ONR Marine Mammal & Biology Program Review

23-26 April 2019

Abstract Book

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Day 1: Tuesday - April 23, 2019					
Time	**Program Thrust**	**PI**	**Institution**	**Award Number**	**Project Title**
830	Mike Weise - Welcome / Program Overview / Update				
900	IER / YIP	Baumann-Pickering	Scripps Institution of Oceanography	N000141512587	Using passive and active acoustics to examine relationships of cetacean and prey densities
930	IER / NOPP	Citta / Quakenbush	Alaska Department of Fish and Game	N000141613019	Movements and habitat use of Pacific Arctic seals and whales via satellite telemetry and ocean sensing
1000	IER	Visser	Kelp	N000141712715	Off-range beaked whale study: Behavior and demography of Cuvier's beaked whale at the Azores
1030	Break				
1100	IER	West	University of Hawaii	N000141712789	The diet composition of pilot whales, dwarf sperm whales and pygmy sperm whales in the North Pacific
1130	IER-S&TD	Shorter	University of Michigan	N000141712747	Can suction cup tags give multi-day attachments for behavioral response studies?
1200	IER-S&TD	DeRuiter	Calvin College	N000141613089	Tools and training to enhance capacity for analyzing high-resolution tag data
1230	Lunch				
130	IER-S&TD	Johnson	University of St. Andrews	N000141612857	Next generation sound and movement tags for behavioral studies on whales
200	IER-S&TD	Andrews / Holland	Wildlife Computers	N000141713046	Development of a multi-week Sound and Motion Recording and Telemetry (SMRT) tag for behavioral studies of whales
230	IER-S&TD	Jorgenson / Teilmann	Aarhus University	N000141712233	Acquisition of oceanographic measurements from baleen whales
300	Break				
315	Mon-Det	Dugan	Cornell University	N000141613156 / N0001418WX00349 / N0001417WX01132	RAVEN-X: A high performance data mining toolbox for bioacoustic data analysis
345	Mon-Det	Freitag	Woods Hole Oceanographic Institution	N000141612599	DMON portable range
415	Mon-Det / PECASE	Seger / Miksis-Olds	University of New Hampshire	N000141612594	Monitoring for shifts in odontocete range and distribution in the Arctic and sub-Arctic Seas
445 - 515	Discussion				

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Day 2: Wednesday - April 24, 2019					
Time	**Program Thrust**	**PI**	**Institution**	**Award Number**	**Project Title**
830	Mon-Det / NICOP	Buchan	University of Concepcion	N000141712606	Whales in Estuaries: Glider surveys and fixed time series for explaining distribution
900	Mon-Det	Nosal	University of Hawaii	N000141612598	Single sensor and compact array localization methods
930	Mon-Det	Roch / Klinck	San Diego State University	N000141712867	Using context to improve marine mammal classification
1000	Break				
1030	Mon-Det	Arranz	University of La Laguna	N000141612973	Estimating beaked whale density from passive acoustic recordings
1100	Mon-Det	Harris / Miksis-Olds	University of New Hampshire	N000141612860	Large scale density estimation of blue and fin whales
1130	Mon-Det	Thomas / Marques / Claridge	SMRU / BMMRO	N000141512648 / N000141512649 / N0001417WX01810	Beaked whale group deep dive behavior from passive acoustic monitoring
1200	Mon-Det	Harris / Thomas	University of St. Andrews / CREEM	N000141512142	Develop a general framework for estimating cetacean density from data collected by slow moving autonomous ocean vehicles, investigating key aspects of survey design, data collection and data analysis
1230	Lunch				
130	IER	Benoit-Bird	Oregon State University	N000141512204	Linking deep-water prey fields with odontocete population structure and behavior
200	EoS-BRS	Southall	Southall Environmental Associates, Inc.	N000141713132 / N0001418IP00021 / N0001417WX01087	Integrating remote sensing methods to measure baseline behavior and responses of social delphinids to Navy sonar
230	EoS-BRS/Stress	Kellar / Calambokidis / Moretti	NOAA - SWFSC	N0001419IP00012 / N000141712887 / N0001419WX00434	Measuring stress hormone levels and reproductive rates in two species of common dolphins relative to mid-frequency active sonar within the greater region of the SOAR range, San Clemente Island, California
300	Break				
315	EoS-BRS	Martin / Henderson	National Marine Mammal Foundation, Inc.	N000141612859 / N0001419WX00428	Behavioral response evaluations employing robust baselines and actual Navy training
345	EoS-BRS/Hearing	Janik	University of St. Andrews	N000141613013	Acoustic startle responses as aversive reactions and hearing indicators in cetaceans
Speed Talks / Posters					
415	IER	Benoit-Bird / Moretti	NUWC - Newport	N0001418WX00352 / N000141712752	The use of Navy range bottom-mounted, bi-directional transducers for long-term, deep-ocean prey mapping
420	IER	Zitterbart	Woods Hole Oceanographic Institution	N000141812811	Dynamic marine mammal distribution estimation using coupled acoustic propagation, habitat suitability and soundscape models
425	IER-S&TD	Zerbini	Foundation for Marine Ecology and Telemetry Research	N000141712275	Tag development and best practices workshop
430	IER-S&TD /NICOP	Zerbini	Foundation for Marine Ecology and Telemetry Research	N000141812749	Assessing performance and effects of new integrated transdermal large whale satellite tags
435	IER-S&TD	Woodward	NOAA IOOS	N0001419IP00014 / N0001418IP00082	Animal Telemetry Network - Data Assembly Center
440	IER-S&TD	Woodward	NOAA IOOS	N0001419IP00015 / N0001418IP00080	Animal Telemetry Network - Argos Fees Program
445	IER-S&TD	Jonsen	Macquarie University	N000141812405	Building the next generation of analysis tools for animal tracking data
450	Mon-Det	Baker	Oregon State University	N000141812808	Dynamics of eDNA
455	Mon-Det	Baumgartner	Woods Hole Oceanographic Institution	N000141812810	The wide-band detection and classification system
500	Mon-Det	Berchok / Bond	University of Washington	N000141812792	Cetaceans, pinnipeds, and humans: Monitoring marine mammals in the Arctic and characterizing their acoustic spaces
505	Mon-Det	Klinck	Cornell University	N000141512240	Field testing and performance of the Long-range Acoustic Real-time Sensor for polar Areas (LARA)
510	Mon-Det	Flagg	Desert Star Systems, LLC	N000141812818	Long-term field validation and software integration for the SonarPoint underwater acoustic recording system
515 - 530	Discussion				
530 - 700	Poster Session				

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Day 3: Thursday - April 25, 2019					
Time	**Program Thrust**	**PI**	**Institution**	**Award Number**	**Project Title**
830	EoS-Phys	McDonald	San Jose State University	N000141612852	Heart rate logging in deep diving toothed whales: A new tool for assessing responses to disturbance
900	EoS-Phys	Madsen	Aarhus University	N000141613148	RATE: Respiratory acoustics to estimate energy
930	EoS-Phys	Fahlman	Research Foundation of the Hospital La Fe	N000141613088	Cardiorespiratory physiology in the bottlenose dolphin before, during, and after breath-holding and restraint
1000	Break				
1030	EoS-Phys	Williams	UC Santa Cruz	N000141712737	Physiological consequences of flight responses in diving mammals: Critical metrics for assessing the impacts of novel environmental stimuli on cetaceans and other marine living species
1100	EoS-Phys	Thompson	Sea Research Foundation, Inc.	N000141512203	Evaluation of non-lethal effects of N2 bubbles on marine mammal health and the potential role of immune activity in facilitating the development of dive related injury
1130-1200	Discussion				
1200	Lunch				
100	EoS-PCoD	Booth	SMRU, LLC	N000141612858	PCoD+: Developing widely-applicable models of the population consequences of disturbance
130	EoS-PCoD	Thomas / Falcone / Moretti	University of St. Andrews	N000141512191 / N000141613068 / N0001417WX00590	A population consequence of acoustic disturbance model for Cuvier's beaked whale (Ziphius cavirostris) in southern California
200	EoS-PCoD	Noren	University of California Santa Cruz	N000141613129	Development of an index to measure body condition of free-ranging cetaceans
230	EoS-PCoD	Aguilar de Soto	University of La Laguna	N000141613017	Assessing resilience of beaked whale populations to human impacts: Population structure and genetic diversity in impacted and semi-pristine areas
300	Break				
Speed Talks / Posters					
330	Mon-Det / NICOP	Panicker / Stafford	University of Washington	N000141812795	Understanding community composition of marine mammals in the northern Indian Ocean using visual and passive acoustic methods
335	EoS-Phys-DURIP	Khudyakov	University of the Pacific	N000141812224	Quantitative gene expression system for state-of-the-art biomarker research and development at an undergraduate institution
340	EoS-Phys	Noren	University of California Santa Cruz	N000141812789	Postnatal development of diving physiology: A review to examine vulnerability of immature beaked whales to hypoxia, hypercarbia, acidosis and decompression sickness (DCS) during their "flight response"
345	EoS-PCoD	Claridge / Moretti / Kellar	BMMRO	N000141812778 / N0001419WX00447 / N0001418WX01925 /	Assessing nutritional stress and pregnancy in Blainville's beaked whale at the Atlantic Undersea Test and Evaluation Center (AUTEC)
350	EoS-PCoD	Costa	UC Santa Cruz	N000141812822	Developing metrics of animal condition and their linkage to vital rates: Further development of the PCoD model
355	EoS-PCoD	Fedak / Miller	University of St. Andrews / SMRU	N000141712757	On-board calculation and telemetry of the body condition of individual marine mammals
400	EoS-PCoD	Schick	Duke University	N000141712817	Web-based visualization of marine mammal health across space and time with application towards marine spatial planning
405	EoS-PCoD	Schorr	Foundation for Marine Ecology and Telemetry Research	N000141812777	Demographics and diving behavior of Cuvier's beaked whales at Guadalupe Island, Mexico: A comparative study to better understand sonar impacts at SCORE
410	EoS-PCoD	Thomas	University of St. Andrews / CREEM	N000141812807	Double MOCHA: Phase II Multi-study Ocean acoustics Human effects Analysis
415	EoS-Stress	Romano	Mystic Aquarium	N000141812779	Further investigation of blow or exhaled breath condensate as a non-invasive tool to monitor the physiological response to stressors in cetaceans
420	EOS-Hearing	Cranford	San Diego State University	N000141812797	Deciphering mysticete audiograms using a prepared skull
425	Education-Outreach	Harris / Miksis-Olds	University of New Hampshire	N000141812173	SeaBASS 2018: Bioacoustic Summer School
430 - 500	Discussion				

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Day 4: Friday - April 26, 2019					
Time	**Program Thrust**	**PI**	**Institution**	**Award Number**	**Project Title**
830	EoS-PCoD / YIP	Goldbogen	Stanford University	N000141612477	Biomechanical and energetic analyses of whale-borne tag sensor data to assess the population consequence of acoustic disturbance
900	EoS-PCoD	Williams	Oceans Initiatve	N000141613058	IPOD: Iterative PCoD for oceanic dolphins
930	EoS-PCoD	Thomas / Watwood / Harwood	NUWC - Newport	N000141812821 / N0001419WX00431	Integrating information on displacement caused by mid-frequency active sonar and measurements of prey field into a population consequences of disturbance model for beaked whales
1000	EoS-PCoD	Schwacke	National Marine Mammal Foundation, Inc.	N000141712868	A model for linking physiological measures of individual health to population vital rates for cetaceans
1030	Break				
1100	EoS-Phys	Costidis	Marine Mammal Solutions, LLC	N000141713146	Investigation into the gross and microscopic anatomy of the endotracheal plexus of cetaceans
1130	EoS-Stress	Houser	National Marine Mammal Foundation, Inc.	N000141712729	Reproductive hormones as related to age, sex, season, and levels of stress hormones in the bottlenose dolphin
1200	EoS-Stress	Champagne	National Marine Mammal Foundation, Inc.	N000141512773	Molecular indicators of chronic stress in a model pinniped - the northern elephant seal
1230	Lunch				
130	EoS-Stress	Atkinson	University of Alaska	N000141613016	Development and validation of a technique for detection of stress and pregnancy in large whales
200	EoS-Stress	Usenko / Trumble	Baylor University	N000141712755	Reconstructing stress and stressor profiles in baleen whale earplugs
230 - 300	Discussion				

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Mon-Det	Freitag	Woods Hole Oceanographic Institution	N000141612599	DMON portable range	31
Mon-Det / PECASE	Segar / Miksis-Olds	University of New Hampshire	N000141612594	Monitoring for shifts in odontocete range and distribution in the Arctic and sub-Arctic Seas	33

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Mon-Det	Harris / Miksis-Olds	University of New Hampshire	N000141612860	Large scale density estimation of blue and fin whales	44
Mon-Det	Thomas / Marques / Claridge	SMRU / BMMRO	N000141512648 / N000141512649 / N0001417WX01810	Beaked whale group deep dive behavior from passive acoustic monitoring	46
Mon-Det	Harris / Thomas	University of St. Andrews / CREEM	N000141512142	Develop a general framework for estimating cetacean density from data collected by slow moving autonomous ocean vehicles, investigating key aspects of survey design, data collection and data analysis	48
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Mon-Det	Flagg	Desert Star Systems, LLC	N000141812818	Long-term field validation and software integration for the SonarPoint underwater acoustic recording system	75

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EoS-Stress	Atkinson	University of Alaska	N000141613016	Development and validation of a technique for detection of stress and pregnancy in large whales	NA
EoS-Stress	Usenko / Trumble	Baylor University	N000141712755	Reconstructing stress and stressor profiles in baleen whale earplugs	126

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Day 1: Tuesday April 23, 2019

2019 ONR
Marine Mammal & Biology
Program Review

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Using passive and active acoustics to examine relationships of cetacean and prey densities

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Background

Ambient sound patterns of biological origin in a frequency range relevant to US naval sonar may alter sonar ranges with a diel, lunar, and seasonal pattern, and likely geographic area. Additionally, organisms in the water column are “bio-clutter” targets that alter sound propagation on these time scales, dependent on the target behavior and density. The identification, description, and quantification of sound patterns and the behavior of the corresponding organisms are of relevance to the US Navy sonar community.

Scatterer, especially krill and deep-scattering layer (DSL) organisms, are prey items for large predators, of which marine mammals are of particular interest to the US Navy. Prey is likely a driver and indicator for marine mammal distribution, abundance, and behavior. The possibility of long-term, autonomous monitoring of prey distribution and behavior using passive acoustic means will lead to an increased predictability of marine mammal presence in an area relevant to the US Navy.

Objectives

We addressed the following hypotheses:
H1: Backscatter organisms modulate or produce sounds that can be monitored passive acoustically.
H2: Long-term spatio-temporal variability of active acoustic backscatter strength and passive acoustic ambient sound are related.
H3: Predator (cetacean) presence and behavior is related to prey (DSL and krill) density and behavior, measurable active and passive acoustically.

Methods

High-frequency acoustic recording packages (HARPs) in a variety of setups (floating devices, moorings) were deployed at several sites in Southern California, specifically the San Diego Trough, Tanner Basin, and in abyssal waters off Point Conception between several days to >1 year to record passive acoustic sound from 10 Hz to 100 kHz. In all configurations, Simrad scientific fisheries echosounders were recording backscatter strength either integrated or nearby the HARP instruments, either with single or multiple frequencies between 38 and 200 kHz. Oceanographic measurements were taken with as simple as a microCAT CTD cast from a small boat to full ship-board surveys and complex mooring systems.

Data analysis was conducted with custom-written scripts in *Matlab* (MathWorks, Natick, MA) and use of *Echoview* (Echoview Software Pty Ltd, Hobart). Statistical analysis occurred with *R* (R Project for Statistical Computing).

Results

DSL organisms, comprising a variety of mesopelagic fishes, and squids, siphonophores, crustaceans, and other invertebrates, are preferred prey for numerous large marine predators, e.g. cetaceans, seabirds, and fishes. Some of the DSL species migrate from depth during daylight to feed near the surface at night, transitioning during dusk and dawn. We investigated if any DSL organisms create sound, particularly during the crepuscular periods. We identified a nighttime chorus between 300 and 1000 Hz with crepuscular peaks in amplitude and highest received levels during DSL ascent and descent. We were not able to localize the origin of the sound and hence unmistakably identify DSL organisms, especially mesopelagic fishes as the source but found a positive relationship between DSL backscatter strength and maximum received level during evening ascent. Additionally, we observed the occurrence of this sound throughout the Southern California Bight; however, starting in late spring more inshore, lasting through early fall and starting early summer more offshore, lasting until early winter. The DSL are globally present, so the observed acoustic phenomenon, if also ubiquitous, has wide-reaching implications. Sound travels farther than light or chemicals and thus can be sensed at greater distances by predators, prey, and mates. If sound is a characteristic feature of pelagic ecosystems, it likely plays a role in predator-prey relationships and overall ecosystem dynamics.

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A one-year observation of passive and active acoustics, as well as oceanographic measures, taken at a moored location 150 nmi offshore Point Conception in 4000 m deep pelagic waters, shows presence of delphinids and baleen, sperm, and beaked whales throughout the recording period with species-specific seasonal occurrence. Variability in small pelagics, krill, and diel vertical migrators is evident from the active acoustic data. Over the course of the year, distinct water masses brought very different animal communities to the site. This was evident in backscatter as well as top predator presence. Common dolphins were associated with time periods of strong DSL occurrence. Risso's and Pacific white-sided dolphins occurred with small pelagics in surface waters during winter/spring. Cuvier's beaked whales had their strongest association with winter/spring California current waters and abruptly left the site when warmer waters reached the region. Other associated soundscape patterns have yet to be evaluated. This study provides valuable information on ecosystem processes in meso- and epipelagic waters on a relatively small spatial scale but very fine temporal scale.

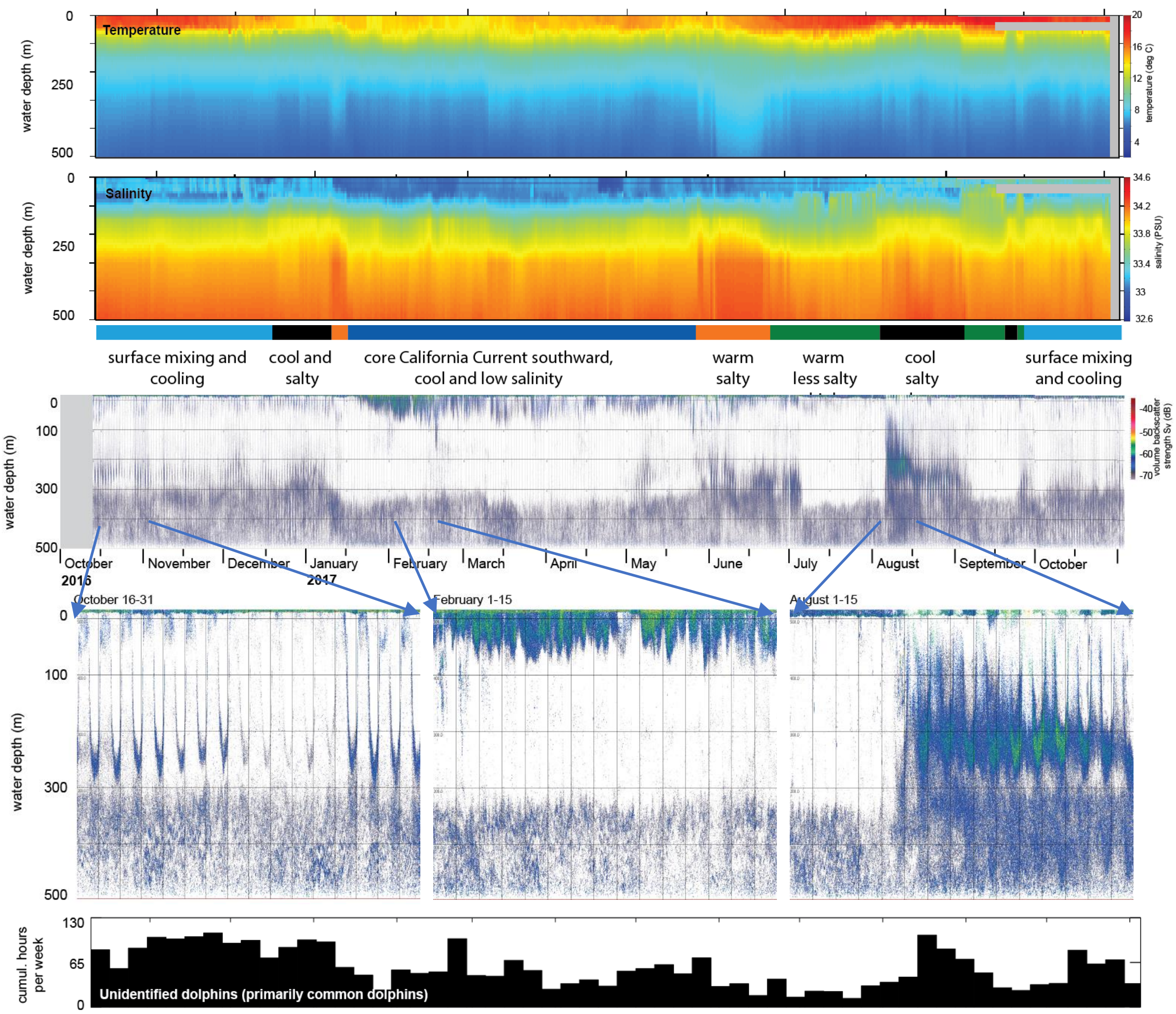

Figure 1. Temperature and salinity profile (rows 1 & 2) over the deployment period and descriptive evaluation of water masses at the offshore site. Backscatter strength (row 3) with distinctly different animal communities in the water column (details in row 4). Common dolphin (row 5), as top predator example, associating most clearly with strong presence of diel vertical migrators.

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Movements and Habitat Use of Pacific Arctic Seals and Whales via Satellite Telemetry and Ocean Sensing

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Background

Oceanographic features such as fronts and stratified layers are known to enhance feeding opportunities for marine mammals. Such oceanographic features aggregate zooplankton, which then attract forage fish. Hence, marine mammals that feed on zooplankton (e.g., filter feeding whales) or fish (e.g., ice seals) may also be attracted to fronts and stratified layers. Oceanographic data (e.g., temperature and salinity) used to identify water masses and the boundaries between them are difficult to collect in remote areas that are often ice-covered. Ship-based oceanographic surveys, often limited to ice-free months and fixed moorings, sample a limited number of locations. The use of animal-borne Conductivity-Temperature-Depth (CTD) satellite-linked tags can alleviate many of these problems by having the tagged animal collect oceanographic profiles, paired observations of temperature and salinity with depth, as it travels.

Objectives

To describe how the movements and foraging of marine mammals are affected by the ocean environment.

Methods

SMRU CTD transmitters were attached to animals captured with and by local hunters. Tags were glued to seals and attached to bowhead whales with subdural anchors.

As an example of how CTD data can be used to explain animal movements, we examined how the movements of seven bowhead whales (*Balaena mysticetus*), tagged during 2016-2018, related to oceanographic variables in the Chukchi Sea in autumn (September-November). We first fit the location data to a behavioral state-space model that switches between two behavioral states, traveling and lingering (presumed feeding). We then modelled behavior as a function of eight different oceanographic covariates that are functions of salinity, temperature, and their vertical gradients, and six environmental covariates that examined the effect of wind and bathymetry.

Results

As of 1 October 2018, we have collected 16,591 CTD profiles; 5,707 in the Bering Sea, 5,978 in the Chukchi Sea, and 1,509 in the Beaufort Sea (Figure 1).

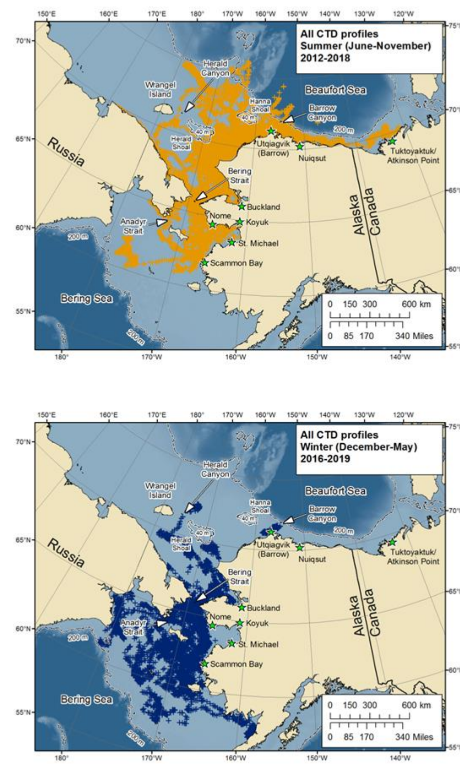

Figure 1. Location of CTD profiles collected summer (top) and winter (bottom).

Of these, 9,500 CTD profiles were from spotted seals (*Phoca largha*), 4,023 from bearded seals (*Erignathus barbatus*), 1,573 from ringed seals (*Pusa hispida*), and 1,495 from bowhead whales. In addition, 5,491 profiles were collected in winter months when ship-

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based oceanographic surveys are rare due to ice cover.

In the Chukchi Sea, bowhead whales generally made square-shaped dives that targeted the seafloor. A total of 267 location estimates were classified as traveling and 506 were classified as lingering. The best approximating model suggested that lingering behavior was linearly related to surface temperature and bottom temperature, such that the greatest amount of lingering occurred when water was close to -1.8 ºC (Figure 2). Lingering was weakly related to the presence of large gradients in salinity. Visual inspection of temperature salinity profiles indicates that whales often target the thermocline/halocline that forms between colder/saltier water of Pacific origin, such as Pacific Winter Water and Bering Shelf-Anadyr Water and warmer/fresher Alaska Coastal Water and Siberian Shelf Water. This result, using empirical data from CTD tags, generally agrees with results we have previously found when using covariate data taken from an ONR-funded pan-arctic coupled ice-ocean model (RASM; http://dx.doi.org/10.1016/j.dsr2.2017.03.009).

Although whales were clearly targeting thermoclines/haloclines, such features were difficult to detect within a statistical modelling framework because whales often targeted the top of the stratification without passing through it. CTD data for animals are limited in that we only detect the environment the animal passes through and may lack information on environments they avoid. Data from oceanographic models can provide information on habitats that animals avoid; as such, a major benefit of CTD tag data will be to improve on such oceanographic models.

In 2019, we plan to examine the role of oceanographic variables in determining the movements of ice seals. We are also seeking funds to use animal-borne CTD data for comparing with and improving upon predictions from an ONR-funded oceanographic model (e.g., http://dx.doi.org/10.1029/2001JC001039 https://www.oc.nps.edu/NAME/RASM.htm).

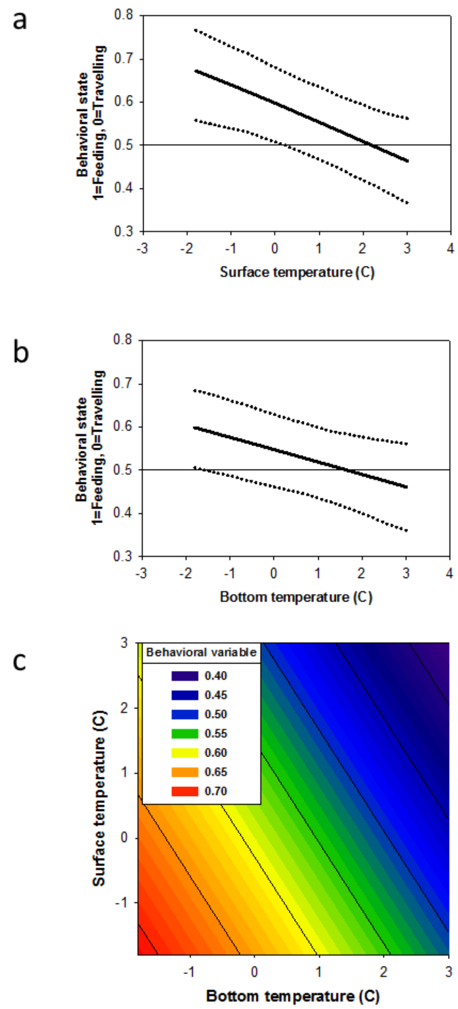

Figure 2. Behavioral state of bowhead whales as a function of surface temperature (a), bottom temperature (b), and the relationship between the two (c). Behavioral state varies from 0 (pure traveling) to 1 (pure lingering). Dotted lines are 95% confidence intervals.

Notes:

Off-range beaked whale study: Behavior and demography of Cuvier's beaked whale at the Azores (North Atlantic)

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Background

One of the prime Life Sciences research topics at ONR involves behavioral responses of marine mammals to man-made sounds. There is a pattern of atypical mass strandings of several beaked whale species that coincide with naval sonar exercises; the cause(s) of stranding remain unknown, but might include strong avoidance responses, resulting in prolonged deep dives. Strandings, but also sub-lethal changes in behavior and habitat-use resulting from sonar exposure pose a significant risk of negative effects at the population-level. The type and severity of behavioral responses can strongly vary with the context of the exposure, and may be subject to previous experience. Therefore, there is a critical need for the study of off-range and natural behavior of beaked whales to enable assessment of the universality and biological significance of on-range responses, to provide a full scientific basis for Navy mitigation measures. The current research will help fill these data gaps and provide a better scientific basis for monitoring and mitigation policies.

