistent improvement in performance of the whistle extraction model, with a maximum increase of 1.69 in the whistle extraction mean F1-score. Our stage-wise GAN also surpasses one single GAN in improving whistle extraction models with augmented data.

Sponsor: ONR
Status: ONGOING

Analyzing whale calls through novel multivariate Hawkes processes

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Background and Objectives

Sound is the primary mode of communication among many marine species. Studying recording of these sounds helps to understand the function of the acoustic signals. With increasing anthropogenic noise in the ocean, understanding its impact on the acoustic behavior of marine mammals is needed. One important type of vocalization is an “up-call,” thought to serve as a contact call between individuals. Motivated by a dataset recorded by a network of hydrophones in Cape Cod Bay, Massachusetts, utilizing automatically detected upcalls in recordings, we aim to study the communication process of the endangered North Atlantic right whale. We propose novel spatiotemporal excitement modeling consisting of *background* and *countercall* processes.

The background process describes the intensity of contact calls, providing inference for the impact of diurnal patterns and noise on acoustic behavior of the whales. The countercall process accounts for the potential excitement.

Results

Call incidence is found to be clustered in space and time; a call seems to excite more calls nearer to it in time and space. We find evidence that whales make more calls during twilight hours, respond to other whales nearby, and are likely to remain quiet in the presence of increased ambient noise.

Tracking algorithms for MAMBAT (Multiple-Animal Model-Based Acoustic Tracking)

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Background and Objectives

The overall goal of the MAMBAT (Multiple-Animal Model-Based Acoustic Tracking) project is to improve and fully automate model-based passive acoustic methods for tracking multiple marine mammals. While an overview of the MAMBAT project will be presented in our accompanying talk, this poster will present the technical details and considerations of the tracking component of MAMBAT.

Methods

We are developing and integrating a non-traditional multi-target tracking framework based on random finite sets (RFS) (Mahler, 2007) to advance multiple-animal model-based localization methods (Nosal, 2013). MAMBAT consists of two main parts: “track-before-localize” and “localize-then-track”, where multiple targets are first tracked in the Time-Difference-Of-Arrival (TDOA) then in the 3-D spatial domain. Based on the multi-target Bayesian approach, specifically on the Gaussian Mixture Probability Hypothesis Density (GM-PHD) filter, the tracker jointly estimates multiple target states and incorporates the missed detections and false alarms in the problem formulation. The MAMBAT framework eliminates the need for detection, classification, or association steps, thereby improving efficiency and objectivity.

Results

The feasibility and performance of MAMBAT are demonstrated using real-world data of a clicking sperm whale from the US Navy’s AUTEC test range.

References

RP Mahler (2007). Statistical multisource-multitarget information fusion. Norwood, MA, USA: Artech House, Inc.

E-M Nosal (2013). Methods for tracking multiple marine mammals with wide-baseline passive acoustic arrays. The Journal of the Acoustical Society of America, 134(3):2383–2392.