Objectives

Here, we aim to advance the knowledge on the natural behavior and population demography of Cuvier's beaked whale in an off-range habitat (the Azores, North Atlantic) to further our understanding of their behavioral response to sonar and enhance the ability for effective, science-based mitigation measures. Building on an existing data-set, this study aims to:

1) Determine natural patterns of Cuvier's beaked whale behavior
2) Investigate Cuvier's beaked whale population characteristics
3) Compare behavioral patterns and population demography between on- and off-range populations through expert collaborations.

Methods

The Azores form a mid-Atlantic deep-water habitat for at least 6 species of beaked whales, including Cuvier's beaked whale. Anthropogenic sound sources in the area are currently still limited, qualifying the area as a relatively undisturbed, off-range and high-quality beaked whale habitat. While deep-water, Cuvier's beaked whale habitat is well-accessible and spatio-temporal patterns of distribution and behavior can be studied from shore.

We use a multi-sensor approach, with concurrent sampling using Dtags, visual observations, photo ID and aerial photogrammetry (UAS). For each focal group, we aim to collect: a) high-resolution data on underwater movement, diving and vocal behavior from *Dtags*, b) location, group size- and composition (age/sex class distribution), social surface behavior and individual presence from visual observations, photo identification and aerial photogrammetry and c) individual length, age-class and body condition from photo ID and aerial photogrammetry.

Results

During two field work efforts in 2017 and 2018 (3rd final planned for 2019), all data streams were successfully collected. We identified a core area with CBW presence. Our Dtag data set now holds six 12-24 h records on males and females. The individuals performed regular deep foraging dives to a maximum depth of 1752 m, interspersed with shallower non-foraging dives, broadly comparable to dive patterns in other regions. Longer-term focal follows revealed a relatively limited range of movement over the course of a day, at the scale of several kilometers. Group size (3.6) was larger than found in other regions (2.6) with the exception of the population near San Clemente Island (3.8; Falcone et al. 2009). In total 31 individuals were identified, including males (5), females (5), sub adults (8) and calves (4). 30% of individuals was resighted within or between years.

Concurrent collection of age-class/sex data (*i.e.* from erupted teeth, body scarring) from photo ID and aerial photography enabled matching, ground truthing and methodological extension of CBW age and sex class determination.

Multiple mother-calf pairs were encountered at sea, and successfully filmed and photographed using the UAS. This allowed for the first measurements of body

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size and condition using photogrammetry of CBW mothers and calves in the wild (Fig. 1).

Two mother-calf pairs potentially showed considerable difference in body condition (Fig. 1). Whereas full assessment requires a substantially larger dataset, these data indicate 1) the potential for UAS photogrammetry to identify differences in CBW body condition, including for essential elements of the population (females with dependent offspring) and 2) the potential to obtain life history, size and condition data on female-offspring pairs, that are typically not available/approachable for tagging. Recapture of mother-calf pairs would enable assessment of calf growth and tracking of mother and calf condition over time. Female-offspring pair photo ID and photogrammetry is also instrumental in enhanced recognition/classification of adult female characteristics for assessment of population demography.

Leveraging effort from our concurrent toothed-whale prey study in the area, CBW foraging dive locations were selected for prey sampling using eDNA as a proxy for cephalopod prey throughout the water column and pelagic/benthic video transects were used to assess foraging habitat flora and fauna. Results are in progress and will enable comparison of prey characteristics within and outside of CBW core foraging habitat.

Our long-term toothed whale foraging and habitat-study identified largely separated core foraging habitat for CBW and Sowerby's beaked whale (*Mesoplodon bidens*). We collected two Dtag records on Sowerby's beaked whales (global first records of their foraging behavior). In combination with the prey-analysis, these data create further strong potential for enhanced understanding of CBW foraging strategy and habitat-needs, using an integrated ecosystem approach inclusive of a comparative analysis of foraging characteristics of sympatric, potentially competitive species. The Dtag records on Sowerby's beaked whales indicate behavioural differences with CBW that may have implications for their sensitivity to naval sonar.

Following from successful sampling strategy, this study will strongly benefit from a longer-term perspective, enabling augmentation of our datasets and the longer-term monitoring required for assessment of individual and population health parameters.

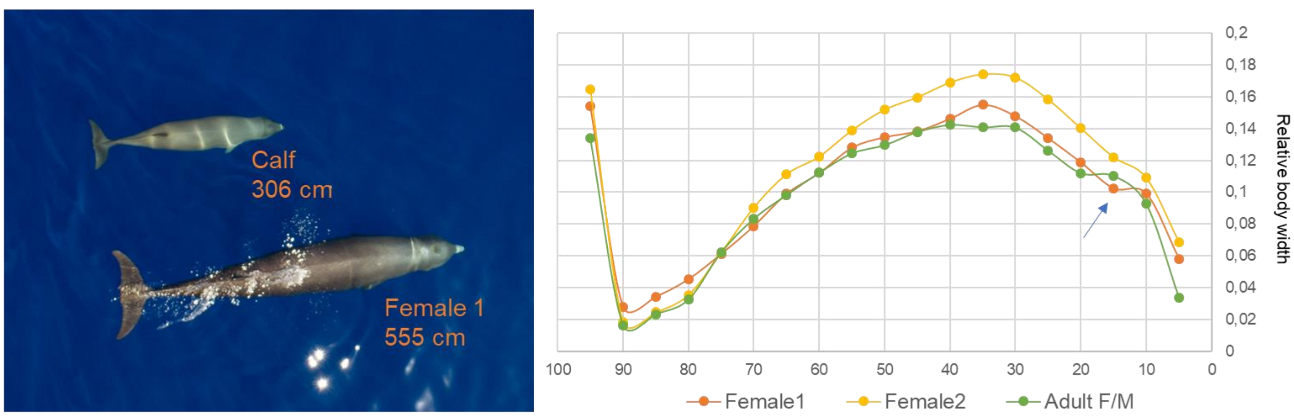

Figure 1. Aerial photogrammetry of Cuvier's beaked whale mother-calf pair revealing length and body condition (left). Body condition (body width/ body length) measured at 5% intervals from tip of rostrum (0) to tail notch (100), for 2 females with calf and one adult without a calf. Note the difference in condition between female 1 (young calf, 306 cm) and female 2 (older calf, 355 cm). Female 1 shows signs of poor condition (indentation behind skull at blue arrow, photo), whereas Female 2 appears to have more than average fat reserves (good condition). Female body condition is mirrored by their calf.

Notes:

Concurrent research effort in the area has also initiated:

- Integrated ecosystem approach; CBW prey analysis from eDNA
- Comparative analysis of beaked whale species foraging ecology and demography

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The Diet Composition of Short-finned Pilot Whales, Dwarf Sperm Whales and Pygmy Sperm Whales in the North Pacific

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Background

Knowledge of a species' diet is crucial for understanding it's behavior and ecology, is relevant to assessing the impact of potential changes associated with anthropogenic activities and is required to understand potential prey limitations. Prey fields appear to mediate the response of cetaceans to underwater noise, and there is a need to determine the composition of prey fields and how prey drives movement, behavior and habitat use. Additionally, prey limitation has been identified as a marine mammal stressor and a greater understanding of diet is needed to evaluate how this potential stressor may contribute to cumulative effects. Assessing diet for many species of cetaceans is difficult, given that most foraging occurs far below the surface and that stomach contents of stranded animals are rarely available.

Short-finned pilot whales (*Globicephala macrorhynchus*), dwarf sperm whales (*Kogia sima*) and pygmy sperm whales (*K. breviceps*) may be susceptible to the impacts of underwater noise, yet nothing is known of the diet of either short-finned pilot whales or dwarf sperm whales in the central Pacific. Similarly, prior to 2009 no information was available on the diet or foraging behavior of pygmy sperm whales from any location in the central Pacific. Stomach contents are available for stranded or by-caught dwarf sperm whale and short-finned pilot whale specimens from the central Pacific as well as additional stomach contents from pygmy sperm whale strandings occurring since 2009. This project proposes to describe the diet composition of both pilot whales from the prey remains of stranded and by-caught specimens, allowing for the first investigation of pilot whale diet composition from the central Pacific. Additionally, dwarf sperm whale diet composition will be evaluated for the first time from this area and potential niche partitioning between pygmy and dwarf sperm whales will be investigated by building on our knowledge of pygmy sperm whale diet through the examination of prey remains from additional specimens obtained since 2009. The identification of each prey item to the species level and size and mass estimates of prey will allow for a detailed description and comparison of diet composition as well as provide insight into the foraging behavior and ecology of these whales in the North Pacific.

Objectives

1. To identify prey remains obtained from the stomachs of pilot whales, dwarf sperm whales and pygmy sperm whales in the North Pacific. Prey remains will be identified to the level of species or lowest taxonomic level possible.

2. To describe the feeding habits of pilot whales in the North Pacific, signifying the first description of diet composition from this species in the central Pacific.

3. To describe and compare the diet composition among dwarf and pygmy sperm whales in the North Pacific. This would represent the first description of dwarf sperm whale diet from the central Pacific and allow for an investigation into potential niche partitioning among Hawaiian Kogiidae.

Methods

Stomach contents were collected from 5 mass stranded short-finned pilot whales in 2017. Additional stomach contents are available from pilot whales that were stranded or by-caught in the central Pacific between 1991 and 2016. Similarly, contents have also been collected from stranded or by-caught dwarf sperm whale specimens in the central Pacific between 1990 and 2016. Additional stomachs collected from pygmy sperm whale strandings since 2009 will also contribute to this project.

All stomach contents were initially frozen or fixed in formalin, and in some cases samples were thawed and transferred to ethanol for long-term storage at the Hawaii Institute of Marine Biology or at the National Marine Mammal Laboratory (NMML).

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In order to evaluate diet in pilot whales and dwarf and pygmy sperm whales, contents were rinsed through a progression of sieves with decreasing mesh sizes. After sorting, cephalopod beaks, fish bones and crustacean remains will be identified to the lowest possible taxon using the private reference collection of W.A. Walker and the fish bone, otolith and cephalopod beak reference collections housed at the NMML, Seattle, WA. The total number of each species of cephalopod was estimated as the number of lower beaks present. The total number of each fish species was estimated based on the greater number of left and right cranial bone elements or otoliths. Crustacean abundance was estimated using the number of individual carapace remains in each stomach.
Prey item condition allowing, lower beak rostral lengths (cephalopods), carapace lengths (crustaceans) and otolith lengths (fish) were measured to the nearest 0.1 mm using an optical micrometer or vernier calipers. Where possible, prey size was estimated by applying appropriate regressions to these measurements in order to determine individual prey weight. In cases where length/weight regression equations for a particular prey species are unavailable, individual weights were estimated through comparison with appropriate sized museum specimens.

Pigmentation (darkening) of the wing portions of lower beaks will be examined to establish adult from juvenile squid. Beaks were considered to be from adult squid when wing pigmentation is complete.

Results

Diet composition for short-finned pilot whales was summarized from the stomach contents of five individuals that mass stranded on Kauai in 2017. A total of 1560 prey items were identified among the five stomachs. Cephalopods were present in all five stomachs and fishes were present in two of the five. Five fish species representing five families and at least 30 species of cephalopods representing 16 families were identified among the prey remains. Cephalopods dominated the diet of Hawaiian short-finned pilot whales, representing 99.4% of the diet by number. The most abundant cephalopod prey families by number and by mass are Enoploteuthidae (contributing 12.2% by number and 6.0% by mass), Onychoteuthidae (12.8% by number; 13.8% by mass), Histioteuthidae (26.5% by number; 9.9% by mass) Lepidoteuthidae (8.3% by number; 12.7% by mass) and Cranchiidae (13.7% by number; 10.6% by mass).

Hawaiian pygmy sperm whale diet is dominated by cephalopods, contributing 78.7% to the diet by number and 93.4% by mass. Similar to pygmy sperm whales, preliminary results obtained to date from Hawaiian dwarf sperm whales indicate a diet dominated by cephalopods with fish and crustaceans also contributing to diet. However, the most numerically abundant cephalopod families appear to differ when compared between pygmy and dwarf sperm whales. Histioteuthidae and Cranchiidae were the most abundant cephalopod prey families observed among pygmy sperm whale stomachs, with Enoploteuthidae more numerically important to the diet of dwarf sperm whales. Analysis of additional stomachs for all species is in progress and is anticipated to provide further information on habitat usage and niche portioning among Hawaiian whales.

Notes:

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Can suction cup tags give multi-day attachments for behavioral response studies?

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Background

The ability to measure fine scale animal behavior and acoustics over multiple days while minimizing impact of the tag attachment to the animals will significantly enhance the understanding of the effect of acoustic disturbance on cetaceans. Improved reliability and increased duration of suction cup tag deployments are particularly relevant for Navy funded behavioral response studies. Reliable multi-day attachments will enable increased pre-exposure periods, the measurement of enduing reactions to the experiment, and allow lengthier (and more realistic) exposures. The creation of a viable multi-day suction cup attachment system will provide an alternative tagging method for high data-rate archival tags with a reduced impact to animals when compared to skin penetrating attachment methods.

In this project, we propose to evaluate the potential for multi-day suction cup attachments of high fidelity acoustic and movement tags. While the results of this work can be applied to a variety of tag electronics, the proposed tags will be designed around the DTAG4 developed at the University of St. Andrews. The low-power, compact, electronics of the DTAG4 will enable the collection of continuous sound and movement data for up to 4 days. The small payload dimensions of the electronics provide more flexibility for the hydrodynamic housing, flotation and suction cup array, enabling the exploration of a larger design space. In this work we propose to test two questions: 1) do performance benefits of flow control features translate into longer and more secure attachments I the field? and 2) can material selection and increased contact area between the tag and the animal be used to resist sliding tag failure modes that result from drag loading on free swimming animals?

Objectives and Methods

In this work, we will build on previous studies to first create and then systematically evaluate the field performance of enhanced suction cup tags. This technology will facilitate more authentic behavioral response studies (BRS) and generate new knowledge about the behavior of marine mammals over longer time scales.

Objective 1: Design and fabrication of a multi-day suction cup tag

The net hydrodynamic forces created by the tag housing play a significant part in early tag detachments, and have the potential to impact the behavior of small cetaceans. Tag induced forces can be reduced by decreasing the size of the tag, utilizing hydrodynamic shapes for the tag body, and/or implementing control elements to modify the flow in and around the tag. Three promising tag designs from previous work will be refined to accommodate the smaller DTAG4 electronics and explored in this work.

A) Minimum volume housing: The housing will envelope the electronics minimally without consideration of flow.
B) Minimum drag housing: Volume constraints will be relaxed to implement a larger hydrodynamic housing to reduce drag.
C) Minimum total force: Building on Design B we will introduce flow control elements both to reduce the drag loading and control the amount of lift generated by the tag.

Computational fluid dynamics simulations (CFD) will be used to iteratively refine the housing designs and compare performance of design features. A uniaxial force testing machine will be used for controlled loading of the cup attachment designs to both shear and normal forces with both wet and dry surface conditions. A combination of force, displacement, camera, and pressure data will be used to quantify the performance of the attachment systems.

Objective 2: Performance evaluation with animals in professional care

Tags will be fabricated based on the results from the swimming trials (8 to 24 hours) and during prescribed swimming tasks at variable speeds to quantitatively and qualitatively examine:
1) Performance of the tag attachment: duration of attachment, tag movement during attachments, performance of the tag during different behavioral states.

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2) Impact of the tag on the animal: how did the animal respond to the cup attachment, did the animals respond differently to the tag designs, and what was the impact of the suction cup attachment on the skin?

Objective 3: Experimental evaluations of multi-day tag attachments with wild animals

Field versions of the tags from Objectives 1 and 2 will be used to collect data from multiple humpback whales in the Stellwagen Bank National Marine Sanctuary. Three to five tags will be fabricated for testing with humpback whales. During these field trials we will examine:

1) Duration limit of the multi-day DTAGs.
2) Animal behavior over multiple diel cycles.
3) Demonstrate capability to measure daily sound exposure budgets from environmental and opportunistic noise sources.

Results

Objective 1: Custom housings for the DTAG4 electronics were fabricated. The housing was designed to use the standard DTAG3 suction cups, and CFD simulations were used to refine the housing shape to reduce drag loading. Suction cups with two durometers were evaluated on rigid and compliant surfaces using the universal testing machine, Figure 1. Peak normal failure forces were smaller on the compliant surface (60 vs 30 *N*).

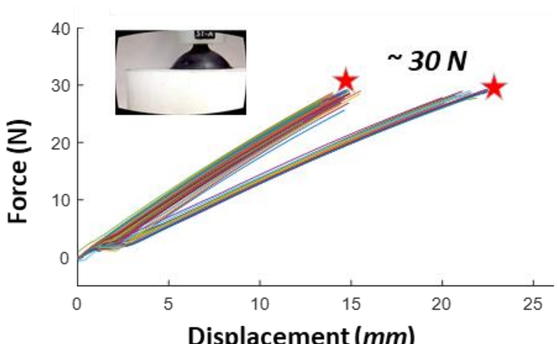

Figure 1 Results from cup testing on a compliant surface. Two different durometers of cups were tested and had similar peak failure forces.

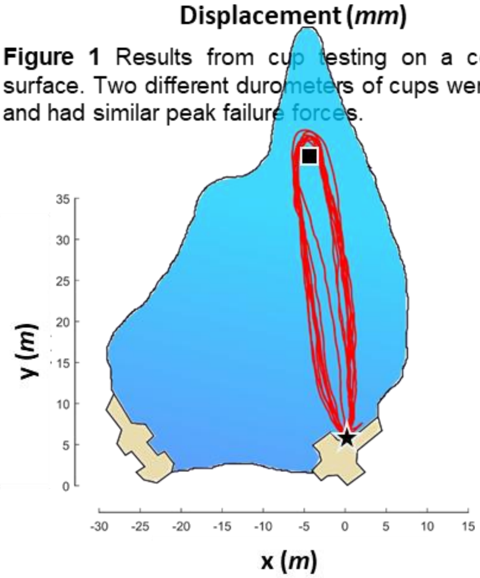

Figure 2 A representative track generated from tag data during a controlled swimming trial in the main lagoon at Dolphin Quest, Oahu.

Objective 2: Before field deployments, tags were successfully tested on animals in professional care. Two animals wore the tags during variable speed swimming trials, Figure 2. The tags remained attached to the animals during all the trials, and minimal impact to the animal's skin at the attachment was observed.

Objective 3: During the summer 2018 field work, nine tags were deployed on humpback whales. Four DTAG3s and five of the tags developed for the proposed work. All tags were deployed with the same silicone suction cups. None of the DTAG3s remained attached for longer than 24 hours. In contrast, the new design remained attached for an average of 40 hours. A representative track from one of the animals is shown in Figure 3.

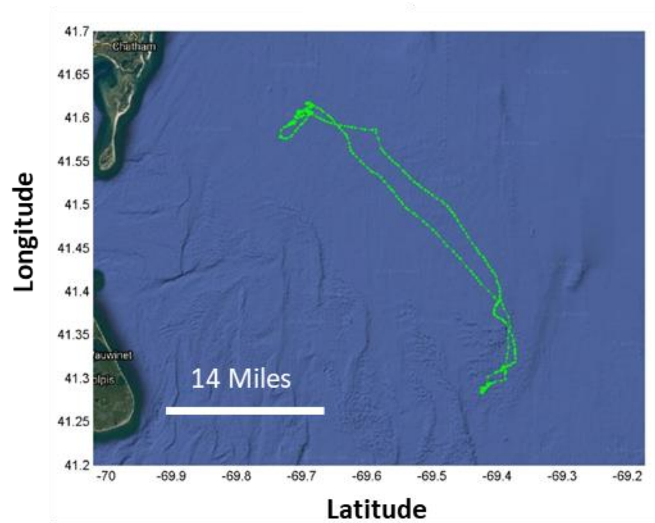

Figure 3 A representative track from a tagged humpback whale off the coast of Massachusetts.

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TOOLS AND TRAINING TO ENHANCE CAPACITY FOR ANALYZING HIGH-RESOLUTION TAG DATA

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Background

Bio-logging studies, where data are collected via animal-borne tags, are growing rapidly in number and scope, driving a need for freely-available, easy-to-use, flexible software tools and training to facilitate analysis and interpretation of the resulting data. Data from high-resolution tags are essential for assessing marine mammal behavior in relation to acoustic disturbance, as well as for acquiring baseline behavior for environmental risk models - both critical U.S. Navy interests. Although marine animals are the main focus of U.S. Navy interest in this field, we note that the same tags are increasingly used on terrestrial mammals and birds, so there is an opportunity to transition tools and methods from Navy-funded research into this larger scientific domain. While high-resolution tags offer exciting opportunities to observe animal behavior in unprecedented detail, analyses of these data are time-consuming and rely on a small cadre of highly-skilled scientists, creating a bottleneck in dissemination of these key findings.

Objectives

We aimed to create software tools to facilitate analysis of tag data and help increase the output and reliability of studies involving high-resolution tags. Before this project, the most comprehensive and authoritative set of tools for high resolution tag data were a set of Matlab scripts developed by Mark Johnson and others, freely available at https://www.soundtags.org/dtags/dtag-toolbox/.

Our project builds on this foundation, retaining the quality of the tools and their compatibility with all versions of the DTAG, but expanding their functionality in several ways. First, we enhanced the tools to enable use with other tag types (for example, Little Leonardo, OpenTag, Wildlife Computers Daily Diary, CATS tag, and Acousonde). We also developed new visualization and statistical tools critically needed for exploring and extracting information from dense multi-sensor tag data. In tandem, we documented the tools, creating detailed help files for each tool as well as a complete user guide, complementing these with a short course to train researchers in the use and analysis of high-resolution tag data. Finally, although the current Matlab tools are freely available, Matlab itself requires a costly license. We provide complete, additional versions of the tool kit in free software (R and Octave) to allow researchers to focus limited budgets on scientific output rather than proprietary software. To summarize, project objectives were:

1. Develop tools for tag data processing and analysis that are flexible, accessible, open-source, and that work with multiple tag types and software platforms (Matlab, Octave, R).
2. Develop and provide software tools that facilitate visualization and interpretation of complex tag data.
3. Develop and provide software tools that simplify processing and statistical analysis of multi-sensor tag data.
4. Devise and implement a multi-day workshop to provide training for tag data analysis using the tool kit.

Methods

We assembled an experienced team, as well as several undergraduate student researchers, to develop software tools, write documentation, and prepare and present workshops. Matlab, Octave, and R versions of each software tool were written and tested, with detailed documentation embedded and also posted on the project website. Our team developed and presented materials for workshops to introduce researchers to the tool-kit and provide hands-on practice at using it with real data. We created a wiki website, animaltags.org, to host all project materials (including software, documentation, presentations from workshops, and tutorials) and to facilitate feedback from users.

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Results

The tag software has been made available at http://www.animaltags.org and https://github.com/stacyderuiter/TagTools/, accompanied by documentation and workshop materials (lecture slides and practical exercises).

Tools are grouped into four modules. Tools for file reading/writing, calibration, and data validation make up the first module. These tools read in data files from multiple tag types, associate the raw tag data with appropriate metadata, and perform calibration and validation (for example, converting to an animal-centric frame of reference, and converting from raw measurements to standard scientific units). Finally, users can save calibrated data and metadata together in a well-defined standard file format (netCDF) appropriate for archiving. While much of the module is complete, we are continuing to expand the range of tag types supported, and to provide a simple and user-friendly interface for editing metadata.

The second module of the tool kit includes data processing tools. For example, functions are provided to compute derived metrics like jerk, overall dynamic body acceleration, and minimum specific acceleration; to detect events; or to compute average or summary statistics for events such as dives or prey captures.

The third module of the tool kit provides tools for visualization of tag data. Ongoing work includes visualization tools to allow users to explore and annotate tag data interactively.

The fourth module contains tools for statistical analysis, for example Mahalanobis-distance-based dimension reduction and change-point detection, three-dimensional track reconstruction, and a rotation test to quantify changes in event rates.

Two conference posters raised awareness of the tools among potential users; these were presented at the 6th International Bio-logging Science Symposium (Constance, Germany, October 2017) and the 22nd Biennial Conference on the Biology of Marine Mammals (Halifax, Canada, November 2017). In addition, three hands-on workshops reached a total of 87 attendees from 13 countries: an initial three-day workshop in St Andrews, Scotland in August 2017 was followed by a four day workshop in Aarhus, Denmark (October 2017) and a one-day workshop in Halifax, Nova Scotia (22nd Biennial Conference on the Biology of Marine Mammals, November 2017). A project paper entitled “Automated peak detection method for behavioral event identification: Detecting *Balaenoptera musculus* and *Grampus griseus* feeding attempts” and led by student David Sweeney is currently in peer-review.

After the first workshop, 86% (25 of 29) of participants completed a survey, which allowed evaluation of the workshop and motivated adjustments for future efforts. 100% of participants replied “Yes” to the question, “Do you expect to refer to your notes and the Wiki in future, and/or share them with others?”, and 84% indicated interest in attending a possible future advanced workshop. They also confirmed that there was a good balance of theory and practical work (with 76% saying balance was just right). Suggestions of topics participants wished had been covered highlighted areas of focus for future work: acoustic data analysis, statistical analysis, and data import/export. All survey results were taken into consideration in design of the two subsequent workshops.

This project has synergies with past and ongoing ONR- and Navy-funded efforts. The MOCHA project (https://synergy.st-andrews.ac.uk/mocha/) developed some of the statistical methods that are implemented in the tool kit and discussed and practiced in the workshops. Some of the software for processing and analysis of tag data builds on the ONR-funded SMRT Tags project. Tools from this project are being used in, or built upon for, the Atlantic Behavioral Response Study and the project “Cuvier’s beaked whale and fin whale behavior during military sonar operations: Using medium-duration tag technology to develop empirical risk functions”.

We hope that the tools provided by this project will help increase the output and reliability of studies involving high-resolution tags.

Notes:

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NEXT GENERATION SOUND AND MOVEMENT TAGS FOR BEHAVIOURAL STUDIES ON WHALES

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Background

Studies of the impact of human-sourced sound on marine mammals have been aided by multi-sensor tags such as the DTAG that simultaneously sample noise exposure and fine-scale behavior. However limited recording and attachment durations of these tags on cetaceans make them unsuitable for monitoring responses to longer sound exposures such as Navy sonar exercises. The 2-3 week longevity required to sample these events raises significant hurdles for tag design. In addition to the difficulty of attaching tags reliably for such long durations, it may be expensive to recover tags making it desirable to transmit essential data via low-rate satellite telemetry. Moreover to minimize impact on the host animal, the tag size is strictly limited, necessitating very power efficient electronics.

This project combines technology from the Sea Mammal Research Unit (SMRU) at St Andrews and Wildlife Computers Inc (WC) with the goal of producing a multi-week tag for behavioral response studies capable of recording continuous data and telemetering highly compressed summaries. In a prior analysis we concluded that telemetry throughput is likely to be low due to the limited battery volume in a low-drag shape. Given this, the tag is envisaged primarily as an archival device with telemetry serving as a recovery aid in which the location of the tag and an indication of the data it holds are sent repeatedly. However, *in situ* data summary algorithms can be added matched to the achievable telemetry throughput.

Objectives

This project, with a companion project by Wildlife Computers, will develop and field test a long-term sound and movement recording and telemetry (SMRT) tag for use on cetaceans. SMRU will contribute hardware, software and data analysis tools for this device. Goals are to:

1. Develop, and support the integration of, a low-power sound and movement (S/M) sensor board into a WC GPS and telemetry tag.
2. Develop embedded software to compress and summarize S/M data for satellite telemetry.
3. Adapt DTAG analysis tools for long-duration high-resolution data acquired by the SMRT tag.

Methods

The project involves 3 parallel developments: (i) the S/M board hardware and its integration into the WC tag environment, (ii) on-board software to collect sensor data and generate summaries for Argos transmission, and (iii) software for post-deployment analysis of the tag data. The S/M board performance is critical for the success of the project. It must be capable of continuous synchronous recording of multiple sensor channels (sound, acceleration, magnetometer, and WC-provided environmental data) with very low power consumption, noise and board size. The design builds-on a long-term DTAG developed for seals at SMRU. In this project, the design was further miniaturized and functions added for interoperability with the WC tag. Bench tests with the prototype S/M board revealed design flaws that affected reliability and noise pickup. These were rectified in a 2nd version which met specifications, and 23 units have now been transferred to WC. Close consultation with WC throughout the design process has facilitated system integration.

Embedded software for the S/M board compresses data for storage and generates brief summaries of animal behavior and the sound environment. This work leveraged algorithms created in a prior ONR project for automated analysis of acceleration, dive and sound data. These algorithms are robust to calibration and orientation uncertainties typical of biologging data. Post-processing software has also been adapted to accommodate the large data sets archived by the S/M board. This software enables rapid calibration and evaluation of the sound and motion data to verify tag operation and infer animal behavior.

Results

The S/M board has been evaluated at 3 levels: on the bench, integrated in the SMRT tag, and deployed on

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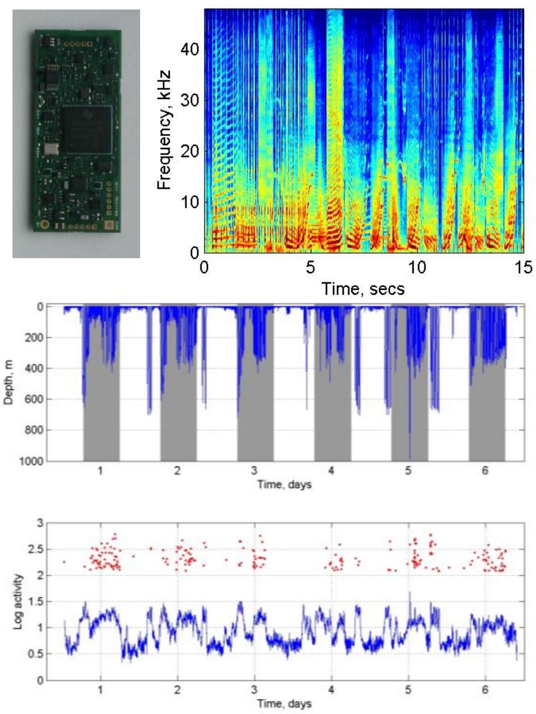
wild animals. In bench testing, the board met design goals. It can record continuously for 2 weeks on a single AA lithium battery at a 48kHz sound sampling rate while attaining a self-noise comparable to cetacean DTAGs. End-to-end testing of the full SMRT tag showed robust performance once some interoperability issues were resolved. Field testing took place in Hawaii in April 2018 on short-finned pilot whales. Tags were attached with 4 trans-dermal pins and included a radio-controlled release. For this first field test the tags recorded all sensor data but did not compute summaries to avoid risks associated with this complex software. Summary and reporting



software will be added in subsequent field tests.

Upper: S/M board and vocalization exchange recorded from pilot whales. Middle: dive profile, shaded at night. Lower: mean activity and transients (red) from prey capture, sudden acceleration, or social contact.

Four of the five tags were recovered and these all contained continuous data recordings that ended when tags released after approx. 5 days. S/M board operation in the field was excellent. Vocalizations and ambient noise were recorded with high fidelity. Motion data was also of high quality indicating a rigid animal attachment. Some minor issues were identified including temperature sensitivity in the magnetometer which has been corrected by post-calibration. Flow noise and strumming sounds were evident in two tags indicating that attachment location and orientation can impact performance.

The field data is being used to test summary and reporting software. Statistics reported by this software encompass diving behaviour (number, duration and shape), activity level and ambient noise. These data are compressed into a single Argos message (29 bytes). Activity is reported as both an average and the number of acceleration transients (e.g. due to foraging or startle). Sound levels are reported as lowest and highest 5%iles in 6 octave bands to characterize continuous and peak noise exposure.

Data collected in the trials tracked diving and detailed echolocation foraging behavior over multiple day/night cycles which has not been possible previously with pilot whales. They also provide vocalization rates as a function of behavior including highly stereotyped calls that may be signature whistles. The 500 hour recording time of the four SMRT tags could only be obtained with suction cup tags by tagging many more individuals. Thus risk of discomfort and harm due to the trans-dermal attachment must be weighed against potentially fewer attachments, an ethical trade-off that must be decided on a case-by-case basis. The SMRT tag is now approaching readiness for Navy research use and will enable much needed longer-duration studies of sonar responses.

Notes:

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Development of a multi-week Sound and Motion Recording and Telemetry (SMRT) tag for behavioral studies of whales

MJ Holland and RD Andrews

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Background

The need to understand the relationship between anthropogenic noise and whale behavior has led to many investigations into the responses of marine mammals to sound, and these studies have relied heavily on sound recording multi-sensor tags such as the DTAG. The DTAG has been an essential tool in behavioral response studies because of its ability to measure both the exposure intensity and the animal's responses on a synchronized time-line. However, in order to determine how these short-term responses translate into fitness consequences, it will be critical to obtain longer-term records than have been possible with the current designs of sound recording tags, which have been limited to recordings of less than 3 days due to electronics constraints and the attachment ability of suction cups. Therefore, we will develop and demonstrate a multi-week Sound and Motion Recording and Telemetry (SMRT) tag.

The technologies we propose to develop should significantly improve the ability of researchers and the Navy to monitor marine mammals around sonar operations. The ability to record the movements and sound production of cetaceans along with sounds received by the animals while precisely locating them in space and time over longer periods of time than is currently possible will vastly improve the Navy's ability to monitor cetaceans before, during, and after sonar exposure or other activities with the potential for disturbance of cetaceans.

Objectives

Develop a multi-week Sound and Motion Recording and Telemetry (SMRT) tag. Our proposed SMRT tag provides a unique combination of capabilities: multi-week sound recording, Fastloc GPS positioning, reliable medium-term cetacean attachment and data synopsis telemetry, which will make it useful for many types of cetacean studies, including behavioral response studies.

SMRT Tag Requirements (Sensors and Features): GPS for tracking animal at higher resolution than Argos localization; Argos transmitter for limited data transmission during deployment and after pop-off, primarily data about tag status that assists in prioritization of recovery; UHF receiver for detecting release command from user-held remote-release device; Depth (0.10 meter resolution); External (i.e. Water) Temperature (0.01 degree resolution). Wet /Dry Sensor; 1 GByte low data rate (i.e. 4 Hz sample rate) archive memory; 3D Accel @~ 50 Hz (Provided by Mark Johnson SM Module); 3D Mag @1Hz (Provided by SM Module); 64 GByte high data rate archive memory (Provided by SM Module); Hydrophone – single channel (sound processing provided by SM Module).

Methods

In order to develop a tag that meets the requirements above, our approach is to continue the collaboration between Wildlife Computers (WC), Dr. Russel Andrews (MarEcoTel) and Dr. Mark Johnson (Univ. of St. Andrews and Sea Mammal Research Unit, SMRU), working towards the design and development of prototypes that will undergo significant field testing, with iterative improvement cycles. The SMRT tag will incorporate a sound and motion (SM) module provided by Dr. Johnson, and we will work collaboratively to successfully integrate the SM module into the SMRT tag, including developing test procedures and inter-board communication software.
In order to turn our SMRT tag design goals into a physical design, our overall work plan was to conduct the necessary electrical and mechanical engineering tasks in parallel, construct complete prototypes, conduct laboratory and simulated field conditions testing, and then deploy the best possible prototype tags on cetaceans.

Results

The initial design and development work culminated in the production of a prototype deemed ready for field trials with cetaceans. The physical prototype design for field trials was the third design, arrived at after construction and testing of the first two physical prototype designs identified elements of the design that could be improved. The prototype #3 design included all of the WC electronics components, as well as the SMRU SM module, which could sample sound at 48 or 96kHz, acceleration on 3-axes at 200Hz, and a 3-axis magnetometer at 50Hz. Audio gain is adjustable, as is the range of the accelerometers (full scale of 8G). Sampling at 96kHz, the SM module's memory chip is likely to fill in approximately 7 days, depending on the compression

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achieved. For most species, sampling sound at 48 kHz will suffice for most purposes, and in that case the memory will likely stretch to 14 d. Extensive lab testing included simulations of a variety of field conditions.

The tag's outer shape is optimized for performance on the whale, primarily to limit the forces that result in premature loss of the tag. The tag package was designed to interface with a tag holder at the end of a hand-held pole and remain secure during tag deployment operations from a boat. When floating in the water, the tag is oriented so that the GPS and Argos antennas are clear of the water. The tag includes an interface to the attachment anchor assembly (baseplate) via a remotely-releasable connection. This remote-release component is refurbishable by WC. The primary lithium batteries can be replaced by WC. Laboratory testing demonstrated that the tag can withstand deployment and attachment forces, pressures equivalent to 2000 m depth, and extended immersion in salt water.

Prototypes were constructed for field trials with short-finned pilot whales off the island of Hawaii, working with our collaborator Dr. Robin Baird and colleagues of Cascadia Research Collective. We anticipated deploying the tags and leaving them attached for approximately seven days, which is when the SM module memory would likely fill with our chosen audio sampling rate of 96 kHz. After that, we planned to find each tagged whale and release the tag via the UHF signaling remote-release controller. However, if it wasn't possible to re-approach a tagged whale around day 7, then the tag would continue to collect low-rate depth and acceleration data with the WC sensors, and Fastloc GPS data for position estimates. At day 10, the tags were programmed to sever the link between the baseplate and the tag body so the tag would start floating for eventual recovery. For the final design, we anticipate using standard length (6.7 cm penetration) LIMPET darts for attachment, for these initial field tests we used short (4.5 cm penetration) darts.

In April 2018, we deployed five SMRT tag prototypes on short-finned pilot whales in Hawaii. We successfully used the remote-release to recover one tag 5.9 d after deployment, and three tags prematurely detached from the whales 4.5 to 5.4 d after deployment. All four of those tags were recovered, data were downloaded and analyses were conducted to identify areas for improvement. One tag suffered an electronics failure 6.8 d after deployment, which we later diagnosed as a tagware code problem that we fixed.

Overall, the initial field trials of this medium-term SMRT tag design were very successful. We have completed a thorough analysis of the performance of the four tags that were recovered, including the deployment process and performance of the tag while attached and while floating on the water. Data were shared with Dr. Mark Johnson as he develops improved data synopsis algorithms for telemetry as well as data handling and analysis tools for off-line processing of archival data collected by SMRT tags. Small mechanical changes were made to improve the deployment process; most other improvements for SMRT version 2 involved tagware code changes. Version 2 has successfully undergone significant lab testing, and we plan another field trial targeting odontocetes in Hawaii in April 2019, followed by trials in southern California, targeting beaked whales and fin whales.

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The use of CTD transmitters on baleen whales

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Background

There is an increasing need for comprehensive oceanographic sampling, and the use of marine mammals for augmenting spatial and temporal coverage, especially in remote and inaccessible areas, is an attractive approach. Electronic ocean sampling tags that relay profiles of temperature and salinity via satellite have been deployed extensively on seals. Data loggers that record conductivity temperature and depth (CTD) have not previously been used on baleen whales thus deployment techniques and general performance should be assessed before the technique can be widely implemented. Two field seasons (2017-18) were conducted to test a new CTD data logger on bowhead whales.

Objectives

The objective in 2017 was to evaluate a newly developed CTD tag with an electrode cell, and compare the performance with that of a commercially available tag that uses an inductive cell. The objective of the 2018 field season was to augment the sample from the CTD tag used in 2017 and to test a revised sampling scheme supported by a larger battery capacity.

Methods

An electrode cell CTD Argos satellite-linked tag was developed for this study by Wildlife Computers (WC). The WC tag was compared to an inductive cell CTD Argos satellite-linked tag manufactured by the Sea Mammal Research Unit (SMRU).

The inductive cell in the SMRU tag was encapsulated in epoxy and presumably less sensitive to biofouling and more robust to physical stress than electrode sensors. However, the inductive cell may be affected by the mass of nearby objects e.g. the large body of a whale. Locations for the SMRU tags were estimated via the Argos location system alone, whereas the WC tag provided both Fastloc GPS and Argos locations. The WC tags were programmed to capture the deepest profile for every 6-h period (4 profiles per day) and were set to transmit each profile eight times. The fixed depths chosen for WC tags were based on the Levitus World Ocean Atlas (WOA) standard depths. Each Argos transmission was programmed to contain 8 depth pairs of salinity and temperature. Consequently, if a dive was deeper than 125 meters, the profile data were transmitted in two separate messages to the Argos satellites.

The SMRU tags were set to record the two deepest profiles every 2 hours, resulting in 24 profiles captured per day and each Argos message contained up to eight temperature and salinity pairs. The SMRU tag used the WOA standard depths, with 8 predefined depth levels as well as 6 ‘broken stick’ depths. Each CTD profile were split into two Argos messages with the eight predefined depths in the first message and the 6 broken stick measurements plus the shallowest and deepest depth triplets were sent in the second.

All the CTD transmitters were mounted a rectangular stainless-steel base plate. The distal part of the plate had a rounded extension that included a hole for the attachment of a stainless-steel spear. The single point attachment allowed the tag to rotate 360 degrees. Tags were deployed with a pole.

Results

We instrumented nine bowhead whales in Disko Bay, West Greenland, in 2017 with two different CTD loggers. One bowhead whale was fitted with both tag types and the tags were positioned on approximately the same height over the water level on opposite sides of the whale, with equal opportunities to transmit data (see Fig. 1). Another 10 WC tags were deployed in 2018.

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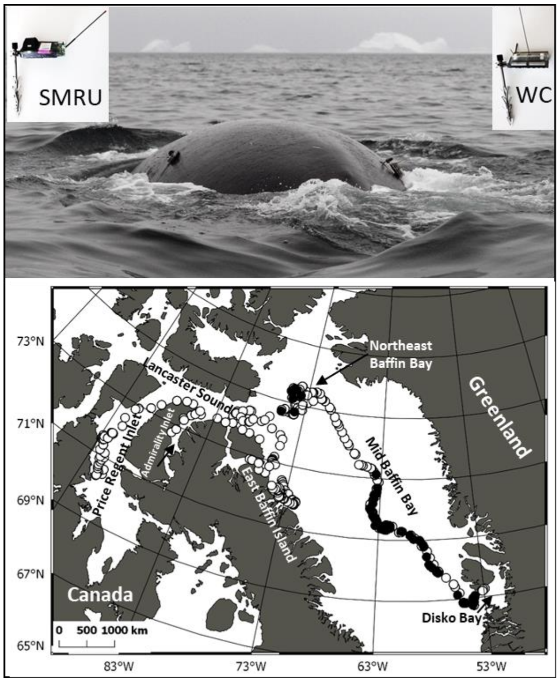

Figure 1. *A) Photo of the back of a bowhead whale instrumented with two CTD satellite tags. B) Map of the positions received by the two tags on the same whale. The SMRU tag is shown with black dots (7 May to 29 June) and the WC tag with white dots (7 May to 23 August). Note the unexplained breaks in the track from the SMRU tag. Only positions with associated CTD profiles are shown.*

Average duration of transmissions from tags lasting >10ds in 2017 was 98 ds for the WC tags with reliable practical salinity units (PSU) reocrds for 73% of the days, and 71 ds for the SMRU tags with 80% good PSU measurements. In 2018 the WC tags had an average duration of 121 ds, but salinity profiling was only reliable for a disappointing 18% of the days.

There was a small offset between salinity values measured by a calibrated CTD and WC tags compared to the SMRU tags. On average, the WC tags measured 0.002 (SD=0.03) PSU lower compared to the calibrated CTD. SMRU tags all measured slightly higher salinity with an average offset of 0.06 (SD=0.03) PSU. Temperature measurements by the WC tags measured an average offset of 0.005 ± 0.05°C and were not significantly different. The temperature from the SMRU tags measured an average offset of 0.1 ± 0.2°C and was significantly different.

Because Argos messages can only contain up to eight salinity and temperature pairs, we registered that at least 10% of the total profiles from the five WC tags were incomplete as only temperature and salinity measurements from depths of ≥150 m were received.

For the shallower profiles, the five SMRU tags had a higher vertical resolution, with more data from the depths in the upper part of the water column (≤ 20 m) and no data were available from the SMRU tag for depths >200 m even when the WC tag provided data for 200 to 300 m in the same region.

When comparing the longevity of two tags deployed on the same whale, the WC tag transmitted data until 23 August, while the SMRU tag stopped transmitting on 29 June. When both tags were functioning, the WC tag provided a higher number of CTD profiles (135 vs. 80), equalling 2.6 and 1.5 CTD profiles/day, respectively. It is, however, important to note that the position of the antenna and the tag on the whale may influence the performance of the tags to an unknown degree.

A cross section of the salinity and temperature regime in Baffin Bay and adjacent waters collected from the WC tag between 7 May and 23 August 2017, demonstrated the contrast between the warm saline water at depths greater >100 m in eastern Baffin Bay in relation to the colder less saline water in western Baffin Bay and the Canadian High Arctic. Furthermore, the profiles show that surface waters (<50 m) were warmer in the western region. From both salinity and temperature profiles, it is evident that data from the WC tag provided better coverage, both in time and depth, than the SMRU tag.

The problems with the CTD profiles in the 2018 deployments are being analyzed and a modified version will be deployed in 2019.

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Raven-X: A scalable high performance computing framework for the analysis of large bioacoustic sound archives

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Background

The U.S. Navy is regularly required to acoustically record and monitor ocean noise levels and marine mammal populations in U.S. waters, commonly inside naval sonar training ranges, to comply with regulations promulgated by NOAA/NMFS. Data generated within the scope of these monitoring efforts (e.g., cabled installations with fixed or mobile hydrophone systems) exceeds several hundred terabytes each year. A multi-species analysis, which is typically mandated by NOAA/NMFS, often requires running semi-automatic detectors and classifiers sequentially on local computers. This processing is relatively slow and it is difficult to insert algorithms from the research community. For these reasons, analysis of large acoustic data sets has been identified as a limiting factor, and needs to be addressed to keep up with the growing size of collected sound archives. Over the last several years, the U.S. Navy has provided funding to the Bioacoustics Research Program (BRP) at Cornell University to develop hardware and software solutions to address this issue. With funding acquired through an ONR/NOPP grant in 2011, a baseline high performance computing (HPC) system and the initial Matlab HPC Toolbox (Raven-X) was developed in order to bring next-generation acoustic data mining to the bioacoustics community. Raven-X has supported a variety of U.S. Navy analysis efforts and features some of the most popular detection and classification algorithms used by the U.S. Navy and research community.

Objectives

The major objective of this project was to (1) create an open architecture, adding several common algorithms to Raven-X (2) update the HPC processing models to provide a scalable, hardware independent solution that supports advanced analytics and (3) make tools available to the research community, especially to Naval computing environments.

Methods

Raven-X is a collaborative development project currently managed in GIT. A relatively simple object-oriented (OO) interface allows users to take serialized, detection and classification algorithms and include them as a data-mining routine. This offers an alternative to writing code in faster lower level languages, which can be time consuming and challenging. Researchers performing basic and applied work, can quickly prototype algorithms in Matlab and deploy them across a distributed computing platform making minor modifications to comply with the Raven-X interface. Raven-X supports a variety of detectors for harvesting sounds from marine mammals. These include the General Power- Law (GPL) detector (Helble *et al.*), Silbido (Roch *et al.*), ERMA (Klinck and Mellinger) and various other algorithms, such as matched-filtering and connected region analysis (CRA) (Dugan *et al.*). Various acoustic measurements can be extracted on each detected event (Fristrup *et al.*) (Mellinger *et al.*), and used for post processing and advanced analytics. Additionally, Raven-X provides the ability to read standard and custom sound types (wav, aif, flac, navy-dat). Data- mining outputs can be saved in a multitude of file types (Raven Pro, Tethys, xls, csv or custom format).

Hidden to the user, Raven-X uses a collection of complex routines that work to balance out system resources, efficiently distributing sounds and data processing across the computing hardware. Recent design enhancements minimize the overhead on memory resources, allowing algorithms to scale more evenly while offering the ability to record large amounts of data, such as acoustic measures, used for doing advanced analytics. Unique algorithm distribution allows this technology to run on desktop computers, enterprise level, as well as cloud-based server environments.

Results

Throughout the lifecycle of the Raven-X development, models are constantly refined and tested using existing and new detectors. For this review tests were performed on a single workstation and multi-server HPC system at BRP, see Figure-A and Figure-B respectively. Running the GPL algorithm, 42 features were extracted for each detection event and logged to the system output. Referring to the figures, Raven-X provides consistent allocation of system resources (CPU, Memory). For very large archives, Raven-X may log over a billion data points – processing models proved scalable and stable. Testing has indicated that system memory is a limiting factor for maintaining stable operating margins with high sample rate cases, (e.g. 32 channels x 96kHz). However, with sufficient system memory, Raven-X

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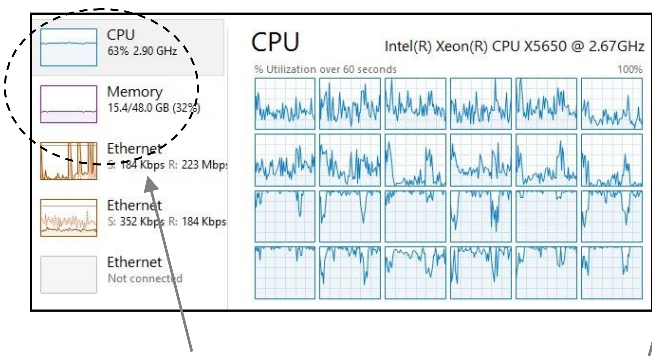
provides a successful solution for processing these datasets. Testing also showed that a distributed system architecture is ideal for handling high sample rate sounds where operators can adjust and transfer workload according to the data requirements.

In fall 2018, Raven-X was deployed at the SPAWAR laboratory, Point Loma, San Diego, CA. Due to the sensitive nature of the data and meta-data, sounds were not available prior to integration and most of the development was done at the SPAWAR facility. Considerable time was spent developing a robust package to read Navy range-specific, multi-channel, time-code hydrophone recordings (M3R DAT format). At the conclusion of three weeks, the new Raven-X DAT package successfully decoded various combinations of time-code formats spanning from 2007 to 2017. As a proof of concept, a fin whale matched filter was applied to various data sets and detections checked for accuracy. The GPL humpback whale detector was successfully used across all 2017 low frequency datasets that were available at SPAWAR.

In collaboration with Marine Acoustics Inc. work is ongoing to provide a customized Linus version of Raven-X for installation at the Naval Ocean Processing Facility, VA. MAI will log noise metrics in near real-time for post processing analysis. A version of Raven-X, without Navy specific functionality, is currently being prepared for public release.

Raven-X running on a Workstation HPC

Raven-X running on a four (4) node



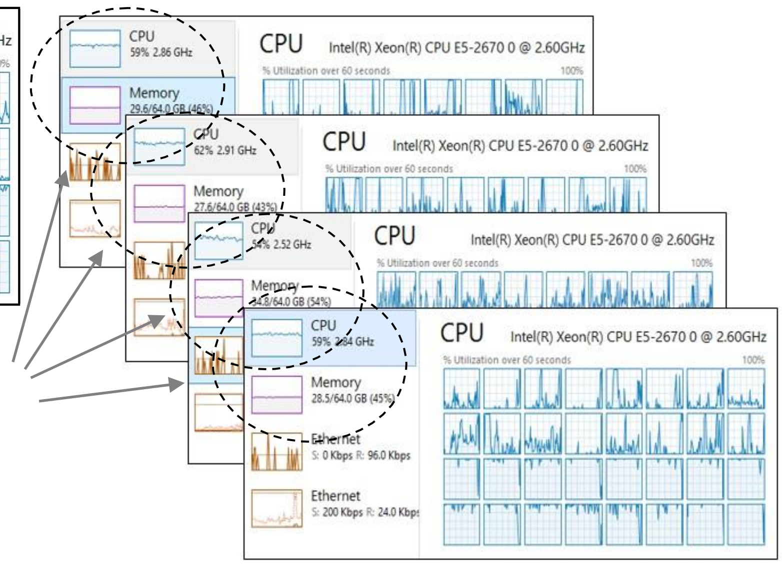


Figure A. Workstation Performance.

Figure B. HPC Performance

Two different computers running the GPL algorithm using Raven-X. Dataset consists of 32 channels from the Navy array. *(Figure A)* Raven-X running on a single workstation 24 processors, 64% CPU utilization, 32% memory usage. *(Figure B)* Raven-X running across (4) four computers on Cornell's HPC system, 128-processors. The HPC machine will run approximately 5x faster than on the workstation[1]. By using the new design, Raven-X provides a consistent resource utilization that is scalable across machines, keeping C*PU, Memory and Network* relatively the same. (*data, Courtesy Marine Acoustics Inc.*)

1 Computer workload varies based on many factors and the system architecture must be designed to accommodate data requirements.

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DMON Portable Range and Autonomous Recorders

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Background

Environmental regulation and ongoing marine mammal research have created a need to understand the movements and behavior of cetaceans in areas where the US Navy uses active acoustic systems for performance evaluation and training. While ranges such as AUTEC, PMRF and SCORE have permanent, bottom-mounted, acoustic receiver systems that can be utilized for detection and localization, not all tests can be done in these areas. Thus, there is an emerging requirement for a portable passive acoustic array that can be deployed for days to months in alternative locations to assess the local marine mammal population, at least to the extent feasible using acoustic methods.

This project has developed an easily deployed and recovered array of acoustic instruments that utilize chip-scale atomic clocks that enable accurate localizations of marine mammals using time-difference of arrival (TDOA) methods. The system is based on the DMON, a versatile acoustic instrument capable of both recording and processing audio from integrated low-, mid-, and high-frequency hydrophones. The DMON has been demonstrated in a variety of applications over the past years, including on Slocum gliders, wave gliders and buoys.

In summary, when combined with acoustic ranging and communications, the portable range will provide a passive acoustic localization tool that will enable detection and tracking of species of interest to the Navy in locations where there is no existing infrastructure.

Objectives

There are two main objectives for the program. Objective one is to develop the 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. Objective two is to continue the DMON work with additional development of the moored autonomous recording system. The components that comprise the new DMON Portable Range (DPR) and the autonomous recording system have been developed or are in use at WHOI, including the DMON, WHOI Micro-Modem, the chip-scale atomic clock (CSAC), plus and optional small surface buoy capable of receiving the real-time detection data from the DMON. The DMON2 development was separately funded by ONR, and is used with both the DPR and the autonomous recorder. This project has focused on integration of these components into the portable range that can be used for a variety of science and mitigation applications.

Methods

The original DMON design by Mark Johnson was recently modified to create a recorder that is larger but easier to fabricate and maintain (Figure 1). The majority of the circuitry is the same, but the form-factor, connectors and layout were revised to make it similar to the WHOI Micro-Modem family of processor boards. The housing was also changed and made larger to accommodate different types of replaceable

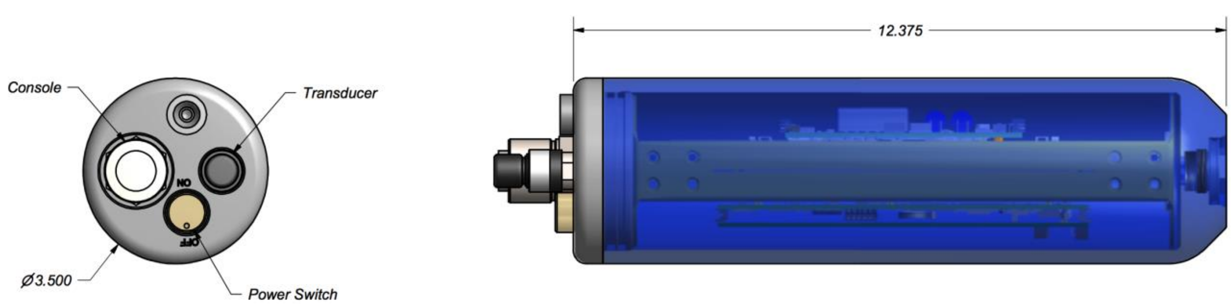

Figure 1 DMON-2 housing with two small rechargeable batteries for short-term deployments. The diameter is 2.5 inches and the unit is just over 12 inches long.

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batteries, both rechargeable lead-acid and lithium primary.

The portable range is intended for easy deployment and recovery and thus it includes a WHOI Micro-Modem that incorporates an acoustic release built into the end cap of the modem (Figure 2). Thus, the unit does not need an additional acoustic release, and it can be surveyed from the deployment ship for geolocation when the portable range is used for tracking. Obviously, the accuracy of tracking results using the array of recorders depends on how deep the bottom is and how long the mooring line is, but for coastal areas less than a few hundred meters deep the DMON can be on a short cable off the bottom to minimize errors due to tidal currents. However, for flexibility, the DMON housings as built for this project are suitable for 2000 m, though the modems with acoustic releases were fabricated for 1000 m.

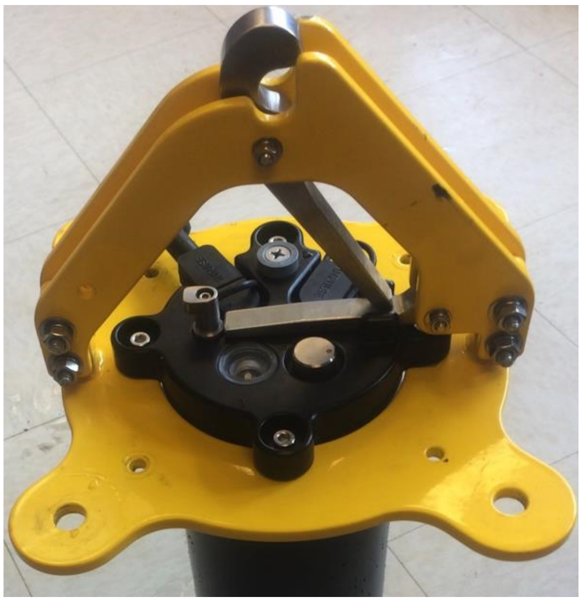

Figure 2 Acoustic release mechanism on the Micro-Modem.

Additional modularity is made possible by use of a multi-channel remote hydrophone head that can be place away from the housings and oriented as appropriate. This has been used on gliders and AUVs in the past.

Results

The original DMON firmware was modified to allow the on-board clock to be set from an external time source, and also to utilize an external pulse-per-second (PPS) signal. The PPS is generated by the CSAC timing module, and it is disciplined well before a field trial and then synchronized before deployment. The use of the external PPS to time-stamp recorded data records was validated using hundreds of hours of test recordings across eight different units with a synchronized source in the lab. The time synchronization is further guaranteed and can be checked by recording the PPS on one of the three available digital channels as a check.

The final validation steps for the DMON2-based portable range will be performed in shallow-water near Woods Hole in 2019. The engineering test will include deployment of four DMON2 units with acoustic releases. The units will be surveyed acoustically and their geo-locations calculated using methods we have used for localization of bottom transponders for mooring tracking. Known signals (FM sweeps) will then be transmitted from a surface vessel, with a GPS recording the actual position. The DMON units will then be recovered and the data processed to yield time of arrival, and then compared with the known transmission time and location to confirm that the time-of-flight measurement is correct. The position of the acoustic source will then be calculated to confirm the geolocation accuracy. Future work in 2019 will include a deployment near a site off Martha's Vineyard for evaluation of marine mammal recording, classification and tracking in a representative environment.

Notes:

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Monitoring for Shifts in Odontocete Range and Distribution in the Arctic and Sub-Arctic Seas

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Background

The ecological dynamics of the eastern Bering Sea shelf are a product of complex oceanographic processes in which physical processes are ultimately linked to biological production. The eastern Bering Sea is an important feeding ground for numerous migratory and resident cetacean populations which feed on a variety of organisms including plankton, crustaceans, fish, and other marine mammals. However, distribution and abundance of marine mammals in the Bering and Chukchi Seas are poorly described due to severe weather and costs for offshore, ship-based surveys.

Major changes in the eastern Bering Sea stocks of marine mammals have correlated with temporal shifts in environmental characteristics and physical forcing mechanisms (Livingston et al., 1999). Small climatic shifts in air and sea temperatures affect the time, extent, and duration of ice cover; thereby also impacting primary production, secondary production, bottom temperatures of benthic habitats, and migratory routes of marine mammals including bowhead and beluga whales (Grebmeier & McRoy, 1989; Grebmeier & Dunton, 2000). An acoustic baseline of odontocete distributions supplements visual surveys and provides more knowledge of how the ecosystem responds to variations in physical, climatic, and lower trophic level conditions. This is critical for continued long term marine mammal mitigation and monitoring efforts in the face of climate change throughout the Arctic Corridor.

Objectives

The major goal of the program is to develop knowledge of odontocete distributions along the Pacific Arctic corridor. Specific objectives include:

1. Documenting the distribution of odontocetes from the Bering Sea to the Chukchi Sea and developing predictive models of species occurrence.
2. Identifying any shifts in odontocete ranges or distributions along the Arctic corridor over time.
3. Relating observed shifts to key environmental parameters.

Methods

Passive Aquatic Listeners (PALs) and Acoustic Water Column Profilers (AWCPs: ASL Environmental Sciences, Inc., Victoria, BC) with 125, 200, and 460 kHz transducers were deployed as part of larger vertical mooring assemblages by NOAA's Ecosystems and Fisheries-Oceanography Coordinated Investigations (Eco-FOCI) Program (http://www.ecofoci.noaa.gov) in the Bering Sea at three locations from Sept 2007 to Sept 2018. Non-acoustic environmental data were obtained by accessing online databases. Ice data were obtained from the National Snow and Ice Data Center (NSDIC) which provides daily concentrations of ice from satellite data at a 12.5 km resolution. Sea Surface temperature was extracted from NASA as mapped daily from 40km x 40km boxes of 4 micro nighttime data.

Odontocete vocalizations in the PAL data were manually classified using Ulysses custom GUI software in MATLAB (Drs. Aaron Thode & Jit Sarkar). AWCP data were processed in EchoView (Myriax, Tasmania) and with custom MATLAB scripts. The occurrences of each species were Bernoulli distributions (presence/no presence) and were tested as response variables with 26 predictor variables using Generalized Linear Models (GLMs) with logit link functions. Generalized Additive Models (GAMs) were explored to determine whether any variable's degrees of freedom (DF) signified smoothing was needed (DF>1). If there existed high degrees of freedom in any highly significant variable (p-value < 0.05) in the GAMs and GLMs, Mixed Models (GLMMs, GAMMs) were explored. These environmental models were made following Zuur et al. (2009) and Miksis-Olds & Madden (2014) with custom R code.

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Results

Three species of odontocetes were acoustically detected in the Bering and Chukchi Seas that have previously been known to inhabit only waters south of the Aleutian Archipelago (Seger & Miksis-Olds, 2019). These include northern right whale dolphins (*Lissodelphis borealis*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and Risso's dolphins (*Grampus griseus*) (Fig 1). The coupling of a cold climatic (benthic-driven food web) regime with a negative phase Pacific Decadal Oscillation (PDO) (warm waters in the Gulf of Alaska / North Pacific) coincided with these acoustic observations.

Generalized Linear Models identified several environmental factors, and combinations thereof, that were uniquely predictive for the acoustic presence of each odontocete species along the Arctic Corridor. For example, beluga whales were detected early in the calendar year when daylight was shorter, sea surface temperatures were colder, ice cover was more expansive, and sound levels at 200 kHz were less intense. Their occurrence was also highly correlated to the acoustic presence of other species. The best environmental model for belugas whales included two interaction terms (Julian day with sea surface temperature, and sea surface temperature with percent ice cover) and is

$$PRES\_DL \sim DAY\_J + SST + ICE\_PERC + KHZSV + DAYS\_IA + DAY\_L + O\_SPPf + DAY\_J * SST + ICE\_PERC * SST.$$

Explanatory variables that were significant predictors of acoustic presence for at least two odontocete species in the Arctic corridor included sea surface temperature, percent ice cover, number of days since and until ice cover, daylight length, acoustic presence of other species, and water columns dominated by large crustaceans. Year was a significant predictor of acoustic presence for killer and sperm whales and its predictive power was most pronounced between 2011 and 2016, which aligns with the same temporal coupling of the cold climatic Bering Sea regime with the negative PDO discussed previously.

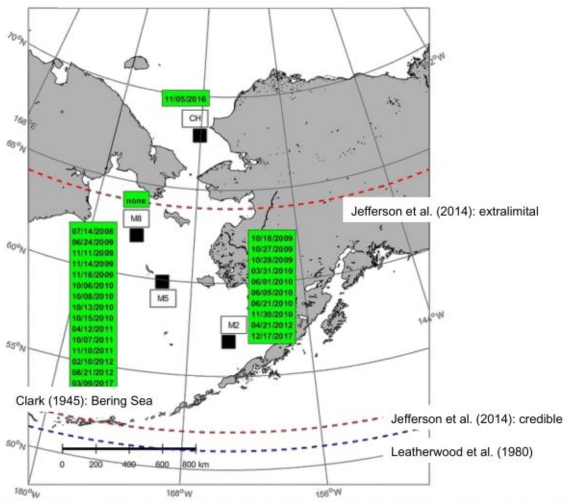

Figure 1: Map with dates of acoustic detections attributed to Risso's dolphins at sites M2, M5, and CH with dotted lines denoting historical range extents.

Grebmeier JM, Dunton KH (2000). Benthic processes in the northern Bering/Chukchi seas: status and global change. Impacts of changes in sea ice and other environmental parameters in the Arctic. 2000 Aug: 18-93.

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Livingston PA, Low LL, Marasco RJ (1999). Eastern Bering Sea ecosystem trends. Large Marine Ecosystems of the Pacific Rim: Assessment, Sustainability, and Management, K. Sherman and Q. Tang (eds.), Blackwell Science, Inc.. pp 140-62.

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Notes:

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Day 2: Wednesday April 24, 2019

2019 ONR
Marine Mammal & Biology
Program Review

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Whales in Estuaries: Glider surveys and fixed time series for understanding distribution

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Background

One of the current research thrusts of the ONR Marine Mammal Biology Program is Integrated Ecosystem Research, with particular emphasis on sensing and tag development. This study seeks to understand the environmental sources of variability in the distribution of marine mammals over a range of space and time scales, examining physical and biological factors. This is the basis for not only understanding, but also predicting the distribution of marine mammals. The study approach is multidisciplinary, and employs glider surveys and moored instruments, which allow us to examine broad temporal (tidal, synoptic, seasonal, inter-annual) and spatial (~50 km) scales. Data integration can be broadly used as a demonstration of the efficacy of glider surveys and fixed time series for developing predictive models of large whale occurrence that is in line with the “Integrated Ecosystem Research” theme of the ONR Marine Mammal program. Although we do not propose the development of a tag or a sensor in this project, we will be taking a glider hydrophone package developed with previous ONR funding, i.e. the digital acoustic monitoring instrument (DMON; Baumgartner et al. 2013) and combining it with other study methods (moored instruments). We will be using existing sensors and instruments and combining them in a novel approach to develop new applications for these existing technologies. The focus on estuaries in this project is relevant. The questions we ask about marine mammal distribution in this project have been examined in Eastern Boundary Current systems, but have not been specifically examined in estuarine systems to the best of our knowledge. Filling this knowledge gap is relevant given the relatively high number of reports on the presence of large whales in estuarine systems and the importance of estuaries for human activity, including port and naval infrastructure.

Objectives

1) Examine the effect of circulation and hydrography on the spatial distribution of phytoplankton (fluorescence) and large whales in the Corcovado Gulf. We will consider spatial variability associated with island wakes, effects of sills, constrictions, and bathymetric depressions, and effects from headlands, over the medium spatial scale (~50 km).
2) Examine the effect of temporal variations in circulation and hydrography on the temporal distribution of phytoplankton (fluorescence), zooplankton and large whales in the Corcovado Gulf. We will explore temporal variability over tidal, synoptic, seasonal and intra-annual scales.

Methods

This project seeks to examine the factors that drive baleen whale distribution in estuarine systems, using the Patagonian Inner Sea as a natural laboratory, by coupling the acoustically-detecting presence of whales with oceanographic data from ocean gliders (in real-time) and fixed bottom-mounted instruments.

A Slocum G1 glider was deployed during our first field season (April 2018) over a two-week period (Figure 1). The glider was equipped with a fluorescence, chlorophyll-a and turbidity sensor, a temperature, salinity and depth sensor (CTD), and a digital acoustic monitoring device, (DMON) for passive acoustic monitoring. A CTD profiler was also used to obtain CTD profiles of the water column at the deployment site. A second deployment is planned for April 2019.

Fixed instruments were deployed on a bottom-mounted mooring with an acoustic release at 170 m water column depth (Figure 1). Instruments included: 600kHz Acoustic Doppler Current Profiler (ADCP) at 125m; an Acoustic Zooplankton and Fish Profiler (AZFP) at 140m; a Songmeter hydrophone at 165 m and a CTD-O sensor at 165 m. This mooring was deployed in January 2018 with a turnover in October 2018. The mooring will be maintained until January 2020. Data available from a previous design of

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this mooring (without the AZFP) from 2016 will also be included in analysis.

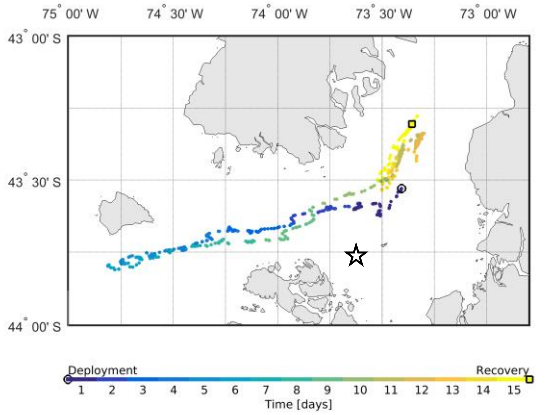

Figure 3. Map of study area with glider deployment track and fixed instrument mooring position

Results

Here, we share the preliminary results from this on-going project. The first year of data collection has been completed, which includes 12 months of moored instrument data collection and one glider deployment. A second glider deployment will be carried out during April 2019 and the mooring will be maintained until January 2020.

All glider data (oceanographic sensor and DMON) during the 2018 deployment was displayed real-time at http://dcs.whoi.edu/. This is the first time the DMON was used outside of the United States and Canada. The glider sensor (fluorescence, chl-a, turbidity, temperature and salinity) data has been processed and we show preliminary data plots. The real-time passive acoustic monitoring with the DMON was carried out successfully and calls were reviewed in real-time by SJB; this work will be presented. Detailed analysis of the passive acoustic data from the 2018 deployment is underway.

In terms of the mooring data, this project built upon a previous version of the mooring, that had a fluorometer but not an AZFP and was deployed in 2016 at the same site. The 2016 mooring data has been analyzed and will be presented. Analysis of the data from 2018 is underway.

This project allowed us to improve the mooring design and build technical capacity within our research group for building moorings that integrate both oceanographic and passive acoustic monitoring instruments. This project also allowed us to acquire an AZFP, which is the first instrument of this kind in the southeast Pacific.

We also provide a summary of the graduate students working on this project.

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Single sensor and compact array localization methods

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Background

This project aims to develop and implement and automated, model-based localization methods for single hydrophone systems and compact arrays. When possible, tracking results are used to study marine mammal behavior and bioacoustics. The outcomes will directly benefit practitioners and researchers using single hydrophone and compact arrays for PAM. They will also benefit projects with larger arrays in cases with weaker, more distant, and/or directional sources which are received on only a few (or single) sensor at a time.

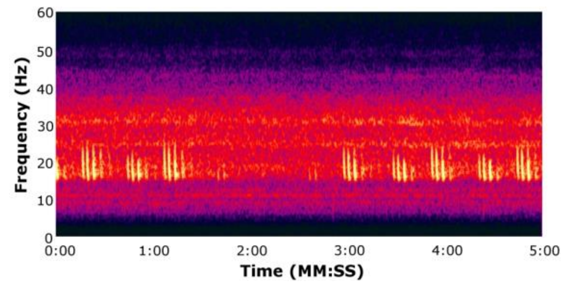

Figure 1. Fin whale calls recorded at ACO demonstrating multi-path arrival structure that is used to estimate range and depth.

Objectives

The specific objectives of this work are to:

1) Develop fully-automated time-of-arrival methods to locate calls using single sensors.
2) Modify and demonstrate model-based localization methods for compact arrays.
3) Explore the potential full wave-field methods to localize marine mammals using single and compact arrays.
4) Apply the methods developed in (1)-(3) to real-world datasets to demonstrate their applicability and to study marine mammal behavior and bioacoustics.

Methods

Sub-project 1 (with Brendan Rideout, PhD student). We developed a model-based ranging algorithm that uses the multipath structure of call arrivals (Figure 1) on a single bottom-mounted hydrophone in deep water to estimate the range and depth of calling animals. The method effectively passes a template of the modeled arrival structure over all detections to identify ranges and depths that produce a match between data and model. An important is improvement over prior related single-sensor methods is that the implementation aims to be fully-automated and robust in the presence of multiple animals and arrivals.

Sub-project 2 (with Yvonne Barkley, PhD student). We modified model-based wide-baseline localization methods for use with short-baseline arrays. As with wide baseline methods, ambiguity surfaces are formed by comparing modeled arrival times with data, and stacking surfaces over pairs of hydrophones and, in the case of a moving platform (e.g. towed array) over array position.

Sub-project 3 (with Anders Høst-Madsen, EE professor at UH, and Jeremy Young, PhD student). In some cases with overlapping calls from multiple animals it is necessary/helpful to separate sources with single sensors. We developed a Viterbi-inspired maximum-likelihood algorithm to separate click trains based solely on inter-call-interval for animals that emit call with regular inter-call-intervals (such as clicking odontocetes).

Results

Sub-project 1. Simulations were conducted to investigate performance and refine the algorithm, including in the presence of environmental mismatch and multiple calling animals. We processed 10+ years of data from the Aloha Cabled Observatory (ACO) hydrophone (bottom-mounted at 4.7km depth, 200 km N of Oahu). Figure 2 shows the results from one day. Several unexpected features in the processed data led us to revisit our sound propagation model, including looking closely at a

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seismic survey that passed nearby ACO in Fall 2018; efforts in this direction are ongoing. Another ongoing effort aims to estimate bearing (in addition to range and depth) in areas of azimuth-dependent bathymetry, which we anticipate will improve the results at ACO (at the expense of increased computational load).

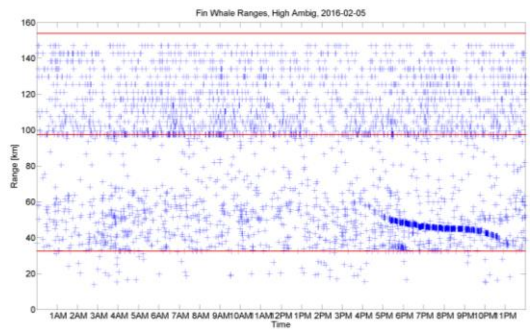

Figure 2. Estimated ranges from ACO on February 05, 2016 using the fully-automated single phone ranging method. Red horizontal lines show the ranges at which the Direct, Bottom-Surface, and Bottom-Surface-Bottom-Surface rays no longer connect. Detection threshold was set low to avoid missing detections at the expense of false alarms. One clear fin whale track emerges on this day at 5pm between 60 and 40 km range. Tracking after detection will eliminate false alarms.

Sub-project 2. The compact-array method was applied to two line array datasets; one vertical array with dolphin whistles and the other a horizontal (towed) array with sperm whale clicks (Figure 3). The compact array localization method has the advantage over some commonly-used methods (e.g. traditional time-motion analysis of crossed bearings) in that animal depth, sound speed inhomogeneities, hydrophone position uncertainty and other sources of error can be accounted for, and error estimates on location can be obtained directly and automatically.

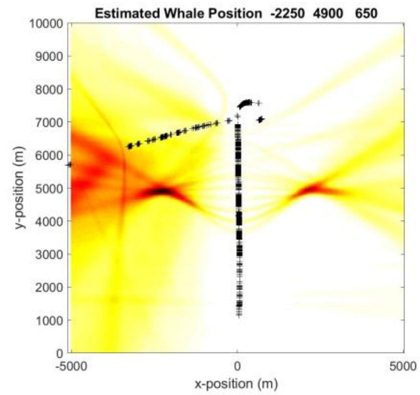

Figure 3. Ambiguity surface from compact array localization method showing areas of high probability of animal occurrence in red. The array in this example was a line array towed behind a ship. Black crosses indicate the location of the ship at the time of each detection.

Sub-project 3. The timing-based separation method was rigorously tested and refined on simulated data (Figure 4). It is currently being modified for and applied to a real-world sperm whale dataset, and modifications are being made to enhance separation by including additional features such as slowly-varying amplitudes and frequency content.

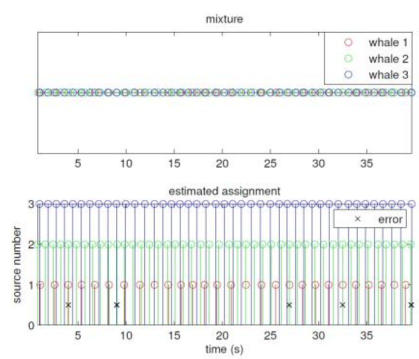

Figure 4. Example of source separation results for a simulated example with 3 clicking odontocetes.

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Using context to improve marine mammal classification

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Background

Traditional techniques for classifying passive acoustic detections of marine mammal calls to species rely on the classification of either single calls or groups of calls that all are assumed to come from the same species.

Objectives

We believe that exploiting contextual information and using deep learning techniques can advance classifier performance, providing better monitoring capabilities to the Navy. Examples of contextual information include location cues that can separate acoustic streams from different groups of animals, call type and timing, oceanographic variables, and historical monitoring data.

Methods

In the first year of this project, we examined multiple deep neural network architectures and demonstrated that deep learners could outperform existing technology in two different areas. We also began experiments on the use of location information to improve classification.

In the first set of experiments, we used a stock convolutional neural networks with minor modifications and data from the Detection, Classification, and Density Estimation (DCLDE) 2013 conference challenge to increase robustness to learn North Atlantic right whale (*Eubalaena glacialis*) upcalls. We compared the performance of our detector to that of other algorithms applied to the DCLDE 2013 test data. We then applied our detector to data from different geographic locations to determine whether it was generalizable and potentially could be used to monitor North Atlantic right whales across a large portion of their range.

In the second set of experiments, we examined the ability of a residual block deep convolutional neural network to learn to predict whistle contour peaks from spectrogram representations of toothed whale calls. We leveraged the detailed whistle annotations from previous ONR-sponsored work that were used in the DCLDE 2011 data to train our network, and incorporated data augmentation techniques into the iterative training process. We used the current estimate of the model to identify whistles with time-frequency peaks that, as measured by the recall metric, were difficult to predict. We used these difficult to detect whistles to generate similar synthetic examples (data augmentation) from which the model could learn. We then processed these time-frequency peaks with a previously published whistle detection algorithm to generate whistle annotations.

We developed an algorithm to automatically group calls based on similarities between time-difference-of-arrival based ambiguity spaces corresponding to successive calls. We increased ambiguity between calls to account for potential caller movement. In the future, this information will be used to segregate call streams that are fed into classifiers, allowing the concurrent use of multiple calls from the same general area to improve classification results.

Results

The North Atlantic right whale detector outperformed relatively recent classifiers that participated in the DCLDE 2013 classification challenge (Figure 1). Furthermore, we examined the detector that was trained on data from the Massachusetts Bay in other right whale habitats (off the coasts of Virginia, North Carolina, Maryland, and Georgia) where right whale upcalls are much rarer. We focused on recall rate vs. false positives per hour because we have established that in at least one laboratory, it is economically viable to have analysts validate detections when there are no more than 20 false positives per hour. In most habitats, we were able to recall nearly 75% of the calls while meeting the false positive per hour criterion.

The whistle contour detector (Figure 5) learned to predict time-frequency peaks associated with whistles better than existing methods and, unlike many instances in the literature, did not require special cases for eliminating interfering signals, such as echolocation clicks. We focused on the precision-recall of the entire system: the deep network, which predicted time-frequency peaks, and an existing ONR-funded graph-based peak assembly and disambiguation algorithm that outputs sequences of potential whistles as time-frequency lists. In experiments over a subset of the DCLDE 2013 data, the new algorithm had a precision of 95.7% and a recall of 83.7%. By contrast, when applied to the same data, the previous method that had a precision of 67.9% and a recall of 67%.

Progress on the localization task is described in our companion abstract by Kaitlin Palmer (poster presentation).

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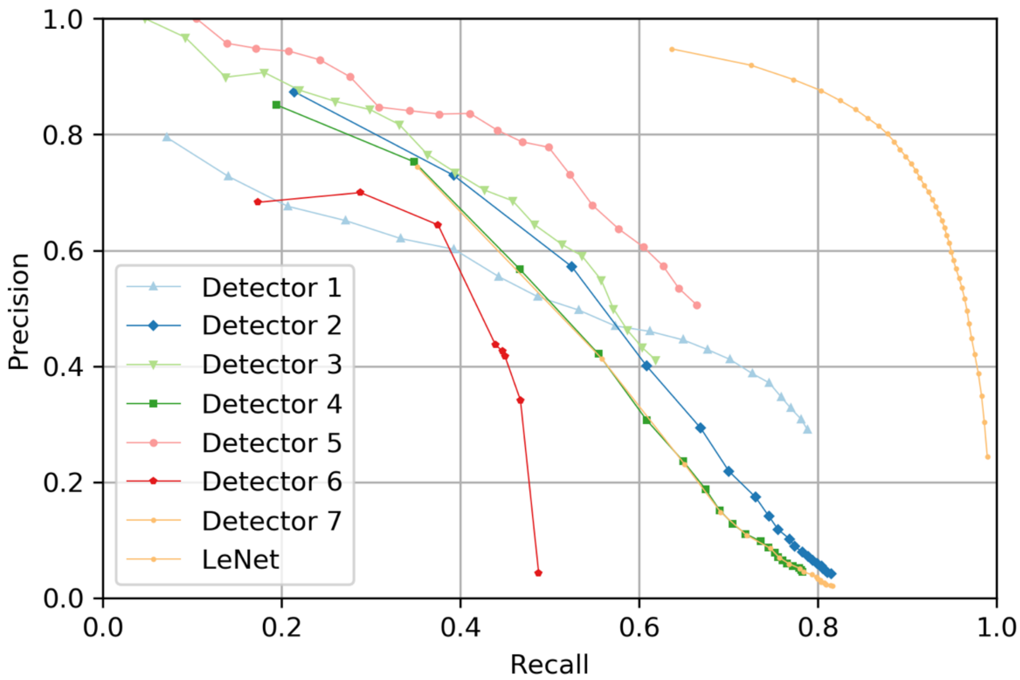

Figure 4 – Comparison of precision and recall performance at different thresholds between our proposed deep net architecture, which is derived from LeNet, and detectors entered in the DCLDE 2013 classification challenge. Better performance is toward the upper right.

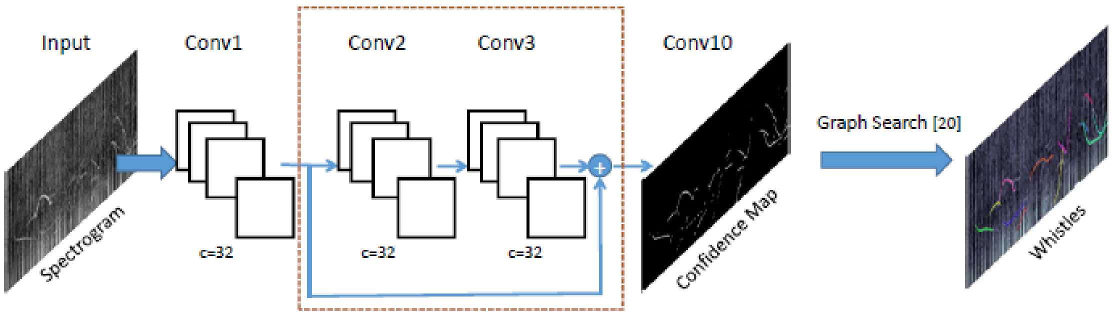

Figure 5 – Our deep convolutional residual network predicts time-frequency peaks in a confidence map, eliminating clutter without special processing. These peaks are fed into an existing algorithm.

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Estimating beaked whale density from passive acoustic recordings

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Background

Beaked whales emit characteristic frequency-modulated clicks that facilitate their detection and species identification from acoustic recordings (Johnson et al. 2004, Zimmer et al. 2005). Several studies have attempted to estimate beaked whale density from the acoustic detections of these species (Kusel et al. 2011; Marques et al. 2009; Moretti et al. 2010; Ward et al. 2013). However, comprehensive research to test these acoustic methods in study areas where independent data on the species' density is available for comparison, remain to be performed. Such research remains a challenging task to this day due to the typically-oceanic distribution of beaked whales and their low detection rates in line-transect surveys (Barlow et al. 2013). In the island of El Hierro (Canary Islands, Spain), populations of Blainville´s and Cuvier´s beaked whales have been shown to be found year-round in the lee of the island and can be monitored both visually and acoustically. The high sighting rates recorded from land observation stations and the low cost associated with the coastal operations needed here, make this area a unique field site for evaluating acoustic detectors and density estimators. The ability to estimate beaked whale density from passive acoustic detections, in real-world scenarios, is critical for the US Navy to comply with legal requirements under the Marine Mammal Protection Act, as beaked whales have been observed to mass-strand in concurrence with mid-frequency navy sonar operations (D´Amico et al. 2009). This project has, therefore, implications not only on the long-term monitoring of potential impacts of naval activities on these taxa, but also on the evaluation of the number of beaked whales potentially at risk in order to minimize possible exposures in real-time mitigation situations.

Objectives

The main objectives of this project are to 1) evaluate key acoustic parameters required for beaked whale passive-acoustic density estimation, and 2) to use these parameters to calibrate and improve the accuracy of density estimators by using passive acoustic data in combination with reliable ground-truth density estimations obtained from concurrent visual surveys.

Methods

We estimated beaked whale density (number of animals present in the survey area for a given day) within the bay of Las Calmas (SW off El Hierro, Spain), comparing two complementary datasets recorded simultaneously. One was obtained through visual observations from a land-based stations; the other was obtained from passive acoustic detections recorded by drifting buoys. These datasets were evaluated both by themselves and including independent data on beaked whales' distribution in the study area. Methodology used to obtain each dataset is described as follows.

Acoustic dataset

Passive acoustic monitoring: Four drifting GPS-tracked passive acoustic sensors, Soundtrap STD (144 kHz sampling rate), were deployed daily at 200m depth and ~3-4km apart, using a stratified sampling design in the leeward of El Hierro island. Soundtraps were then recovered each day before noon and acoustic records were downloaded and analyzed using an automated beaked whale click detector within the acoustic software PamGuard (v1.15.07). The true number of beaked whale clicks was obtained from exhaustive visual screening of the automated detector's outputs. Beaked whale density was estimated for a given day by a cue-counting method that uses number of individual beaked whale clicks recorded as cue, following Marques et al. 2009.

Beaked whale vocal behavior: Average click rate (number of echolocation clicks per second) of Blainville´s was estimated using an auxiliary dataset obtained with suction-cup attached

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digital tags ‘DTags’ (Johnson & Tyack 2003, Warren et al. 2017). For Cuvier’s beaked whales we used click rates published by Warren et al. (2017) and a combined average click rate was estimated for both species. GEEs were then fitted to these click rates in order to model any potential influence of social parameters (minimum group size, gender and presence of calves) on their click production patterns.

Detection function of whale clicks: The acoustic detection function was estimated using trials of clicks produced at a known range and recorded concurrently with DTags and passive acoustic sensors in the study area in 2008 and 2010. A multi-covariate model was used to derive the detection function and to identify parameters that may influence detectability such as range of the whale to the buoy, detection algorithm and depth or type of receiver. To control for environmental conditions, CTD casts were conducted in 2008, 2010 and during the present project. To account for the non-uniform beaked whale distribution in the study area (Arranz et al. 2011), independent data on the animals’ distribution was used. This was obtained from simultaneous double-platform point-transect land-based surveys overlooking the same study area. A probability density function of beaked whale sightings with respect to seabed depth was then modelled, using adapted distance sampling methods (Arranz et al. 2013), and applied to estimate the average detection probability of the acoustic sensors.

Visual dataset

Probability of visual detection: As ground-truth to calibrate the acoustic density estimates, we considered a Mark Recapture Distance Sampling approach to analyse the visual data and included distance, group size, sea state, visibility and seabed depth as covariates to correct for detectability bias. The visual detection function was calculated from beaked whale sightings logged during the double-platform land-based surveys that were conducted simultaneously to the acoustic survey. Density was estimated in a daily basis based on a Horvitz-Thompson-like estimator. The proportion of time that Blainville´s and Cuvier´s beaked whales spend at the surface, and during which they were available to be detected by the land-based platforms, was estimated from dive profiles obtained from the DTags. Beaked whale abundance was extrapolated to the whole study area (178 km^2) from the density estimate. Variance of abundance was estimated as the 95% confidence interval, calculated using nonparametric bootstrap without replacement and with day as the sampling unit.

Results

A total of 274h of simultaneous acoustic and visual beaked whale surveys were conducted during 40 field days, from August 2016 to May 2017. Overall, 781 beaked whale sightings were recorded and 949h of acoustic recordings were collected. Based on the varying environmental conditions throughout the study period, we subsampled a set of 8 days of data, encompassing 206h of concurrent acoustic and visual data, for further analyses.

Acoustic density estimates: In total 32,870 beaked whale click detections were identified, 68% of them considered to be ‘certain beaked whale clicks’ and 32% ‘uncertain’. Detected click rates averaged 147 clicks per hour [95% CI [103, 192], CV 0.82]. Variation in detection rates was not significant amongst acoustic sensors. Combined click production rate for Blainville´s and Cuvier's beaked whales was 0.40 clicks/second (95% CI [0.32, 0.50]). Mean acoustic detection probability, pooled over the 4 Soundtraps and the subsampled period, was P = 0.14 (95% CI [0.13, 0.15], CV 0.25). The estimated abundance of beaked whales in the bay using this passive acoustic data and over the subsampled period resulted in either 10 animals (95% CI [8, 12], *CV* 0.7) or 7 (95% *CI* [5, 9], *CV* 0.7;), depending on whether the ‘uncertain’ clicks were considered as beaked whale clicks or not.

Visual density estimates: Mean observer´s visual detection probability was P = 0.47. Combined availability of beaked whales to be seen at the surface was 0.26 (95% CI [0.23, 0.29]). The resulting estimated abundance of beaked whales in the study area and within the the same period using visual land-based data resulted in 9 animals (SE 1.1).

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

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Background

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

Objectives

This effort developed and implemented a methodology for estimating blue and fin whale density from passive acoustic data recorded on sparse hydrophone arrays in the Equatorial Pacific Ocean at Wake Island.

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

Methods

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

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

OBS data have also been used to inform method development. An array of 24 OBSs was deployed off the coast of Portugal for 12 months. Both range and bearing to fin whale calls can be estimated using the

OBSs (Harris et al., 2013). Therefore, using this array, density results obtained using bearing data can be directly compared with density results obtained using standard distance sampling.

Results

Results from this effort reflect 1) fragmented detection area over which density estimations were assessed (Fig. 1), 2) annual differences in estimated animal density at Wake Island (Fig. 2), and 3) estimated fin whale source levels from NE Atlantic and Central Pacific populations.

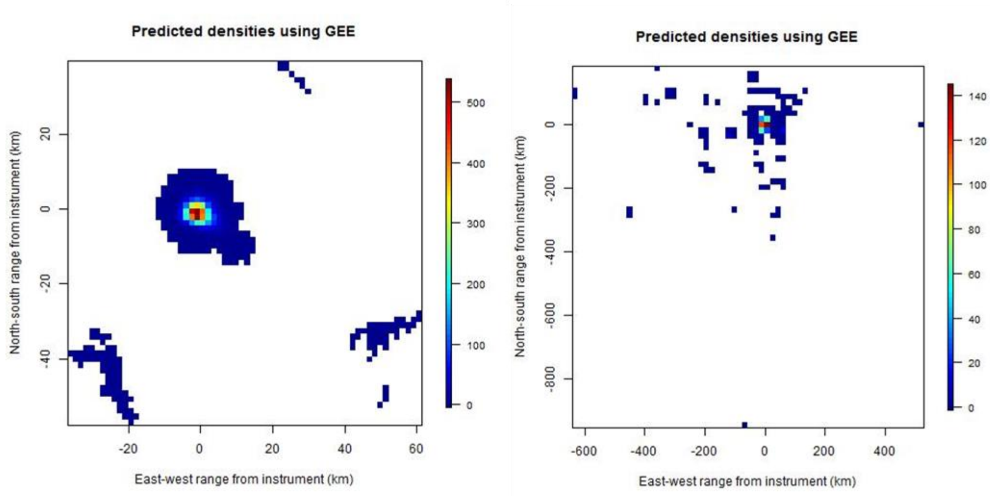

Figure 1. Two estimated geo-referenced plot from the 2010/2011 season (left) and 2012/13 season (right) at Wake Island showing the irregular and patchy area that is being monitored given assumptions based on measurements of source level, noise level, propagation loss, and detector performance. The colors depict the estimated call density (calls/km^2).

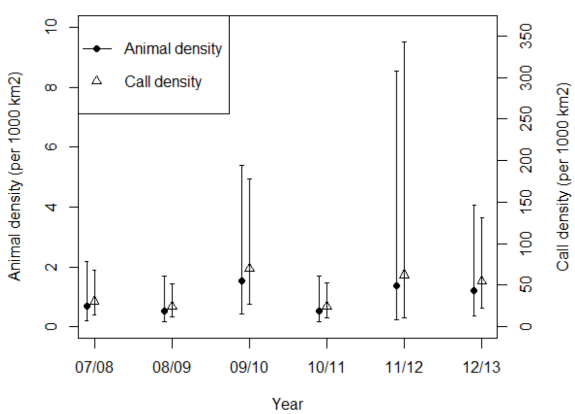

Figure 2. Predicted fin whale animal and call densities by season at Wake Island with associated 95% confidence intervals.

References:

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Notes:

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GROUPAM: Beaked whale group deep dive behavior from passive acoustic monitoring

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Background

While much information is known about individual beaked whale behavior at depth from Digital acoustic recording Tags (DTags), little is known about beaked whale *group* dive behavior at depth. This makes it difficult to interpret the results of single animal exposures to anthropogenic noise, and their impact on long-term foraging success and population health. The overall goal of this study was to provide novel information on the foraging dive behavior of groups of Blainville's beaked whales (*Mesoplodon densirostris,* Md) using Passive Acoustic Monitoring (PAM) at the Atlantic Undersea Test and Evaluation Center (AUTEC).

Objectives

1. Create a new detection, classification, localization and tracking (DCLT) method capable of utilizing AUTEC PAM data to track individual clicking beaked whales within group deep dives and with the potential for real-time implementation within the current M3R signal processing architecture. Apply to archived data to derive tracks.
2. Create a model relating acoustic footprint statistics (e.g., click detection counts, number of hydrophones detected, etc.) on hydrophones to group size and parameterize it using surface visual observations.
3. Create a statistical model of beaked whale group deep dive behavior using the results of (1) and (2). The model will include behavioral dynamics of individuals within the group, group foraging strategy and habitat preferences.

Methods

1. PAM-based tracking of individuals in groups.
Building on previous work, a new tracking algorithm was developed (Baggenstoss 2015), and a custom-designed graphical user interface (GUI) was written in MATLAB for whale DCLT. Over 150 hours of acoustic data were collected during field tests in October 2015 and July 2016 and this, together with archived data were analyzed using the GUI to determine the presence of potential vocal groups, and to localize individuals where possible.

2. Modeling group size.
A set of visually-verified observations of Md groups were obtained from previous validation exercises at AUTEC made between 2005 and 2008; these were paired with passive acoustic detection data from the M3R system. To increase sample size, acoustic-only data was included for a few group dives where detailed manual analysis gave a reliable estimate of group size. A statistical model (Generalized Additive Mode, GAM) was developed of group size as a function of explanatory variables (various acoustic footprint statistics) and model selection used to determine which variables were most useful in predicting group size.

3. Within-group movement modeling.
Distance between pairs of animals was modelled as an autoregressive Gaussian distribution that was a function of time since start of vocalizations and group size. A state-space model of 3D movement of individuals within groups with state-switching, allowing for irregular sampling and positional error, was attempted but not completed within the time-frame of the project.

Results

1. PAM-based tracking of individuals in groups.
A total of 29 groups were tracked with sufficient resolution for within-group movement modelling. Of these groups, 10 were single individuals, 10 were pairs and 9 were triplets. An example track is shown in Figure 1.

2. Modeling group size.
A total of 51 groups were used in the GAM (41 from visual sightings, 10 from acoustic-only data). Group size varied from 1 to 6 whales.

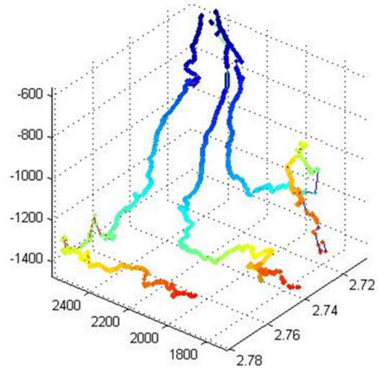

Figure 1. Tracks of 3 individuals from 10/1/2018 (color describes time into dive where blue is the beginning of dive and red is at the end).

The AIC-best model included just one explanatory variable: click rate – i.e., total number of clicks detected during the dive (Figure 2). As a demonstration of the utility of predicting group size of each dive, the fitted model was used to predict group size for dives in 3 time periods in 2011, and this was used as part of a dive-counting algorithm (Moretti et al. 2010) to estimate daily animal density at AUTEC (Figure 3). Results have been submitted for publication (Jorge et al. Submitted).

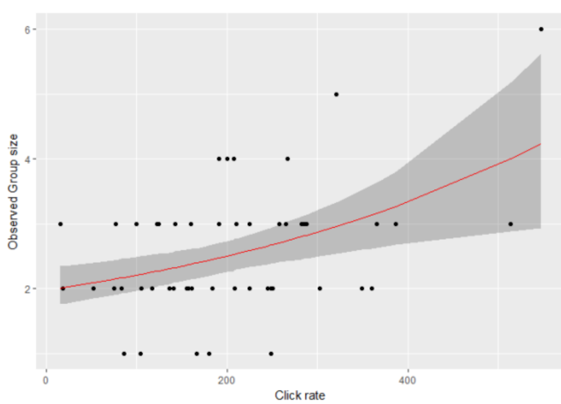

Figure 2. Observed group sizes and corresponding click rate (black dots) with the modeled relationship (red line) and 95% CI on mean group size (dark grey area).

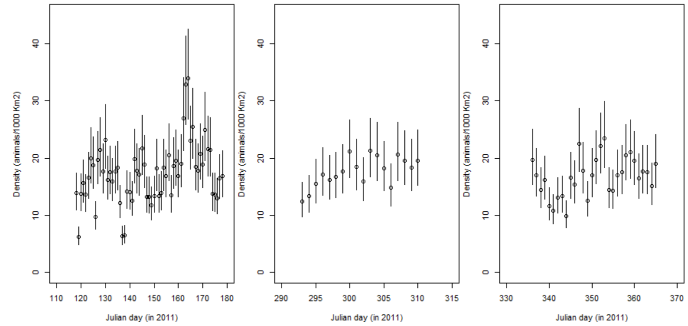

Figure 3. Daily beaked whale density estimates for 3 separate periods in 2011.

3. Within-group movement modeling.

Groups were observed at 2 distinct foraging depths (950m and 1300m) – these may represent different prey types. Individuals tended to start clicking at depth in close proximity, separate during the dive, and then come back together before ascending in silence (Figure 4). A manuscript describing within-group behavior is in preparation.

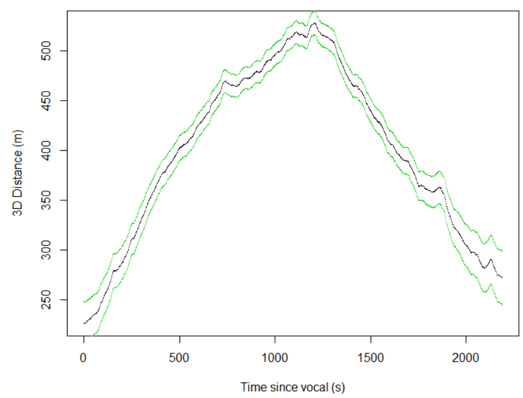

Figure 4. Modelled distance between pairs of animals as a function of time since onset of vocal part of dive. Green lines show 95% CI.

Notes:

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

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

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Background

Autonomous underwater vehicles such as ocean gliders and vertical profiling floats have the potential to play a key role in future marine mammal monitoring efforts. When equipped with hydrophones, these vehicles can collect passive acoustic data from marine mammals. The major advantage of gliders and other autonomous vehicles over existing passive acoustic monitoring approaches is their ability to provide both broad spatial and temporal coverage of a survey area. This is in comparison to moored stationary instruments that provide good temporal coverage but limited spatial coverage, or towed hydrophones from vessels that can have extensive spatial coverage during a relatively short timeframe. However, while a variety of methods have been developed to estimate animal density from acoustic data collected by fixed or moving platforms, methods for estimating cetacean density from autonomous ocean vehicles require development.

Objectives

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

1. Evaluate whether ocean glider data can be analyzed in a design-based framework using existing data from surveys completed in three areas of naval interest: Gulf of Alaska (GOA), Mariana Island Range Complex (MIRC), Hawaii Range Complex (HRC).
2. Quantify ocean glider/profiling float survey effort and evaluate encounter rates of example species at the same three areas of naval interest.
3. Develop a methodology for estimating the probability of detecting cetacean vocalizations on ocean gliders/profiling floats using data collected as part of existing projects at both the Southern California Offshore Range (SCORE) and the Undersea Test and Evaluation Center (AUTEC).
4. Estimate animal densities (or call densities if average call rates are not available) of example species at the three areas of naval interest using ocean glider data.
5. Develop an experiment that does not rely on a large Navy range (such as SCORE) to estimate a species' probability of detection by an ocean glider.

Methods

All deployment tracks from the GOA, HRC, and MIRC sites have been evaluated to assess the degree to which the planned track lines were adhered to. Example species have been selected (Blainville's and Cuvier's beaked whales, fin whales and Risso's dolphins) and encounter rates for these species have been calculated at the GOA, HRC, and MIRC sites. A literature review has also been conducted to compare encounter rates of the example species with encounter rates from previous visual surveys in these areas.

Data collected at AUTEC have been used to develop methods to estimate the probability of detecting vocalizations on an ocean glider. For Blainville's beaked whales, the range hydrophones at AUTEC were used to localize vocalizing individuals, effectively setting up detection trials for the ocean glider, which were modelled using a logistic regression analysis. This approach is also being implemented for fin whales using localizations at the SCORE range. Alternatively, in the event that localization is not successful for a given species, acoustic detections across hydrophone arrays can be used for spatially explicit capture-recapture methods (SECR) (Borchers, 2012) or a simulation approach can be implemented using existing acoustic tag data and propagation modelling (e.g., Küsel *et al*., 2011). A simulation approach was used to produce detection probability estimates for the Blainville's beaked whales at AUTEC, to compare to the trial-based estimates. We have also demonstrated an SECR analysis to estimate detection probability of Cuvier's beaked whales using a drifting non-profiling hydrophone array with a co-deployed glider and profiling float. This small field effort was conducted (in July/August 2016) with the aim of estimating detectability of cetaceans

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from a glider without using an instrumented Navy range. Finally, the detection functions generated for the ocean glider will be used to estimate local call densities on the AUTEC and SCORE ranges, and at the Catalina study site. If suitable call rate data are available, animal densities will be estimated at these sites as a demonstration of the methods.

Results

Results will be presented from all five objectives.
Objective 1 results have shown that gliders can be used in a design-based framework, despite deviation from their planned tracks. Objective 2 results demonstrate that the relative slow movement of the glider in comparison to the animals means that time must be considered when defining survey effort. Density estimation methods also need to account for animal movement, otherwise results may be biased – this has informed the methods being used in Objective 4. Regarding Objective 3, the AUTEC Blainville's beaked whale detection probability analysis suggests that detection probability at zero horizontal distance from a glider is not certain (Fig. 1) and detection probability increased with glider depth. Objective 5 used a three-dimensional localization algorithm to track Cuvier's beaked whales, estimating a mean foraging depth of 950 m (Fig. 2).

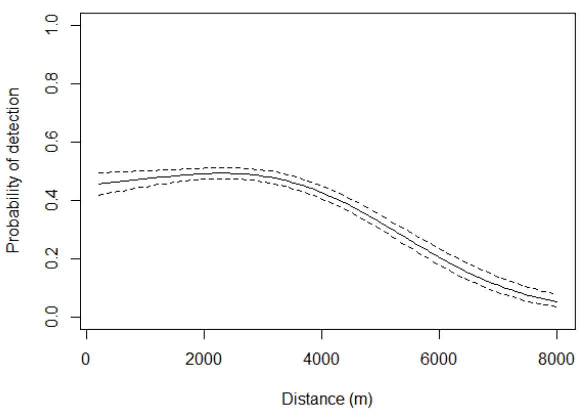

Fig. 1 Detection probability as a function of range for individual Blainville's beaked whale clicks using a logistic regression. Model standard errors are displayed (dotted lines).

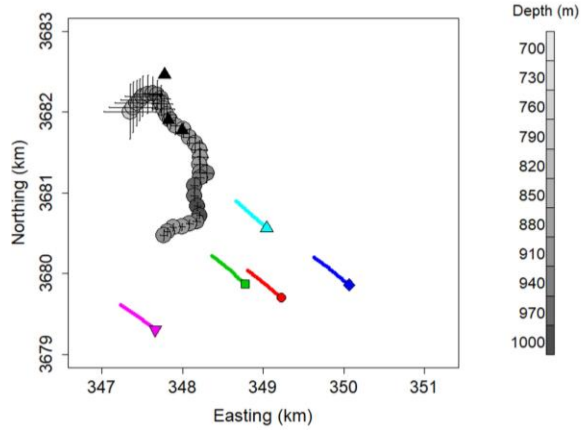

Fig. 2 Estimated 3-D tracks of a 35-min dive of a Cuvier's beaked whale (gray circles) detected on five drifting recorders (colored lines indicate buoy drifts over this time period). Grayscale indicates estimated depth of the track. Localizations based on surface reflections are illustrated as black triangles. Location error bars indicate two standard deviations from the Bayesian posterior distributions.

References:

- Borchers, D.L. (2012) A non-technical overview of spatially explicit capture recapture models. *Journal of Ornithology* 152 (Suppl 2), S435-444.
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Notes:

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

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Background

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

Objectives

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

Methods

Active acoustic sampling integrated into a deepwater AUV were combined with ship-based acoustic sampling to provide measures of potential beaked whale prey throughout the water column. Active acoustic sampling was complemented and ground-truthed utilizing a suite sampling methods including jigging, nets, and imaging along with measurements of the physical habitat. Beaked whale habitat was quantified using a combination of synoptic visual observations from the ship and passive acoustics from a towed array on the ship, sensors placed on the deepwater platform, and with the AUTEC range hydrophone array. These data were assessed in combination with historical patterns of habitat use from other studies in the same area In combination, these tools allow us to examine the relationship between beaked whales and their prey and asses the importance of areas in and around the AUTEC range to the foraging success of these sensitive predators.

Figure 1. The deepwater AUV used to measure the deepwater distribution of beaked whale prey is prepared for deployment.

Results

Within the AUTEC range, significant differences were observed in both mesopelagic and bathypelagic resources. However, total biomass and estimated squid size did not vary within the

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TOTO regions. To examine the potential drivers of habitat use in beaked whales, we included a variety of environmental and prey metrics as independent variables in a discriminant function analysis with sampling zone as the grouping variable. All of the variables in the first function, together explaining 78.5% of the variance, included, described prey within the depth range that beaked whales have been observed foraging. The most important were the density of 'squid' targets and the patchiness of 'squid' targets at 100-m scales. Abaco was most similar to samples from the highly used West zone, suggesting prey features in both regions drive the consistent patterns in beaked whale foraging observed throughout the region.

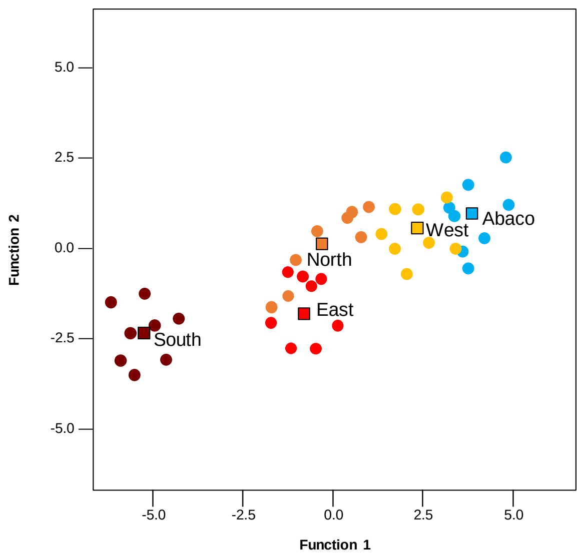

Figure 2. Results of a discriminant function analysis incorporating environmental and prey variables to predict sampling region. Increasing values of both functions are correlated with increasing habitat use by beaked whales. Our analysis suggests that prey resources at Abaco are significantly better than areas on and around the AUTEC range.

We combined our results from the Bahamas with analysis of the Navy's southern California (SOAR) range. In all three locations, including both temperate and tropical US Navy ranges, historical observations of a high degree of submesoscale variability in foraging activity by two species deep-diving beaked whales (*Ziphius cavirostris, Mesoplodon densirostris*) were used to guide sampling of potential prey resources. Across locations and dominant marine mammal species, we found that habitats preferred by foraging beaked whales had a high degree of spatial structure in squid at 100m-scales and significantly larger squid than less preferred areas within the depth range that beaked whales forage. Striking differences quantified in prey characteristics between regions at depth, however, were often not reflected in differences observed in surface layers and there were no consistent differences in total prey biomass between preferred and less preferred foraging habitats. Several marine mammal taxa have evolved remarkable physiological and behavioral characteristics to feed at depths exceeding 1000m. The convergent occurrence of this physiologically challenging and energetically costly strategy in obligate air-breathers strongly suggests that there are valuable prey resources available at these depths. The presence of biological heterogeneity at scales less than 100m contributes to the increasing evidence that our existing view of that the deep ocean as homogeneous and static is vastly oversimplified. This provides a more robust biologically-meaningful framework for how we study and interpret the behavior of deep-diving predators. Our results support the growing understanding that biological 'hotspots' are critical in driving predator behavior in pelagic ecosystems and underscore the importance of understanding resource distribution in effectively managing populations.

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

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Background

Oceanic delphinids are generally not endangered, nor have they been observed in mass-stranding events associated with Navy sonar, but these taxa include some of the most common protected marine mammal species (e.g., common dolphins *Delphinus sp.*, bottlenose dolphins *Tursiops truncatus*) exposed to sonar in high numbers in some Navy operational areas. Consequently, they represent a large proportion of predicted negative effects of sonar operations (e.g., behavioral harassment) for many Navy environmental compliance assessments. Their response probabilities have been inferred from laboratory measurements and/or from anecdotal field observations in uncontrolled contexts, each of which have significant limitations. Within this pilot project, we developed innovative methods to measure both broad and fine-scale group and individual observations and conduct the first-ever experimental behavioral response studies.

We developed a novel integration of established methods to quantify behavior in four delphinid species (long and short-beaked common, bottlenose, and Risso's (*Grampus griseus*) dolphins) frequently exposed to Navy mid-frequency active sonar (MFAS) off California. We evaluated potential responses to simulated (3-4 kHz) MFAS using controlled exposure experiments (CEEs). The nature of the resulting data is categorically different from previous response studies involving tagging of single individuals, with focus on both broad scale and finer-scale group movement patterns, behavioral cohesion and other social factors, including group acoustic behavior. Beyond the fact that retaining tags on individuals of these species has proven difficult or infeasible to date, these social species typically occur in groups and therefore behavioral similarity or variance between group members needs to be quantified to describe their responses to disturbance.

Objectives

This was truly a pilot effort in that no previuos studies had developed and applied integrated visual, photogrammetric, and passive acoustic methods to quantify even the behavior of these frequently fast and ephemeral species, much less measure behavioral responses to sonar. This project was also strategically coordinated with a study of physiological stress hormones during baseline and noise exposure conditions in order to evaluate physiological effects of sonar exposure. Collectively, the pilot field efforts had the following objectives:

1. Develop integrated methods to simultaneously track group movement and behavior using shore- or vessel-based visual observers, aerial photogrammetery, remote-deployed acoustic recorders.
2. Apply group-sampling methods using integrated technologies to better characterize typical (undisturbed) behavioral parameters for these species.
3. Obtain direct measurements of group behavioral changes and stress hormone responses in these delphinids, if any, resulting from experimentally controlled simulated Navy MFAS.

Methods

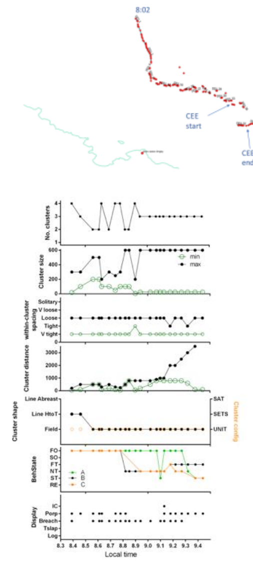
Our novel behavioral approach consisted of three complimentary data collection systems to measure aspects of baseline behavior and quantify responses to MFAS signals projected during controlled exposure experiments using a vertical line array sound source. These data collection systems include:

1. Shore- and vessel-based visual sampling
2. Unmanned aerial systems (UAS) for photogrammetry
3. Remote-deployed passive acoustic sensors
4. Biopsy sampling to obtain samples at strategic times following noise exposure as part of the companion physiological/stress response study.

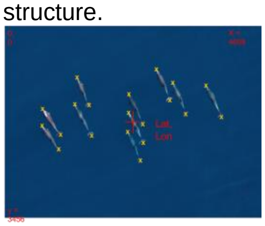
Results

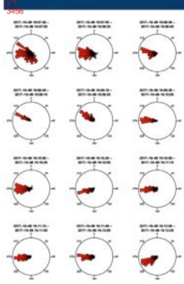
Three field efforts were conducted in 2017-18, with over 100 different groups across all focal species sampled using these different methods. Initial efforts focused on testing and evaluating field methods and identifying optimal weather and animal configurations to optimize the changes of success for each research element. Examples of the types of data obtained within each data collection system are given below.

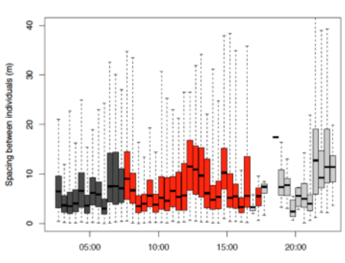
Shore-based theodolites and high-power binoculars were used to generate tracks and speeds of overall group movement (Fig. 1a) and detailed aspects of social dynamics within groups (Fig. 1b).



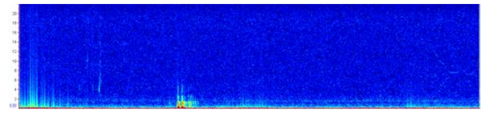
UAS-based photogrammetry analysis measured >335,000 individual dolphins across all periods. Individual head-tail positions were mapped with high resolution in real space (lat and long coordinates) each second (Fig. 2a), enabling detailed measurements of individual heading (2b), spacing (2c) as well as swim speeds to quantify behavior and individual length measurements to describe group structure.

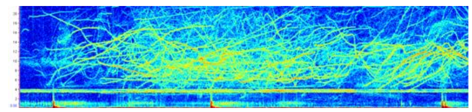


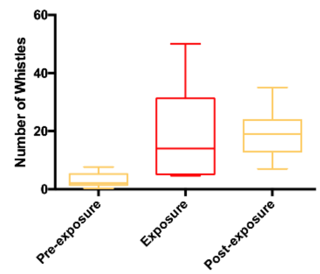




Passive acoustic measurements of group vocal behavior were analyzed by multiple independent observers and scored during specified periods (Fig. 3a, 3b) in order to compare group vocal rates for before, during, and after CEE exposure periods (Fig. 3c).







Once methods were proven to be effective, CEE sequences were conducted (n=20 total across all focal species) in both no noise (control) and MFAS exposure conditions. This pilot project developed methods and evaluated the feasibility of conducting CEEs for these challenging species and was not expected to generate sufficient sample sizes to definitively evaluate response. However, these data represent the first-ever behavioral response data in known MFAS exposure conditions for these species. Preliminary results indicate strong responses in some, but not all conditions, and suggest some species and context-dependent differences, which should be investigated further. Given the success in collecting fine-scale movement, behavioral, acoustic and physiological data in known conditions from this pilot effort, and the initial insights into response characteristics when they did occur, we are well positioned to add to this operational Navy sources in subsequent field efforts.

Notes:

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Measuring stress hormone levels and reproductive rates in small dolphin species relative to mid-frequency active sonar exposure within the greater region of the SOAR range, San Clemente Island, California

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Background

The National Environmental Policy Act, the Marine Mammal Protection Act, and the Endangered Species Act require Federal agencies to address potential impacts of major activities like sonar use. This assessment is hindered by a lack of knowledge of the physiological responses to these activities and whether potential impacts represent biologically significant events. One approach to evaluating the significance of a response is through characterization of hormones associated with the generalized stress response and activation of the hypothalamic pituitary- adrenal axis (HPA) in which stress hormone (e.g., cortisol) production is a hallmark. Prolonged or chronic activation of this pathway leads to numerous pathologies including those that impair reproduction.

Important for this study is that blood samples typically used to measure hormone levels are exceedingly difficult to obtain from free-ranging cetaceans but dart biopsies of skin and blubber tissue offer a practical alternative. Previous ONR-funded studies have shown that a) the pharmacokinetics of hormones in the blubber are tightly associated with those in the blood, and b) they are strongly indicative of physiological responses to stressors on the timeframe of hours to days.

Objectives

The intent of this project is to integrate physiological measurements and population-level metrics into the Population Consequences of Disturbances (PCoD) model in efforts to evaluate the potential effects of sonar by measuring blubber hormone levels in free-ranging small pelagic dolphins within and adjacent to the U.S. Navy's Southern California Anti-submarine warfare Range (SOAR). In addition, blubber hormone levels are being measured from samples collected directly after controlled experimental exposures (CEE) to sonar, leveraging from a concurrent behavioral response study of the same animals to assess: 1) exposure to mid-frequency active sonar (MFAS – a potential disturbance), 2) measures of physiological stress (potential link between disturbance and population effects), and 3) reproductive rates (the population consequence).

Our specific objectives include:

1) Collection of new samples (those in addition to the samples collected previously during standard NOAA Fisheries survey operations 2006-2018) and obtained during the CEEs.
2) Determination of pregnant females.
3) Quantification of stress hormones.
4) Estimation of the relative exposure of each sampled animal to sonar activity that preceded sample acquisition.
5) Evaluations of the relationship between sonar exposure levels, blubber cortisol concentrations, and pregnancy rates.
6) Examination of relationships between the hormone measurements and behavioral response to sonar (behavioral data generated from the main CEE effort).

Methods

Remote biopsy sampling is being used to collect over a thousand skin and blubber samples from small delphinids in the California Current including those inhabiting the areas in and adjacent to the SOAR range, off the coast of San Clemente Island, California and as far away as the southern waters off Baja California, Mexico, where it is generally thought that there is limited sonar use. In addition, 201 biopsies were collected during the CEEs and control periods. Procedures for conducting these CEEs are delineated in our companion project *"Integrating remote sensing methods to measure social delphinid baseline behavior and responses to Navy sonar"*. During each event, distinct dolphin groups are exposed to a

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simulated sonar source with a duration of no more than 10min.

Biochemical analyses of skin biopsies are being used to infer sex, pregnancy state, and physiological stress. The hormone analysis uses a multi-step, biphasic organic solvent extraction as described previously with quantification via enzyme immunoassays, validated for cetacean blubber extracts. Genetic qPCR sexing assays are being used to assign sex. Sonar activity measurements are being derived from: 1) data archives and Range ship tracks collected as part of the Marine Mammal Monitoring on Navy Ranges (M3R) program on SOAR and 2) SPORTS reports extracted from the Navy database by NUWC.

Results

The CEE effort displayed impressive coordination between participating groups and generated a sample set exceeding 200 samples from exposed and 40 from non-exposed animals. These samples were added to the overall sample set that now totals 1426 samples.

The molecular assays showed that the sex ratio differed substantially between the two sampled Delphinus species; females comprised 36.6% of *D. capensis* samples (n=658) and 64.5% of *D. delphis* samples (n=636). However, the percentages of females that were pregnant at the time of sampling were similar between the two species: 22.1% (n=204) of *D. capensis* and 28.1% (n=303) of *D. delphis*. For both species we found strong geographic patterns in pregnancy, suggesting that some areas were more conducive for pregnant females. As we proceed in the analysis of the potential effects of sonar on behavior and reproduction it is important to keep this evolving understanding of animals' use in the study areas as important contextual information.

The initial blubber cortisol analysis of the 2017-2018 CEE biopsies have yielded promising data. On average exposed animals had approximately twice the blubber cortisol levels (3.6ng/g-lipid; 0.88ng/g) as non-exposed animals (1.37ng/g-lipid; 0.47ng/g) (t-stat = 3.3, p = 0.007, df = 89). However, there is much overlap between treatment groups and no trend in cortisol concentration as a function of duration from source initiation was observed; a result that is consistent with slower cortisol dynamics in the blubber and individual variation that modify these dynamics, such as body composition, peripheral perfusion, and the innate reaction to this stressor. We continue to process these samples to examine the temporal dynamics and to obtain a more robust understanding for the relationship between sonar exposure, the response as measured in blubber cortisol, and associated reproductive rates: especially important will be to integrate the contextual information acquired by the behavioral work that is being done concurrently with the idea to help explain some of the residual variation in blubber cortisol seen among the sampled animals.

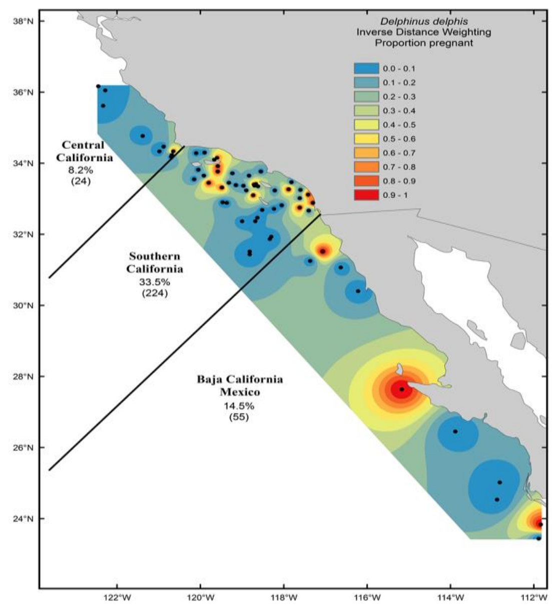

Figure 1. Biopsies of female long-beaked common dolphins (*Delphinus delphis)* and regional estimated pregnancy rates.

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Behavioral Response Evaluation Employing Robust Baselines (BREVE)

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Background

The US Navy has been mandated by courts to conduct monitoring efforts to determine the impacts of US Navy mid-frequency active sonar (MFAS) training on marine mammals. Passive acoustic monitoring (PAM) baseline data has been collected on a sample basis for over a decade at the Pacific Missile Range Facility (PMRF), Kauai, Hawaii. Additional PAM data collections have also occurred every February since 2011 before, during and after a major US Navy MFAS training activity when baleen whale species seasonal presence is high. The training event monitored is a large multiple platform, multiple day, anti-submarine warfare training activity. This provides a rich PAM data set for investigating how behaviors observed during MFAS training compare with baseline behaviors. PAM derived marine mammal behaviors are based upon baleen whale tracks generated from successive localized calls. While this type of opportunistic study does not have a formal experimental design, like Behavioral Response Studies, it has advantages in terms of relatively low cost, low impact on monitored whales, and larger sample sizes

Objectives

The objective of this project is to develop and apply methods to statistically determine baleen whale species' behavioral responses to actual US Navy MFAS training activities using PAM data collected from dense arrays. Successful methods will be rapidly transitioned to US Navy fleet funded marine mammal monitoring efforts for application to all available data. Results from this effort are expected to increase our understanding of impacts of MFAS training on marine mammals by enhancing our ability to quantify the whales' responses.

Methods

The research has two components. First, to establish robust baseline behaviors for multiple baleen whale species with an initial focus on minke whales. This effort leverages existing automated processing capabilities to process/re-process existing large datasets from PMRF and creates outputs such as individual animal acoustic tracks for multiple baleen whale species. Second, we are extending and adapting methods for quantifying behavioral responses to MFAS doses that were developed in an earlier ONR project (MOCHA) to this PAM data. A before, during and after paradigm is being utilized. The goal is to address: 1) If a different spatial distribution of baleen whale PAM derived tracks occurs during training compared to before and after; 2) If the whales' kinematic behavior changes during exposures; and 3) If there are changes in the whales' vocal behavior.

Results

Initial plans were to utilize a single data set from February 2014, iterate various analysis methods and evaluate their effectiveness. The 2014 data comprised of ~ 400 h of data with 126 minke whale tracks in a large study area including the PMRF offshore hydrophone array. However, only 11 minke whale tracks overlapped with MFAS activity, and only 4 of those tracks occurred within the area of the hydrophone array where the best localization accuracy and kinematic data exists. To increase the sample size a second data set for mid Jan and Feb 2017 was included in the analysis (~570 h w/200 minke whale tracks). Iterations in tracking methods and methods to smooth whale headings have occurred over the effort. Semi-automated methods have been developed to estimate the cumulative sound exposure level whales have received from all surface ship hull mounted MFAS transmissions over the duration of a whale track.

Behavioral responses of minke whales to these US Navy MFAS training activities have been investigated in three aspects to date. First, we have documented 28 minke whale tracks with MFAS exposure with estimated cumulative sound exposure levels ranging from 138 to 174 dB re 1$\mu Pa^2 s$. This has implications toward developing a dose-response type curve for the species with a potential response being cessation of calling.

Second, a spatial analysis has been conducted on the 2014 and 2017 data sets which are documented in a submitted paper by Harris et al. which resulted from a presentation at the 2018 Effects of Sound on Marine Mammals (ESOMM) meeting (Harris et al. submitted). The spatial analysis results (Figure 1) indicate a significant redistribution of whale calling, with a decrease both in the middle of the range and in the vicinity of the center of ship activity, and a significant increase in whale calling towards the north of the range. The distribution patterns were also different between the two years. A limitation of PAM data is this could be a silencing of whales in the middle of the range, the whales moving away, or some combination of the two. This is consistent with BRS findings based

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upon a sample size of 2 minke whales with MFAS exposures that exhibited avoidance in response to MFAS activity (Kvadsheim et al. 2017).

Finally, preliminary kinematic analysis of the 2014 and 2017 minke whale speed and smoothed heading changes in 5 min bins was performed using generalized estimating equations with explanatory variables including exposure phase and time to and since the nearest sonar periods in the during phase. Each year is currently being analyzed separately. Results indicate that whales increased their speed during sonar activity relative to the before phase. The turn angles derived from the smoothed whale headings indicate that movement became more directed with time in the during phase. Future iterations of analysis include an additional metric for the whale-ship geometry to investigate avoidance response.

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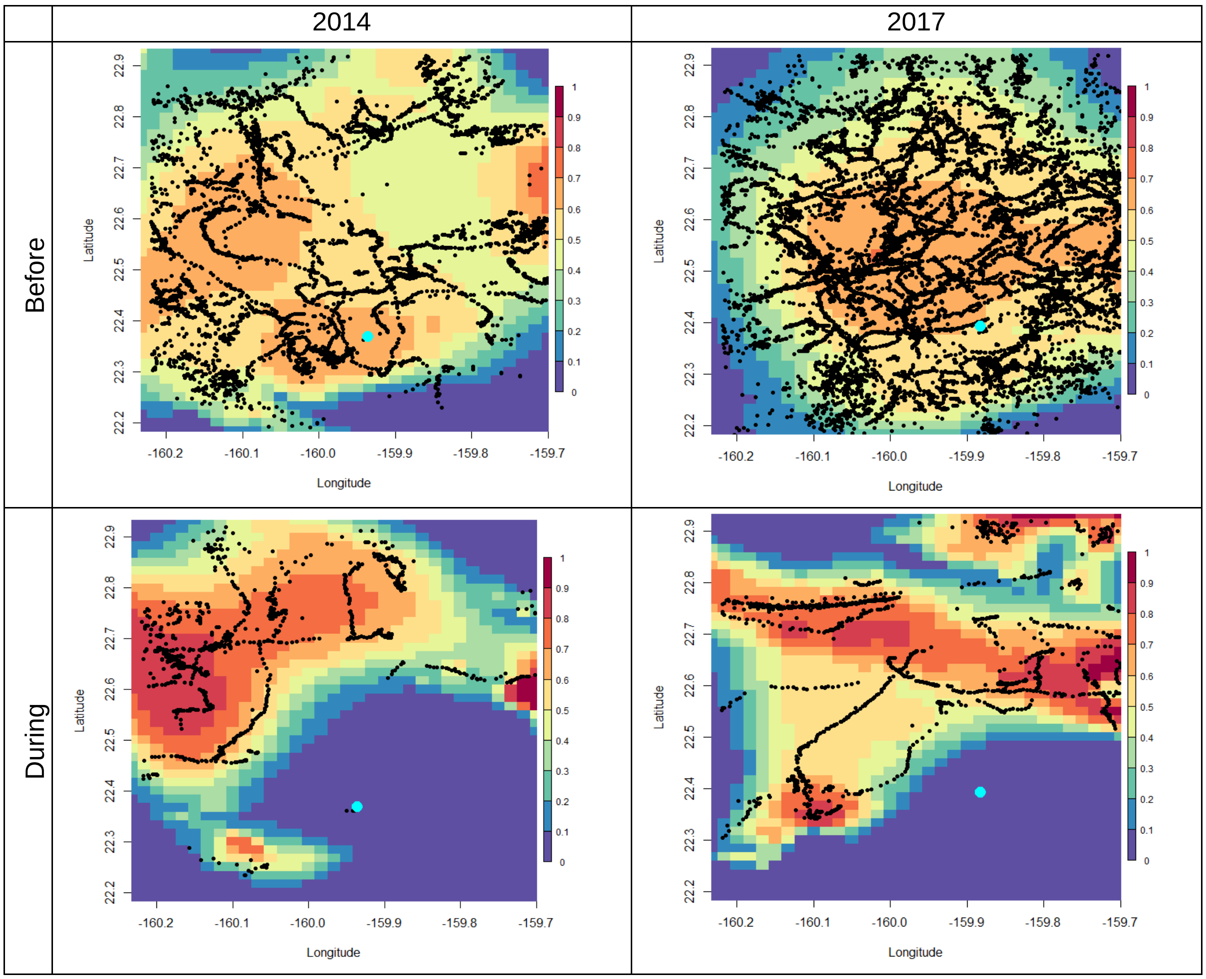

Figure 1: Minke whale tracks (black) overlying prediction surfaces from the selected model. The surfaces represent predicted probability of presence with cool colors representing low probability of presence and warm colors representing high probability of presence. The left plots relate to 2014, the right plots relate to 2017. The top panel show Before and the bottom panel shows During MFAS training activity. The cyan dot represents the estimated mean center of ship activity for that year.

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Acoustic startle responses as aversive reactions and hearing indicators in cetaceans

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Background

Studies on pulsed sounds show that exposure to signals with sharp onset times elicit a startle response in both pinnipeds (Gotz & Janik 2011) and cetaceans (Gotz et al submitted). The startle reflex is a response to sounds that reach a level of 80-90 dB above the hearing threshold within approximately 20 ms after onset (Gotz & Janik 2011). Tactical sonars such as the ones used during the Bahamas stranding incident have sufficiently fast onset times (<1 msec) to elicit a startle response (Evans & England 2001) if an animal is close enough to the source to experience peak levels of 80-90 dB above its hearing threshold. This reflex, which initially results in a sudden flinch, appears to be present in all mammals. In stark contrast to most other sounds, repeated exposure to sounds eliciting the startle reflex in grey seals leads to a sensitization in which flight responses become stronger and more pronounced over time (Gotz & Janik 2011). Extensive research on the neurobiology of the startle reflex has shown that this reflex can also be modified by changes in noise preceding the startle sound. Most importantly, the reflex is inhibited if a quieter pre-pulse is played within a time window of 3-500 ms before a startle stimules (Koch & Schnitzler 1997). The latter modification is called pre-pulse inhibition (PPI) and has been used successfully to determine the audiogram of rodents (Walter et al 2012).

In our study, we investigate the effects of startle sounds on free-ranging cetaceans to gain insights into the potential for sensitization to repeated startle sounds and the potential use of this reflex to determine hearing thresholds. Sensitization to startle sounds could be an explanation for beaked whale strandings in response to sonar exercises (Harris et al 2018). Hearing thresholds for baleen whales are necessary to assess what types of noise have an effect on these species and how to mitigate such effects.

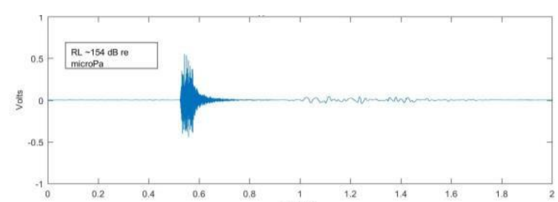
Objectives

1) To characterize startle responses in humpback whales (*Megaptera novaeangliae*) to explore their potential use in audiometry
2) To investigate the link between startle and avoidance responses in Cuvier's beaked whales (*Ziphius cavirostris*) and report whether their avoidance response to startle sounds shows sensitization.

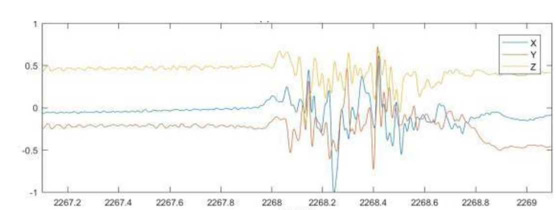
Methods

We conducted playback trials with humpback whales off the coast of Virginia Beach, Virginia and the island of Kauai, Hawaii and with Cuvier's beaked whales in the LIgurian Sea off Italy. All animals were equipped with DTAGs prior to the start of playbacks. DTAGs provide received levels of startle pulses at the whale and information on sudden muscle contractions, pitch, roll and movement of the animal in response to the signal. A vessel followed tagged whales with a playback source to produce the startle sounds and to observe behavior of the animals from the surface in addition to the tag record. Startle pulses were delivered in bouts of 6-10 pulses within 60 s. Whales were exposed repeatedly to such bouts when possible. Repeated exposures were separated by at least 15 minutes.

A)



B)



Results

We conducted repeated playbacks on 13 tagged humpback whales and 3 Cuvier's beaked whales. Humpback whales were exposed to startle pulses of rms received levels between 103 and 162.8 dB

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re 1 µPa with a peak frequency at 1 kHz. Startle responses (Fig. 1) were only seen in two animals and each only startled to one pulse in one of the bouts played to them (at 149 and 154 dB re 1 µPa respectively). Our data suggests that humpback whales either a) have a modified startle response, b) are able to adjust their hearing thresholds in the presence of aversive acoustic stimuli as shown for toothed whales (Natchtigall & Supin 2015) or c) may have on average a comparatively poor hearing threshold of > 70 dB re 1 µPa.

Beaked whales received pulses with a 7 kHz peak frequency at 132 to 148 dB re 1 µPa rms while near the surface. They reacted by stopping all clicking for up to 2.5 hours and by going into a deep dive after each playback (Fig. 2). One animal was exposed to low RL pulses at 97-101 dB re 1 µPa in its second exposure and still showed a rapid increase in speed as a reaction. We conclude that rise time is a significant component of a noise stimulus for beaked whales and has the potential to lead to sensitization in avoidance reactions over repeated exposures.

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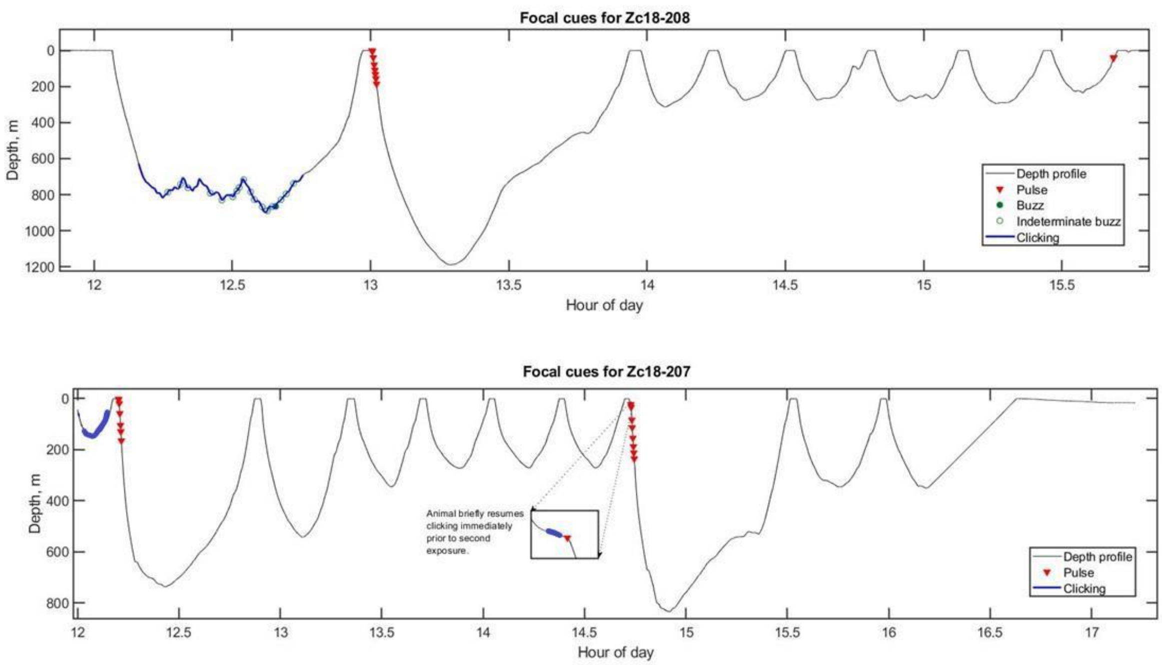

Fig. 2: Dive profiles of two Cuvier's beaked whales during exposure trials. Red triangles indicate individual startle pulses played, blue parts of the profile show times of clicking localized to the tagged whale. The tag for the whale in the upper panel detached seconds after the second pulse playback.

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Approved, DCN# 43-5191-19

The use of Navy range bottom-mounted, bi-directional transducers for long-term, deep-ocean prey mapping

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Background

Navy at-sea tests are conducted under an Environmental Impact Statement (EIS) specific to each operational area. The EIS is based in part on an estimate of the number of behavioral "takes" predicted through modelling. As part of the environmental authorizations, the National Marine Fisheries Service (NMFS) must determine if the effect of such operations, informed by the modelling, has a "negligible effect" on marine mammal populations.

To understanding the cumulative effect of mid-frequency active sonar (MFAS) on beaked whales, which appear to leave an area during such operations, it is important to understand distribution of their prey. Beaked whales appear to be particularly sensitive to MFAS and are of particular interest. These animals forage at great depths, therefore obtaining such data is extremely difficult. This project will evaluate a potential means of leveraging the existing infrastructure of the major Navy Ranges to map the long-term spatial and temporal distribution of deep benthic prey fields and tie these measurements directly to long-term passive acoustic beaked whale foraging dive behavior. This information can in-turn be directly related to MFAS operations with data provided through both passive acoustic MFAS detection and direct ship tracks provide by SCORE.

Combined, these data will inform the beaked whale Population Consequences of Acoustic Disturbance (PCAD) models to predict the cumulative effect of MFAS exposure on beaked whale populations allowing NMFS to make an informed decision regarding "negligible impact".

Objectives

The overall goal of this project is to evaluate the use of Navy Range bottom-mounted transducers for the long-term spatial and temporal monitoring of deep ocean prey.

Methods

Twenty-one of the hydrophones at SCORE are bi-directional. These phones are designed to transmit amplitude modulated, upper-sideband, suppressed carrier, underwater telephone communications in the 8-11 kHz bandwidth. The optimal frequency for scattering from moderately sized squid is 10-50 kHz, which overlaps with the transmit frequencies for the bi-directional phones. Our approach is to transmit an appropriately designed signal from the bi-directional nodes, then record the backscatter from prey within the water column on the transmitting and surrounding hydrophones.

Results

For our first test, we projected a variety of 500 to 1,500 µsec continuous wave (CW) and up-sweep signals score. Each signal was made up of contained 10 pings with and inter-ping interval of 1 second. Each file was played out a bidirectional node from the Range Operations Center (ROC) on North Island and the data recorded on both the bidirectional node and the surrounding hydrophones. The recorded data included a raised band of noise in the UQC band (~8-14 kHz) during the transmission. This was determined to result from amplified noise while the transmitter was energized.

To eliminate inter-ping noise, we were successfully able to inject data into the UQC transmitter (underwater telephone) on San Clemente Island. Each transmission included a gate-pulse (microphone key signal) coincident with the ping. The gate-pulse enables the amplifier only during transmission thus eliminating the raised inter-ping noise.

A field trial using these gated signals is planned for spring of 2019.

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Dynamic Marine Mammal distribution estimation using coupled acoustic propagation, habitat suitability and soundscape models

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Background
The long-term scientific goal of this proposal is 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. The specific aim of the proposed study is to map and predict the large-scale spatial distribution of marine mammal vocalizations based on existing multi-level passive acoustic monitoring data from sparse arrays as well as synthetic data. We will couple acoustic propagation, ambient sound, ocean and habitat models using a Bayesian framework to yield a maximum likelihood distribution mapping.

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Cetacean Tag Development and Best Practices Workshop

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Background
Electronic tags constitute one of the main means of collecting information on cetacean physiology and behavior, track their movement patterns and habitat use, and to understand their responses to anthropogenic threats. As such, tagging has become a powerful tool for managing cetacean populations and providing robust mitigation measures for protecting at risk species.

Electronic tags have been used to provide information required by the US Navy (USN) to comply with a number of Federal environmental regulations and legislation applicable to marine protected species following the Endangered Species Act (ESA) and the Marine Mammal Protection Act (MMPA). As such, tags have been key instruments to assist the USN with the implementation of monitoring programs to assess impacts and minimize risks of their activities, an important component of the regulatory compliance process.

In 2009, ONR organized a workshop to identify needs related to development of cetacean tag and tag attachments and to identify research required to evaluate physical, physiological and behavioral effects (ONR, 2009). This meeting led to a number of studies, which resulted in improvements in tag technology and a better understanding of the effects of tags on cetaceans. A review of these studies was needed to further develop electronic tags for cetaceans and new workshop, sponsored by the Office of Naval Research (ONR), the National Marine Fisheries Service (NMFS/NOAA) and the International Whaling Commission (IWC), was organized.

Objectives
The main goal of the new workshop was to review recent advances in cetacean tagging following the outcomes of the 2009 ONR workshop.

The specific objectives were:
(1) Review follow-up studies, which have assessed the impacts of tag attachments on cetaceans.
(2) Review of advancements in tag attachment technology and identification of areas where further development is required, including approaches to further minimize impacts.
(3) Review 'guidelines for cetacean tagging research' to describe best practices to minimize the potential short and long term effects of tagging studies.

Methods
The workshop was held in Silver Spring, MD, USA from 6 to 8 September 2017. A small group met in Seattle, WA, USA on 19 and 20 June 2018 finalize discussion on specific topics related to the workshop agenda. A total of 43 participants from 9 countries attended the meetings.

Results
The workshop agreed on new tag terminology, which more accurately represented the range of

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available attachment systems. Two broad categories were recognized, invasive and non-invasive tags, with the former representing attachments that penetrate the skin and the latter corresponding to attachments achieved at the surface of the body.

A total of 19 presentations covered a broad range of topics, particularly (a) new developments to tag/tag deployment technology, (b) effects of tags on cetacean behavior, health/physiology, and demographics, (c) new technologies that could be used in future tag development, and (d) tagging best practices. The outcomes of these presentations led workshop participants to identify research needs and to develop 22 recommendations to further advance cetacean tagging technology, tag deployment methods, and to better understand and minimize tag effects and risks to individual animals.

A major outcome of the workshop was the review of the 'Cetacean Tagging Best Practice Guidelines', a document prepared by a group of authors with a multidisciplinary background and substantial experience with tag design, deployment and tagging follow-up studies. A draft document of these Guidelines was shared with the workshop participants prior to the meeting. Discussions at the meeting resulted in a number of suggestions for improvement of the document. The Guidelines are expected to be published as a peer-review paper. These 'Best Practice Guidelines' shall serve as global resource to assist researchers, veterinarians, animal care committees, and regulatory agencies in the interpretation and implementation of current standards of practice and promote the training of specialists in this field. The Guidelines will be recommended for endorsement by the IWC Scientific Committee, which will contribute to disseminate and promote the use of tagging best practices by the IWC's member countries.

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Assessing Performance and Effects of New Integrated Implantable Large Whale Satellite Tags

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Background

Satellite telemetry is widely recognized a key method to study the spatial ecology of large whales. It is also an important research tool for evaluating the vulnerability of these species to anthropogenic threats. Telemetry has been used to provide information required by the US Navy (USN) to comply with a number of Federal environmental regulations and legislation applicable to marine protected species following the Endangered Species Act (ESA) and the Marine Mammal Protection Act (MMPA). As such, satellite tags have been key instruments to assist the USN with the implementation of monitoring programs to assess impacts and minimize risks of their activities.

A recent follow-up study with North Atlantic humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine (GoM) revealed structural flaws in earlier designs of "implantable" (Type I tags as defined by ONR, 2009) whale satellite tags (Robbins et al., 2013). These flaws resulted in tag breakage that may have resulted in relatively short transmission durations, long-term physiological reactions, and potential negative effects on the health of the host whales. The GoM study led to the development of new, robust tags satellite tags produced with different manufacturing processes and fitted with different attachment elements (Zerbini et al., 2017).

Objectives

The main goal of this study is to further assess the performance of new large whale Type I tag designs and their effects on individual animals across multiple species/population and to develop a multidimensional data set to evaluate future improvements in implantable whale tag designs. The study was divided in two phases. Phase 1 included tag deployment on GoM humpback whales in 2018 to compare performance and effects with earlier tag designs. Phase 2 includes conducting similar performance evaluations on Southern right whales (*Eubalaena australis*) and blue whales (*Balaenoptera musculus*) in the period 2019-2021.

Methods

A total of 15 integrated implantable SPOT-6 (Type I) tags produced by *Wildlife Computers* using a new manufacturing process (referred to as DMLS or *Direct Metal Laser Sintering*) were deployed on GoM humpback whales in July and August 2018 (Fig. 1). These tags also included a newer anchoring system design. The performance of these tags was compared with those of integrated Type I tags previously deployed in GoM humpback whales in 2015. This comparison evaluated tag performance in terms of the manufacturing process (tag duration of DMLS tags versus tags in which anchor and transmitter components were integrated by a welding joint) and in terms of anchor designs (duration of a two bladed tip with two sets of retention elements and a three bladed tip with three sets of retention elements, Fig. 1).

Tagged animals were re-sighted intentionally by the Center for Coastal Studies (CCS) and opportunistically by its collaborators and contributors. Documentation obtained during these re-sightings were used to evaluate the tag site and the condition of tagged animals.

Results

Satellite tags were deployed on 8 males and 7 female humpback whales ranging from 11 to at least 41 years of age. All tags were deployed reasonably high on the body (the upper flank, in the vicinity of the dorsal fin or forward on the back of the whale). There were fewer strong immediate behavioral responses to tagging than in prior deployments (40.0% of the deployments

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in 2018 versus 81.8% in 2015) and no extended responses during a one-hour focal follow.

Re-sightings with tag site coverage occurred on 1 - 25 days (mean=8) which spanned 1-94 days after tagging (mean=40). Unlike prior years, there were no tag flaws nor significant cases of tag-site swelling detected, although follow-up coverage was limited in some cases. Overall, observations to date suggest fewer tag-related behavioral responses and physiological effects in 2018 than in prior deployments in the GOM.

One of the tagged individuals was entangled on July 30, 2018, 10 days after tagging, and the tag was prematurely removed by the entangling rope. Another tag did not properly implant, with the anchoring system remaining exposed. These tags were not considered in the analysis of tag performance presented below.

Duration of 13 tags deployed in 2018 ranged from 14 to 196 days with mean and median tag durations of 52 and 40 days, respectively. These tags, which were all manufactured with the DMLS process lasted slightly longer than previously deployed tags that had anchoring systems and transmitter elements welded to each other (mean duration = 45 days, median duration = 36.5 days, range = 0-112 days, n = 24). The differences in mean duration across the two manufacture processes was not statistically significant (p = 0.60).

Duration of tags with a three-bladed tip and three retention elements (mean = 51.7 days, median = 39 days, range = 14-196, n=27) was higher than those equipped with a two-bladed tip and two sets of retention elements (mean = 37.1 days, median = 36 days, range = 0-76, n=10). These differences were also not statistically significant (p = 0.21).

While statistical differences were not detected in tag performance, tags manufactured with the DMLS process are likely more robust than welded tags and no breakage of has been documented. Furthermore, there appears to have been fewer behavioral and physiological effects. Future tag deployment aims to assess tag performance in other large whale species to increase sample size and allow for more robust statistical analyses. These analyses will also consider other variables such as sex, location of the tag deployment on the body and other factors that could influence behavior and tag performance.

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Fig. 1 – Type I tags with three bladed tip and three sets of retention elements (top) and two bladed tip with two sets of retention elements (bottom). Note that retention elements are shown in deployed position in both designs.

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Approved, DCN# 43-5191-19

The Multi-Agency U.S. Animal Telemetry Network

Bill Woodward

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Abstract

Data and information on the behavior and movement of marine animals is collected remotely via acoustic and satellite telemetry techniques is applied broadly to insure compliance with environmental laws including ESA, MMPA and FSA, assess marine animal habitat use, detect changes in their migratory routes in relation to oil & gas activities and climate variability, monitor changing movement patterns with increasing ship traffic to assist with marine mammal avoidance, and improve abundance/population estimates to ensure both the conservation and sustainable management of commercially harvested species as well as responsibly sustained subsistence livelihoods.

The multi-agency U.S. Animal Telemetry Network (ATN) funded by ONR, BOEM and NOAA, was created and approved by the Office of Science and Technology Policy (OSTP) in 2016 to provide unity, stability and continuity to the national infrastructure which facilitates the collection, management and availability of this marine animal telemetry data. To accomplish its mission, the ATN is being implemented in several Phases and on the following three foundational pillars: *1) Building Alliances and Collaborations, 2) Providing Telemetry Data Aggregation, Management, Display and Delivery, and 3) Funding High Priority Regional Baseline Animal Telemetry Observations.*

This talk will briefly touch on the ATN data aggregation, management and delivery vision - the operational ATN Data Assembly Center (DAC) - and on one example of how the ATN, with funding from ONR, is supporting the baseline telemetry observation infrastructure by paying for the cost of Argos satellite tracking services for those marine animal telemetry researchers who agree to display their animal tracks in real time on the ATN DAC website, as well as submit their data to the ATN DAC.

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State-space models for near-real time quality-control of Argos animal tracking data

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Over the past 15 years, state-space models (SSMs) have been increasingly relied on to filter error-prone locations from animal telemetry data. In this context, SSMs predict a series of locations using a model to describe animal movements coupled with a model to describe the error-prone observations. Focusing on locations collected via the Argos satellite system, I develop and evaluate two continuous-time SSMs that can be fit quickly to large, multi-individual data sets. The models can be fit to CLS Argos' Least-squares-derived location estimates, to their newer Kalman filter-derived location estimates, or to data with a mixture of both. The performance of these and other available SSMs is evaluated using animals tagged with both a high-resolution GPS logger and a lower-resolution Argos logger. I show how these tools can: 1) be used to more precisely locate ocean observations made via animal-borne telemetry and touch on complementary tools for inferring behavior along animal movement paths; and 2) be extended to infer behavior along animal movement paths and estimate relationships with environmental covariates.

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DYNAMICS OF ENVIRONMENTAL (E)DNA FOR CETACEAN DETECTION AND SPECIES IDENTIFICATION IN OPEN-OCEAN AND COASTAL HABITATS

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Background

We are developing methods for detection and identification of cetacean species using environmental (e)DNA collected from seawater. Referred to here as 'eDNA metabarcoding', the potential to confirm species identity and, in some cases, population identity, from environmental sampling will complement the interpretation of acoustic and visual surveys now routinely used to monitor cetacean habitat (Baker et al. 2018).

Objectives

[1] Continue assessment and refinement of open-ocean 'focal sampling' of eDNA, including 'at depth' sampling for identification of deep-diving, cryptic species, e.g., beaked whales, with logistical support from Northeast Fisheries Science Center and Pacific Islands Fisheries Science Center; and

[2] Initiate assessment of the spatial and temporal dynamics of eDNA for a coastal community of cetaceans in Cape Cod Bay, with a specific focus on North Atlantic right whales (*Eubalaena glacialis*), using the habitat sampling grid developed by Center for Coastal Studies, with support from aerial surveys and passive acoustic recorders.

Methods

A flow-chart of the methods for eDNA barcoding is presented in Fig.1. In brief, the eDNA is extracted from 1L or 2L samples of seawater after filtration through 0.4 micron or 1 micron polycarbonate filters. Cetacean eDNA is quantified by droplet digital PCR and identified to species by next-generation sequencing and matching to a curated database of reference sequences (Ross et al. 2003).

Sampling strategies differ for the two primary objectives with opportunistic, focal sampling for the open-ocean species and systematic spatial sampling for temporal changes in abundance of right whales in Cape Cod Bay (Fig. 2). Five passive acoustic recorders (MARUs) have also been deployed in Cape Cod Bay with programs to collect sound continuously at 2kHz.

Results

Opportunistic, focal sampling has now been completed in several open-ocean and coastal environments, including the Southern California Offshore Range. To date, eDNA metabarcoding has confirmed identification of 8 species of odontocetes including two beaked whales: Baird's beaked whales (*Berardius bairdii*) and True's beaked whales (*Mesoplodon mirus*). 'At depth' sampling by CTD cast at the Cross Sea Mount, Hawaii, detected false killer whales (*Pseudorca crassidens*) in the absence of any coincident visual or acoustic detection. The haplotype information indicated that the whales were not members of the island-associated populations.

Temporal sampling of Cape Cod bay was initiated on 9 October 2018, prior to the reported arrival of any right whales, and will be repeated on a monthly basis through July, 2019. These monthly spatial surveys will be augmented by samples collected 'in path' of the right whales. Initial analyses of samples collected to date are underway.

Notes:
Fig 1: The workflow for environmental DNA metabarcoding of cetacean species.

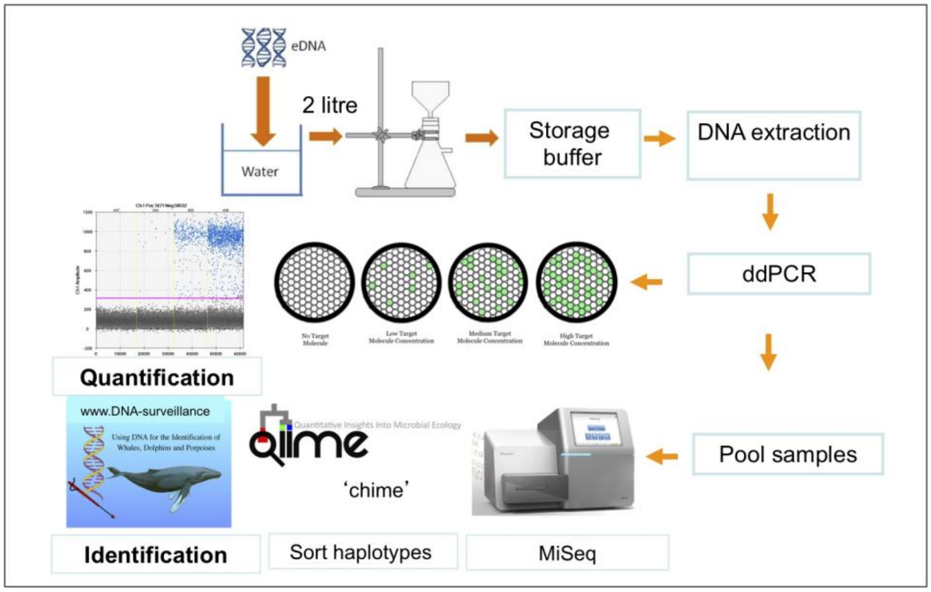
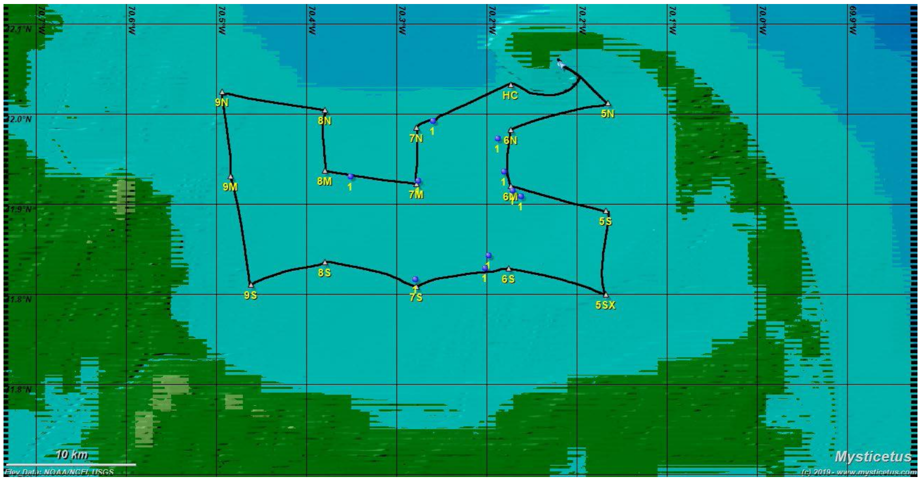


Fig 2: Location of 16 sampling stations (yellow codes) and sightings of right whales (blue dots) during dedicated surveys of Cape Cod Bay on 23 February, 2019, courtesy of Christy Hudak and Charles Mayo, Center for Coastal Studies.



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The wide-band detection and classification system

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Detection and classification of marine mammals using passive acoustic monitoring is traditionally conducted on a species-by-species basis using separate algorithms for each species' characteristic calls. When an application requires the detection of many species simultaneously, this approach becomes inefficient at best, and impractical at worst. Some detection and classification systems seek to detect a class of calls, such as tonal or pulse calls. The low-frequency detection and classification system (LFDCS) is one such system that uses (1) pitch tracking to detect and characterize all tonal sounds in a recording or real-time audio stream, and (2) a separate discriminant function analysis to classify sounds by call type and species. This allows the detection and classification of many species simultaneously in a single processing run. The LFDCS, like all tonal marine mammal detectors, relies on spectrograms produced with the short-time Fourier transform (STFT). These spectrograms characterize spectral content on a linear frequency scale, despite the fact that sound is both produced and perceived on a logarithmic frequency scale. This limits the band of frequencies that can be effectively monitored to roughly 4 octaves, yet marine mammals make tonal and pulse sounds over a range of 12+ octaves. I am working to implement an efficient algorithm to create spectrograms based on the constant-Q transform (CQT), a technique to estimate spectral content on a logarithmic frequency scale, and to incorporate CQT-based spectrograms in a new wide-band detection and classification system (WBDCS). The WBDCS will also incorporate a detection and classification system for pulse sounds (e.g., echolocation clicks, minke whale pulse trains, walrus knocks) that will take advantage of the wide-band processing required for the creation of CQT-based spectrograms. This new system will be conceptually and practically similar to the LFDCS, except the monitored frequencies will extend from 14 Hz to ~50 kHz, the frequency resolution will be more appropriate for pitch tracking tonal calls throughout this frequency range, and time-domain front-end processing will be used to detect and classify pulse calls. This single system will be capable of simultaneously detecting tonal and pulse calls ranging from the low-frequency moans of blue whales to the high-frequency clicks of beaked whale. The WBDCS will be implemented on both a desktop computer for processing archived recordings and on the DMON/DMON2 instrument for in-situ real-time detection and classification from autonomous platforms.

Notes:

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Cetaceans, pinnipeds, and humans: Monitoring marine mammals in the Arctic and characterizing their acoustic spaces

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Background

Many Alaskan marine mammal species are federally protected by both the Marine Mammal Protection Act as well as the Endangered Species Act. They are also an essential part of the diet, traditions, and culture of Arctic Native communities. Mitigation of anthropogenic impacts on protected species requires knowledge of the timing and location of marine mammal distribution, migrations, and movements. Understanding the potential effects of these impacts in the Arctic is becoming increasingly important as climatic conditions shift towards an ice-free ocean (Wood et al., 2015). Additionally, marine mammals are excellent proxies for ecosystem change, since they respond to shifts in abundance and distribution of large zooplankton and small fish taxa. Long-term passive acoustic monitoring provides the only means possible for collection of year-round data on the spatio-temporal occurrence of cetaceans and pinnipeds, anthropogenic noise presence, and ambient noise levels in the harsh and remote environment of the Arctic.

Objectives

The overarching goal of this project is to monitor marine mammal spatio-temporal occurrence and describe the acoustic environments as climatic conditions continue to change across the Alaska region. There are three research objectives of this work, each of which will contribute results that can be compared with those from previous years to address this overarching goal:

1) Maintain the long-term time series of passive acoustic data collected under differing degrees of anthropogenic noise exposure by redeploying the Arctic set of moorings in 2020.

2) Describe the seasonal occurrence of biological (11 species of Arctic and subarctic marine mammals), anthropogenic (vessels and airguns), and environmental (ice) acoustic sources for three years starting with the 2016-17 deployment data.

3) Characterize the acoustic environment at ten mooring locations that represent Alaskan Arctic diversity or are of interest to the U.S. Navy, and describe the main biotic, abiotic, and anthropogenic contributors to these acoustic environments for each season, over a three year period (2016-2019).

Methods

Long-term passive acoustic data were collected via deployment of long-term sub-surface passive acoustic recorder (AURAL[1]) moorings located throughout the Bering, Beaufort, and Chukchi Seas (Fig. 1). The AURALs recorded for an entire year at a sampling rate of 16 kHz, with 16-bit resolution and 16 dB gain, on a duty cycle of 85 min of recording every 5 hours (28%); built in sensors sampled temperature and pressure once per recording period.

Data from the moorings were processed using an in-house MATLAB-based analysis program which operates on binned spectrogram images (Wright et al., 2018). Analysts manually scanned all images and flagged those with signals; multiple species were processed concurrently in two frequency bands (i.e. mid (0-800 Hz) and High (0-8kHz)).

Spectral probability density and spectral percentiles will be calculated using the Acoustic Ecology Toolbox (Dugan et al., 2011). Ambient noise levels and spectral density will be calculated for selected recording periods, per mooring location and season (where no evident sound sources were identified during the seasonal data analysis described above) and will be considered as the baseline ambient noise conditions in each mooring area, season, and year.

Results

Table 1 lists the progress to date on the analysis of the long-term mooring data. Results will be

[1] Autonomous Underwater Recorder for Acoustic Listening, AURALs, Multi- Électronique, Inc., Rimouski, QC, Canada

summarized as percent of 10-min time segments per day with sounds from each species or noise source. An example of what these results look like when compiled over multiple locations and years is shown in Fig. 2; here it is evident that some bowhead whales remained above the Bering Strait throughout the winter of 2017-18 (PH1). Likewise, the presence of bowheads off Barrow during the open water season can be seen at the BF2 site in all years, but particularly in the summer/fall of 2016. Additional results will be presented at the meeting, along with noise metrics, with a focus on differences among species, years, and locations.

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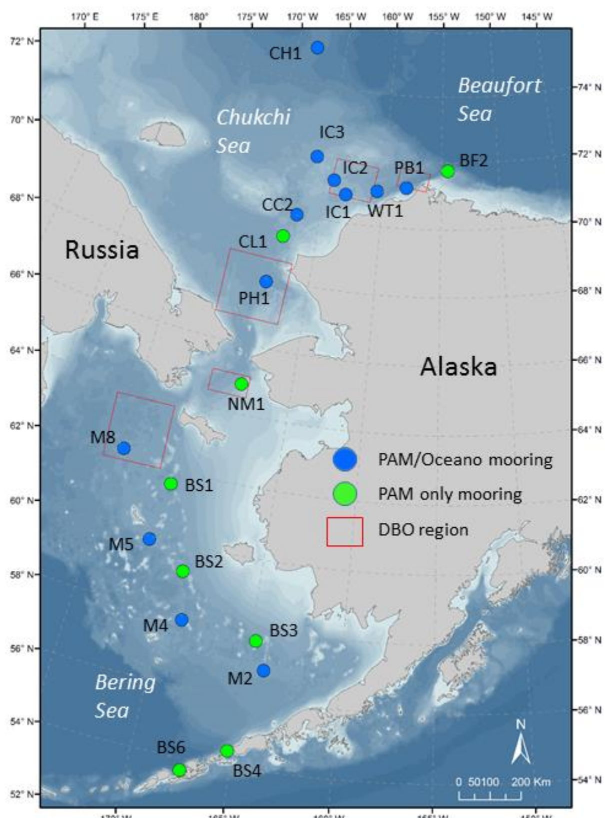

Fig. 1. Long-term passive acoustic recorder mooring locations. Analyses for this project include moorings from NM1 and north.

Tab. 1. Analyses completed to date for the 2016-17 and 2017-18 deployment years (see Fig. 1 for location of moorings).

Mooring	Regular Band		High Band	
	16-17	17-18	16-17	17-18
BF2	x			
PB1	x	x		
WT1	x			
IC3	x	x		
IC2	x			
IC1	x	x		
CC2	x	x	x	
CL1	x		x	
PH1	x	x		
NM1	x		x	
CH1	x	x	x	

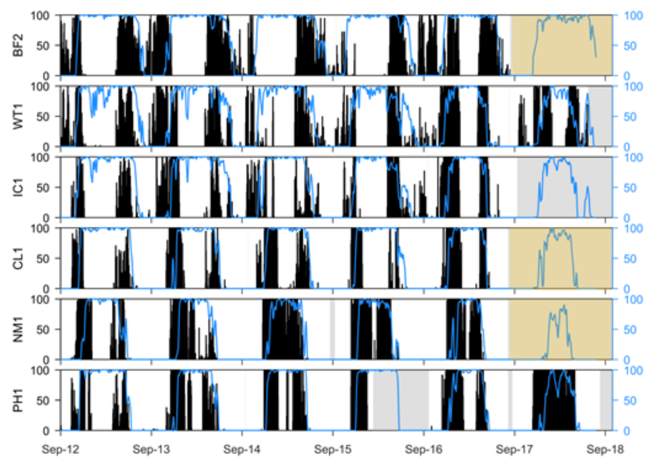

Fig. 2. Bowhead whale daily calling activity (% 10 min bins with calls) from 2012-2018; Blue line = % ice conc. Gray shading = no data. Yellow shading = not yet analyzed.

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

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Background

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

Objectives

With ONR/DURIP funding, we developed the Long-term Acoustic Real-Time Sensor for Polar Areas (LARA). Within the scope of this ONR project, we field tested the system multiple times off the coast of Newport, Oregon, USA and evaluated its performance.

Methods

LARA combines the advantages of both submerged and surface systems. The real-time information system makes stationary passive acoustic monitoring more effective and provides maximum flexibility, allowing for a wide range of applications even in ice-covered areas. In addition, the vertically-moving components of the winch system contains two CTD (conductivity, temperature, and depth) sensors (located at the main instrument housing and the antenna housing, respectively) that monitors oceanographic conditions, allowing estimation of the sound speed profile and thus better understanding and modeling of ocean acoustic propagation conditions in the deployment region. LARA is deployed on a typical oceanographic mooring at a predefined depth (current system limited to 300 meters) to detect and record acoustic signals of interest. The control module in the sensor unit runs a sea-ice sensing algorithm based on the temperature and salinity profile in the upper water column. This algorithm has been proven to reliably detect sea ice in the Southern Ocean. LARA operates an on-board acoustic event detector targeting odontocetes echolocation clicks in real-time. After an event is detected (or at a pre-defined time interval), a command is sent by the sensor unit to the winch via hydro-acoustic modem to raise the sensor unit to about 15 m depth. During this process a temperature and salinity profile is measured. Based on these measurements, the control module decides whether or not to surface the sensor. If the “no sea ice criterion” is fulfilled, the control module sends a command to the winch to surface the sensor unit. To further reduce the risk of damage by ice and other surface obstacles, only the antenna is raised to the surface; the actual sensor unit stays at a safe depth of about 10 m. A bi-directional communication link to shore is established via an Iridium satellite connection.

Results

LARA was deployed multiple times off the coast of Newport, OR, USA. The system was typically configured to record sound at 125 kHz sampling rate and 16-bit resolution at a 50% duty cycle (2 minutes every 4 minute). Several iterations and improvements were necessary to work out encountered challenges. An example of the up-and-down movements of the sensor and antenna unit for a selected period is shown in Figure 1. As illustrated, the system worked as

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expected during the first five cycles. During cycle #6, the communication between the winch and the sensor unit did not function properly. After a 30-minute timeout, the internal watchdog was activated which reset the system and resolved the issue.

Because the sea ice detection algorithm could not be tested in Oregon waters, we implemented a wind speed (sea state) detection algorithm. This algorithm can be used to avoid surfacing the unit in unfavorable weather conditions. 8-kHz ambient noise levels were computed for 12-s windows and the wind speed estimated following the method outlined by Vagle *et al.* (1990). Results are shown in Figure 2. Generally, the estimated wind speeds tracked the NH10 buoy measurements well. In a few instances the estimated wind speeds exceeded the actual wind speeds. These overestimates were primarily caused by biological (dolphin) and anthropogenic (close ship passes) sound sources. However, these occasional overestimates of wind speeds do not negatively impact the operation of LARA.

The quality of the obtained acoustic recordings was excellent and without any noticeable system noises. During the deployments, various biological signals were recorded. Those included vocalizations of blue, fin, and humpback whales as well as various odontocete species. During the deployments, the onboard algorithm detected Stejneger's beaked whale. Those detection were reported back to shore in near real-time when the unit surfaced.

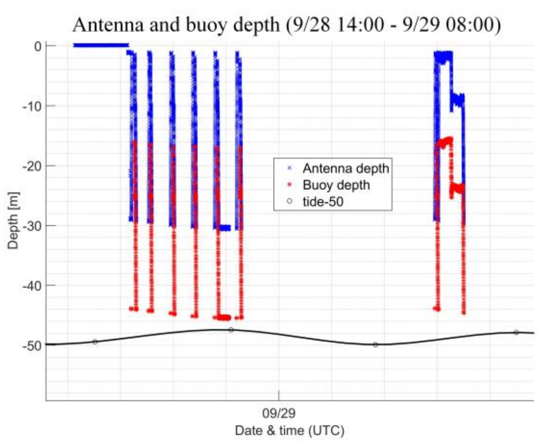

Figure 1. Depth profiles of sensor unit (red line) and antenna unit (blue line) during eight up/down cycles executed between 28-29 September 2018. Black line represents the magnitude of the local tide offset by 50 m.

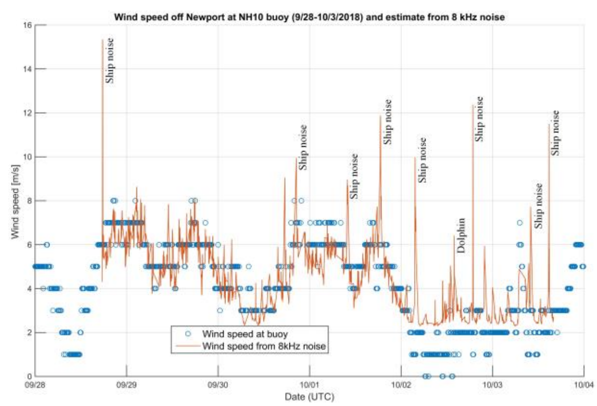

Figure 2. Wind speeds (orange line) measured by the NH10 buoy (44°38'31.20"N, 124°18'0.00"W) and estimates (blue circles) based on acoustic noise level measurements from the LARA system (44°38'45.55"N, 124°22'23.79"W).

NOTES:

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Long-term Field Validation of the SonarPoint™ Sound Source Localizing Autonomous Acoustic Recorder System

Marco Flagg[1] and Thomas Norris[2]

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SonarPoint™ is a modular systems of sound source localization capable autonomous acoustic recorders (AARs). This system can be deployed, plug-and-play style, in many configurations. The main configurations include submerged seafloor arrays with time-synchronization via an acoustic pinger, and recovery using acoustic releases. For semi-permanent deployments (e.g. temporary moorings), a surface buoy module with integrated solar power, GPS time synchronization and a terrestrial or Argos satellite modem is available. A drifting configuration is also possible using the same components as the semi-permanent configuration.

In 2017, SonarPoint™ was tested through short-term (1-2 day) deployments in all three configurations. The current field validation effort concentrates on longer deployments of a week to several months, with a cumulative endurance test of one year. The objective is to fully characterize system performance and test operational reliability, thus reducing the technology risk and providing guidelines for researchers interested in the use of the system. Testing has started including endurance testing in the lab and a test pool. This phase is now completed, and the start of ocean testing in Monterey Bay is imminent, pending our research permit from the Monterey Bay National Marine Sanctuary.

We will review the test configuration and early results of the ongoing test series.

Notes:

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Day 3: Thursday April 25, 2019

2019 ONR
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Heart Rate Logging in Deep Diving Toothed Whales: A New Tool for Assessing Responses to Disturbance

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Background

This study directly addresses two of the ONR Marine Biology Program thrusts: Diving physiology and stress physiology. We are designing a heart rate data logger to deploy on wild toothed whales allowing us to obtain essential diving physiology data for deep diving whales. Due to their deep diving capabilities and proven ability of this team to tag pilot whales in the wild, pilot whales are the ideal model species to develop and validate new techniques for studying diving physiology and the stress response in a wild cetacean. We will investigate the relationship between dive behavior and the heart rate response in pilot whales, and hopefully beaked whales, and document the level of bradycardia, and how behavior influences heart rate in a deep diving toothed whale. This information is fundamental to understanding not only how cetaceans manage oxygen and nitrogen during routine activities, but also how plastic their oxygen store management is, which is key to evaluating their ability to adapt to a changing environment. This project will thus deliver a new physiological sensor capability for future noise effects studies as well as a major advance in our understanding of cardiac and gas regulation in deep-diving whales. These data are critically needed by the Navy to estimate the impact of naval exercises on marine mammals.

Objectives

1) Develop an acoustic and/or surface potential sensor for measuring heart rate. The sensors will be incorporated into a modified Dtag4 and tested on captive cetaceans (porpoises and other available species) to determine the ideal attachment locations (Phase 1).

2) Use the EKG-Dtag4 to measure the diving heart rate, along with the sounds and movements of wild, free-ranging pilot whales, enabling a study of the relationship between dive heart rate, activity, and ventilation rates.

3) Attempt to deploy the EKG-Dtag4 on Blainville's beaked whales off El Hierro in the Canary Islands to uncover how beaked whales regulate heart rate in shallow versus deep dives.

Methods

<u>Phase 1: Develop sensor & integrate into DTag4</u>

We tested a suite of potential heart rate sensors on captive porpoises including: acoustic, ballistic and n-differential ECG electrodes. We evaluated a variety of sensors, sensor combinations, and processing methods to determine which sensor or sensors will allow us to measure heart rate in wild cetaceans. The best performing sensors were incorporated into the Dtag4 (See results for details).

<u>Phase II: Diving Heart rate of Pilot whales</u>

We will use the ECG-Dtag4 to measure the diving heart rate, along with the sounds and movements of wild, free-ranging pilot whales, enabling a study of the relationship between dive heart rate, activity, and ventilation rates. The first round of deployments will take place in March 2019. We will evaluate tag performance after the first field season, make needed modifications, and re-test on captive porpoises before the 2nd field season in Fall 2019. Pilot whales will be tagged off Tenerife (Canary Islands) where a resident population of approximately 350 short-finned pilot whales is found 1-8 km off the southwest coast in water depths of 800 to 2000m. Our goal is to tag approximately 5-7 whales each field season.

<u>Phase III: Heart rate of Beaked whales</u>

If deployments on pilot whales are successful in 2019, we will attempt to deploy the heart rate Dtag on Blainville's beaked whales near El Hierro (Canary Islands) in 2020. Tagging will take place over a one-month field season. Due to the difficulty of spotting beaked whales, an observation post on a high cliff will be used to sight whales. Once a group of animals is located the information will be communicated to a small boat which will then approach for tagging. We will use the ECG-Dtag4 to measure the diving heart rate to examine how beaked whales regulate heart rate in shallow versus deep dives. These data are essential for future modeling studies investigating noise induced DCS

Results

Surface potential methods performed best of the different modalities tested both in the lab and on the porpoises leading us to focus primarily on these sensors. This conclusion was also informed by field deployments of a prototype 2-ECG DTAG described below (Fig 1). Two candidate materials for the sensors emerged from initial selection: silver chloride (AgCl), and a carbon black silicone composite (CB). Both materials have no half-cell voltage minimizing movement artifact. However, AgCl is a mild skin irritant and use of this type of electrode for more than a few hours can lead to poor skin condition. In comparison, CB electrodes are biocompatible and are widely used in ambulatory human ECG recorders so we selected CB electrodes for the field tag. Compared to conventional ECG use in human medicine, the 2-ECG method on cetaceans has two additional constraints: (i) a high dynamic range is needed to cope simultaneously with a small ECG signal due to close electrode spacing and large transient myoelectric and movement signals, and (ii) common-mode feedback to the body, which is conventionally used to cancel electrical interference by controlling for the electrical bias of the surface potential electrodes, cannot be used as this would create a single-point failure mode for the sensor array. As commercially-available ECG acquisition semiconductors both rely on common-mode bias control and have limited dynamic range, we designed a novel low-power surface potential amplifier and sampler that will meet the unique requirements of this application. This design takes significant circuit board area and the final specification for the ECG acquisition board was therefore constrained to support two wide bandwidth surface potential channels. Each of these channels can be used as a 2-ECG, 1-ECG or BIA sensor making the board very versatile.

Dr. Johnson built two EKG-DTAG-4 loggers for deep diving cetaceans. The tags are configured with one differential channel (i.e., two suction cups) and one single-ended channel (i.e., one cup and one water ground). Sampling rate for this is 4500 Hz per channel (more than needed, but improves ability to detect weak signals in noisy data). The tags consist of standard Dtag sensors (hydrophone, pressure, accelerometers, magnetometers), and a snapshot GPS which will be useful correlate speed of travel to heart and respiration rates. The suction cup electrodes are CB and mounted on highly compliant low profile arms (like squid tentacles) to reduce movement artefacts. The spacing of the electrodes can be adjusted, but we will start with 400 mm.

We have deployed a prototype 2-sensor EKG-Dtag3 on 9 porpoises and obtained high quality heart rate data from 5 of the tags for 10, 12, 22, 35 and 40 hrs. These 10+ hour deployments with high quality ECG are extremely valuable for guiding the development of software tools and analysis techniques for handling these dense data, but they also offer a more promising outlook on the potential of surface potential measurements on free-swimming cetaceans (Fig 1).

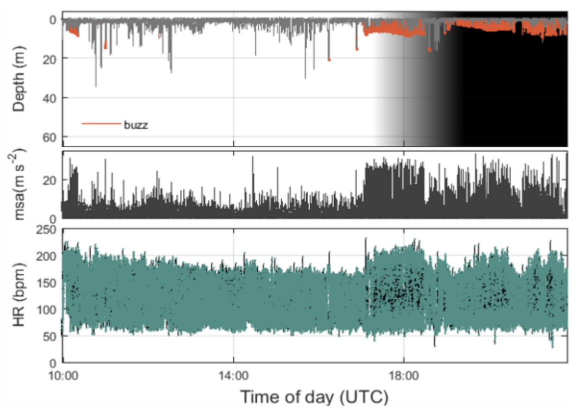

Fig 1: Diving behavior & heart rate of a harbor porpoise instrumented with a two-electrode ECG Dtag3 for 12 hours. A) Diving behavior of the porpoise in relation to daylight. Red dots signify buzzes. B) Minimum specific acceleration indicating that the porpoise was more active during foraging dive. C) Instantaneous heart rate.

Notes:

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RATE: Respiratory Acoustics to Estimate Energy

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Background

Respiratory rate is a vital sin often used to assess responses to natural and disturbed behavior in human and in animals and can reflect changes in minute ventilation and metabolic rate. However, using respiratory rate as the only parameter assumes equal volumes of air are exchanged breath-to-breath. Cetaceans show considerable variation in tidal volume, greatly affecting estimates of metabolic rate from breathing frequency alone, especially in the short-term or during activity (1). Monitoring tidal volume in wild animals is challenging, especially in cetaceans. We sought to estimate free-ranging tidal volumes by applying techniques from human medicine to establish a relationship between recorded respiratory sounds and measured air-flow rates.

Through RATE, we have made the first near-continuous breath-by-breath estimates of tidal volume in wild cetaceans, with major implications on estimating short-term ventilation and gas exchange in natural and disturbed behaviors.

Objectives

1) Estimate respiratory flow rates from breath sounds recorded in bottlenose dolphins, calibrated to pneumotach measurements.

2) Apply the method to monitor free-ranging tidal volumes of tagged wild dolphins.

3) Assess changes in respiratory variables in response to specific exposures or behaviors measured by tag inertial and acoustic sensors.

Methods

We calibrated measurements of respiratory air-flow (with a pneumotach; 2) to sound recorded on bio-logging tags behind the blowhole of bottlenose dolphins (Fig 1a). We developed the method with data from Dolphin Quest Oahu, where we used the pneumotach and tag on dolphins before, during, and after exercise. We focused on inhaled volume as cetaceans often begin to exhale before or as they surface.

Acoustic data from the tags were audited to detect breathing and the quality of each marked breath reviewed to distinguish clear recordings with no interference with the breath sound. We time-aligned the air-flow (pneumotach) and acoustic (tag) records to match data for each breath. We removed the DC component, high-pass filtered, and removed transients in the acoustic prior to taking the Hilbert envelope to obtain the processed sound record, which we then down-sampled to the same frequency as the pneumotach and cross-correlated to time-align (Fig. 1c).

For each trial, we selected five random breaths as training data. We fit the model flow rate = a sound^b between the time-matched measured flow rate (L/s) and sound envelope. We applied this fit to all breaths in the same tag deployment to estimate air-flow rates from the filtered sound envelope (testing data, Fig. 1d). We assessed the method by comparing the measured and estimated volumes and completed various cross-validations.

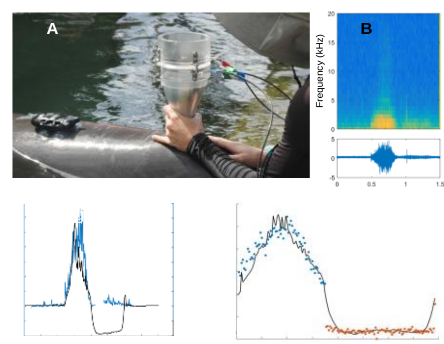

Figure 1. A: Pneumotach and tag on a bottlenose dolphin. B: Spectrogram of the breath sound. C: Time-aligned air-flow (black) and sound (blue). D: Estimated flow (points) and measured flow (black).

To apply these methods to estimate inhaled tidal volumes (VT) of wild bottlenose dolphins tagged in Sarasota, FL in May 2014, we (1) estimated air-flow rates from sound, (2) estimated resulting VT when the animals were held prior to release, and continued to estimate (3) inhale duration, (4) VT, and (5) minute volume from acoustics after animals were released.

Results

We estimated *VT* of 768 inhaled breaths over 36 trials on 6 animals at Dolphin Quest (2013 and 2017). Estimated flow rates and volumes were -0.4±2.3 L/s and -0.6±1.1 L different from measured. At rest, *VT* was on average 8.0±1.5 L; *VT* increased to 13.4±1.0 L when swimming, and

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inhale duration decreased. After exercise, *VT* decreased from 11.6 L to resting levels and inhale duration increased (Fig 2).

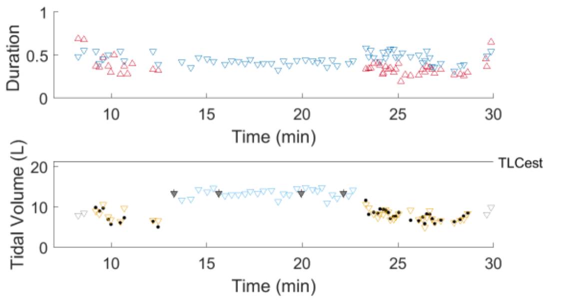

Figure 2. Duration (sec) of exhales (red) and inhales (blue) during an exercise trial at Dolphin Quest and measured inhaled tidal volumes (black points) and estimated inhaled tidal volumes (triangles) when at rest (yellow = pneumotach on, gray = pneomotach off) and during swimming (blue). Filled traingles are meanSD when volumes could not be estimated due to splashing. *TLCest* is the estimated total lung capacity of the dolphin from Kooyman (*3*): $TLC_{est} = 0.135 \times M_b$

Over 22h of tag recordings of 7 wild dolphins, we detected 4699 breaths. 300 were before animals were released, of which 95 had the pneumotach over the blowhole.

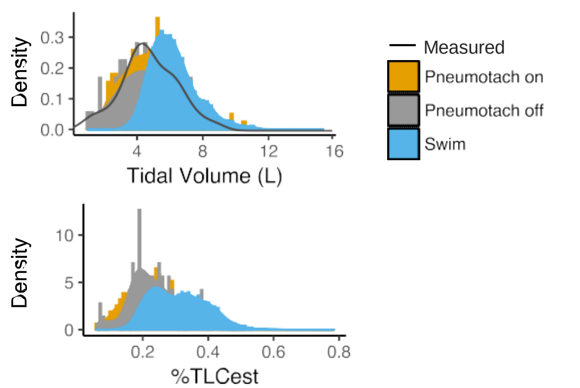

Figure 3. Measured (black line) and estimated tidal volumes of 7 wild bottlenose dolphins when the pneumotach is on, off, and when animals are swimming, expressed as L and % estimated total lung capacity.

At rest, the grand mean measured *VT* was 4.5±1.4 L across all individuals, or 19.1±8.2 ml/kg (mean CV = 0.27). Estimated *VT* was no different from measured (4.5±1.5 L; paired *t*, $t = -0.540$, $p = 0.608$; Fig 3). We found no difference in estimated *VT* between breaths when the pneumotach was on *vs.* off (ANOVA; $F_{1,6} = 0.03$, $p = 0.866$). Overall, estimated *VT* varied from 3.6-5.6 L (IQR; 15-26 ml/kg) when dolphins were held in water (Figs 3, 4).

Following release, we detected 4534 total breaths, 4474 (99%) for which we estimated VT. Tidal volumes of free-swimming, wild bottlenose dolphins were 29±7 ml/kg, 7 ml/kg (33%) higher compared to when held (Fig 3). Swimming *VT* was 31±9% of *TLCest* (Fig 3).

VT estimates had a mean CV of 0.15 when free-swimming; between breaths, *VT* varied from ≅0 to 7.4 L. Assuming *VT* is a fixed value of 0.6xTLC for every breath results in a 32% overestimate of estimated minute volume.

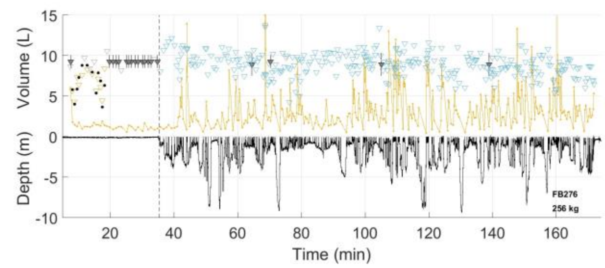

Figure 4. Depth (black solid line), estimated (triangles) and measured (black points) tidal volumes, and instantaneous breathing rate (yellow) for a wild bottlenose dolphin in Sarasota, FL. Vertical line is the release time. Estimates (triangle); orange – pneumotach on, grey – pneumotach off, blue – swimming).

With this method, we can speak to the natural variation in VT through time, as well as specific changes in the context of sound exposure, quantifying biologically significant reactions.

This project was also funded by EU Horizon 2020 MSCA grant 70687 to JVDH.

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Notes:

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Cardiorespiratory physiology in the bottlenose dolphin before, during, and after breath-holding and restraint

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Background

In cetaceans, no published data exists on cardiac contractile responses or ejection fractions during either forced or voluntary dives, with most of the studies being largely focused on cardiac frequencies. Thus current estimates on cardiac output (CO) in cetaceans, and how they may be modified during diving, are based on estimated values from pinnipeds and terrestrial species. As blood flow through the pulmonary and systemic circulation has a decisive effect on gas dynamics, improving such knowledge would significantly enhance our understanding of how they deal with the physiological constraints imposed on these animals. In addition, by studying how apnea and exercise affect multiple cardiac parameters, we can assess how external factors, such as prolonged dive time, increased exercise, or stress response, are likely to affect gas dynamics. For this reason, the objective with this study was to assess the cardiorespiratory changes associated with a static surface apnea in the bottlenose dolphin.

Objectives

Aim 1) Determine CO (heart rate [f_H], and stroke volume [SV]) in resting bottlenose dolphins during different phases of the respiratory cycle.

Aim 2) A) systemic and pulmonary cardiac parameters (f_H SV, mitral and tricuspid regurgitation pressures) were measured before, during, and after trained static sequential breath-holds of up to 5 min. From these data we estimated systemic and pulmoarterial blood pressure by assessing physiological mitral and tricuspid regurgitation. B) We determined respiratory flow-rates, expired O_2 and CO_2, simultaneously with systemic and pulmonary cardiac parameters before, during, and after static breath-holds of up to 5 min.

Aim 3) We determined respiratory flow-rates, expired O_2 and CO_2, and CO (f_H, SV, blood pressure) before and after a “stressful” event, such as physical restraint in shallow water by lifting the floor of a false bottom pool.

Methods

We used a Vivid-i (General Electric) ultrasound machine with a 1-3 MHz wide band phased probe to obtain and register left ventricle outflow tract (aorta) blood flow (Miedler et all, 2015). From the flow traces we determined f_H and SV. Stroke volume was calculated from the velocity time integral (VTI), and the aortic cross-sectional area based on the aortic diameter at the level of the aortic valve. Accurate flow measurement required the Doppler beam to be parallel to the flow of blood and the aortic annulus diameter to remain unchanged during systole. Previously we have shown that the aortic cross sectional area, where the blood flow is measured, is circular and that the diameter at the level of the insertion of the valve (valve annulus) does not change at the different cardiac phases (Miedler et al., 2015). Stroke volume was calculated using the equation: $SV=r^2 \pi$ VTI, where r is the radius of the aortic cross-sectional area. Cardiac output was calculated as the product of f_H and SV.

Measurement were made while animals were resting at the surface and voluntarily breathing for a period of up to 10 minutes. For these experiments, the animals were stationed in the water and were either breathing spontaneously, or were asked to make a maximal respiratory effort (chuff) while continuous measurements were made. The data collected were used to determine systemic blood flow (CO), during different phases of the respiratory cycle, in relaxed dolphins. Corresponding respiratory data allowed us to assess the relationship between respiration and cardiac function.

To obtain continuous measurements of f_H, we also used a suction cup based electrodes attached on the ventral side of the dolphins to measure continuous ECG, respiratory flow and end-expired gases between cardiac function and respiration.

Results

The dolphins exhibited a significant post-respiratory tachycardia and increased SV. Therefore, only data after this Respiratory Sinus Arrhythmia (RSA, Fig. 1) had stabilized were used for analysis and comparison. The average (± s.d.) f_H, SV, and CO after spontaneous breaths were 44 ± 6 beats min^{-1}, 179 ± 31 ml, and 7909 ± 1814 l min^{-1}, respectively. We used the RSA to successfully predict inhaled tidal volume (V_T, Fig. 2) (Cauture et al., 2019; Fahlman et al, 2019). During the apnea the f_H, SV, and CO decreased proportionally with the breath-hold duration, and after 5 min they, respectively, had decreased by an average of 18%, 1-21%, and 12-36%. During Frecovery, the f_H, SV, and CO rapidly increased

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by as much as 117%, 34%, and 190%, respectively. Next, f_H, SV and CO rapidly decreased to resting values between 90-120 s following the surface apnea (Fig. 3). These data highlights the necessity to accurately estimate resting f_H at the surface separating it from the RSA associated with each breath, and questions if the dive response exists or is an artefact of analyzing confounding variables.

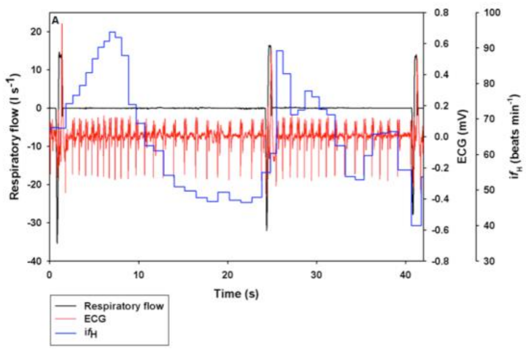

Figure 1. Representative data showing respiratory flow, ECG, and instantaneous heart rate (*(ifH) in a bottlenose dolphin during rest (Cauture et al. 2019)*

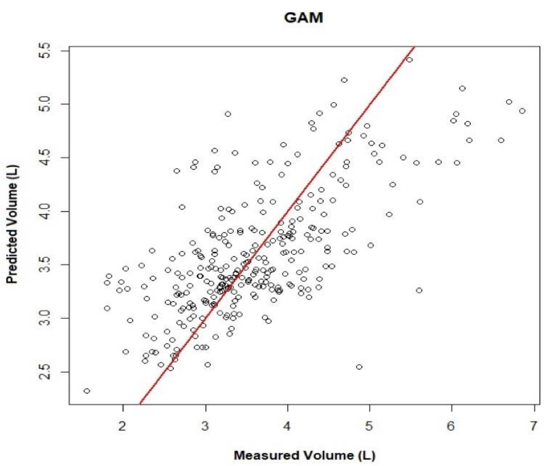

Figure 2. Predicted vs measured inspired tidal volume (r^2 = 0.45). The GAMs model is used to generate the predicted volume, and measured volume is the inspired tidal volume measured using the pneuomtachometer, red line is the line of unity (Cauture et al, 2019).

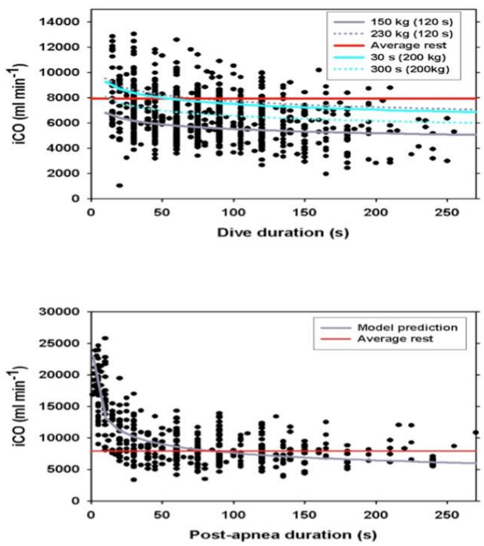

Figure 3. Measured and predicted instantaneous cardiac output (iCO) during and following surface apneas. Red line is the data for a resting dolphins before apnea.

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PHYSIOLOGICAL CONSEQUENCES OF FLIGHT RESPONSES IN DIVING MAMMALS

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Background

The purpose of this study is to determine the physiological consequences of escape responses in cetaceans, and to develop key metrics for accurately predicting short- and long-term energetic impacts of anthropogenic noise on diving mammals. Our approach considers that anthropogenic disturbances that harm marine mammals are associated with extreme, conflicting physiological responses (high speed and elevated stroke frequencies coupled with bradycardia and metabolic suppression) that occur with submerged escape. We focus on cetaceans due to the sensitivity of this group to oceanic noise, their vulnerability to developing decompression syndromes, the lack of biological information regarding anthropogenic threats, and concern over the number of cetacean strandings attributed to Navy sonar (Williams *et al.,* 2018). Using a lab-to-field protocol with trained and wild odontocete cetaceans ranging in body mass from 50 kg porpoises to 3500 kg killer whales, we are testing the central hypothesis that conflicting exercise, diving, and cognitive responses comprising escape reactions override the unique biological adaptations that normally enable cetaceans and pinnipeds to safely complete a dive, and represent a risk to metabolic and cardiovascular homeostasis. We are currently evaluating three commonly reported reactions to oceanic noise by cetaceans, diving deep, performing escape maneuvers, and “cardiac freezing” during submerged flight.

Objectives

Four specific objectives of the project are:

1. **Determine energetic costs and physiological risks of evasive maneuvers by dolphins and whales.** Building on our previous work on preferred exercise in marine mammals, we are evaluating the impact of high-level underwater performance typical of escape reactions by cetaceans.

2. **Build allometric regressions for predicting gait-specific stroking costs for small and large cetaceans.** Comparative measurements of oxygen consumption and stroking mechanics from exercising cetaceans varying in size from 50 kg to 3500 kg are being used to develop allometric regressions and predictive metrics for determining acute and chronic impacts of escape dives.

3. **Assess the physiological effects of metabolic diapause in diving cetaceans particularly for maintaining heart-brain integration.** Using a random dive schedule design with trained bottlenose dolphins we are investigating the inter-relationship between diving bradycardia, and central and peripheral blood flow during submerged exercise by dolphins and whales.

4. **Apply energetic/physiological/physical metrics to predict the cost of escape in free-ranging cetaceans exposed to anthropogenic noise.** To test our overall hypotheses in the field, we are integrating our allometric data and accelerometer/heart rate records from wild cetaceans (narwhals, beaked whales) exposed to experimental noise to evaluate the instantaneous cost and potential for damage to hypoxia-sensitive tissues (e.g., heart, brain) during escape responses.

Together, these studies are designed to develop a suite of metrics (performance costs, evidence of cardiovascular anomalies, relative risk to neural function, atypical behaviors) to define acute and chronic impacts due to oceanic noise. Importantly, the project will accomplish the overall objective of improving the protection of marine mammals during Naval operations by enabling Navy personnel to develop environmentally-sensitive schedules for acoustic activities that account for the most likely lethal and sub-lethal effects on marine mammals.

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Methods
We have developed an electrocardiograph-accelerometer-depth (ECG-ACC-Depth) monitor (Fig. 1) that has been deployed on wild, free-ranging narwhals with collaborators from the Greenland Institute of Natural Resources (Williams *et al.,* 2017a and in prep.) as well as on trained bottlenose dolphins to model beaked whale responses to anthropogenic noise (Williams *et al.,* 2017b).

Fig. 1. ECG-ACC-Depth monitor deployed on a narwhal in Scoresby Sound, Greenland. The tag is placed on the skin by suction cups and falls off after 1- 4 days.

Combined with measurements of oxygen consumption using open-flow respirometry (Sable Systems, Inc., Henderson, NV, USA) and tissue oxygenation/blood flow recorded with a portable near-infrared spectroscopy (NIRS) system (Artinis Medical Services, Netherlands) and heat flux sensors, we are now integrating the behavioral and biomechanical responses of cetaceans with cardiovascular and energetic responses at the whole animal, organ, and tissue levels.

Results
The most recent measurements using the ECG-ACC-Depth monitor were conducted on wild, adult narwhals exposed to anthropogenic noise (seismic pulses). Preliminary results show that primary physiological responses to noise exposure included an increase in the intensity of bradycardia during diving, and a marked increase in respiration rate and heart rate during the post-dive recovery period, which were both correlated to changes in stroking frequency (Fig. 2). These, in turn, instigated an increase in cardiac variability during diving and an elevation in energetic costs with potential implications for challenging overall physiological homeostasis.

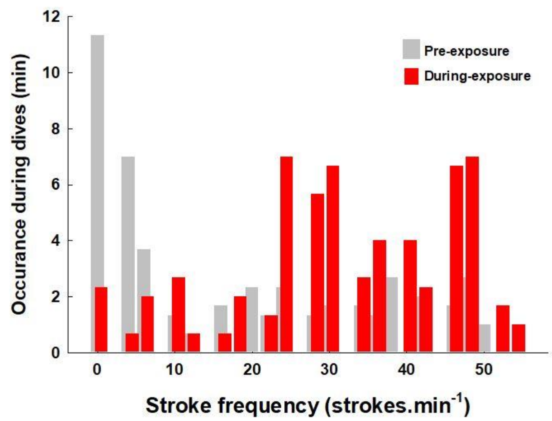

Fig. 2. Changes in the preferred stroke frequencies used by an adult narwhal during 100 - 200 m dives occurring before (grey bars) and during (red bars) exposure to seismic noise events. Zero stroking indicates periods of gliding, which was markedly reduced during escape responses.

Notes:

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Evaluation of Non-lethal Effects of N2 Bubbles on Marine Mammal Health and the Potential Role of Immune Activity in Facilitating the Development of Dive Related Injury

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Background
Dive related injury, such as decompression sickness (DCS), is linked to activation of inflammation (Ward *et al., 1987;* Barack and Katz, 2005). While the occurrence of nitrogen gas (N2) bubbles in diving marine mammals is still under question, little is known about how marine mammals may respond to such a challenge, or even how the marine mammal immune system is adapted to diving under normal conditions. This work builds on previous ONR funded research exploring the relationship between marine mammal health, dive physiology and stressors. Results will increase understanding of how marine mammals avoid dive related pathologies, and aid in interpreting integrated effects of human activities on marine mammal health.

Objectives
This project aims to address the hypothesis that a less reactive immune system serves a protective role against the development of decompression sickness (DCS) in marine mammals by evaluating marine mammal immune responses to N2 gas bubbles *in vitro*.

Aim 1) Measure phagocytosis and respiratory burst in marine mammal blood samples following in vitro exposure to N2 bubbles.
Aim 2) Monitor changes in complement components (complement activation) in marine mammal blood samples following in vitro exposures to N2 bubbles.
Aim 3) Evaluate the role of exercise modulation on the response of immune cells and complement activation to diving and in vitro exposures to N2 bubbles.
Aim 4) Validate the presence of complement proteins in blow for potential use in monitoring immune status in belugas.

Methods Beluga blood and blow samples were obtained from beluga whales (n=7) at the Mystic Aquarium, Mystic CT, as well as wild belugas in Bristol Bay, AK (n=9). Additional beluga samples were provided by SeaWorld San Antonio. Harbor seal samples were collected from stranded animals admitted to the Animal Rescue Program at Mystic Aquarium.

Cortisol was measured at the Endocrinological lab at Cornell University, and catecholamines were measured in house using a Waters (Milford, MA) High Performance Liquid Chromatography system with Electrochemical Detection.

N2 bubbles were introduced to blood samples *in vitro* at 4 flow rates (0.02, 0.06, 0.5 and 1.0 ml min^{-1}), for 3 durations (30 min, 3 min and 30 seconds).

Aim 1: Phagocytosis and respiratory burst were measured using the protocol detailed in Spoon and Romano (2012), developed for use with marine mammal samples at the Mystic Aquarium.

Aim 2: A commercially produced enzyme immunoassay (EIA) for complement protein C5a was validated and used to measure complement activation.

Aim 3: Blood and blow samples were collected from a female beluga following 3.5 minute active and stationary dive behaviors.

Aim 4: Paired blood and blow samples were collected from belugas to confirm the presence of complement proteins in blow.

Results
Aim 1: Belugas displayed increased measures of phagocytosis following the 30 min exposure to N2 at 0.06 ml/min. Increased phagocytosis was also observed following 3 min exposures to 0.5 and 1ml/min. In contrast, no significant changes

in phagocytosis were detected from harbor seals. Analysis of respiratory burst activity was inconclusive.

Aim 2: Harbor seals displayed a significant increase in C5a following 30 min exposures to N2 at a flow rate of 0.02 ml/min (p=0.045). In most cases release samples showed larger and more variable responses to N2 bubbles as compared with admit; with the most similar response between the two conditions occurring during the highest flow rate of 1 ml/min.

The only significant increase in C5a was detected for Bristol Bay belugas following 30 min exposures to the highest flow rate of 1 ml/min (p=0.019). Bristol Bay whales also tended to display larger changes in C5a in response to N2 exposures as compared with aquarium whales, while both groups of beluga display smaller overall changes in C5a as compared with release harbor seal samples.

Aim 3: Norepinephrine was higher following both dive behaviors as compared with baseline, which was expected as norepinephrine plays a role in regulating the dive response. Dopamine was only detected following the active dives and was higher than baseline months. Cortisol was lower following stationary dives as compared with baseline samples, while active dives and baseline were similar.

The active dives themselves did not appear to alter phagocytosis from the individuals' baseline measures, while stationary dives may have resulted in some lowered phagocytic activity. Respiratory burst, in contrast, shows increased activity following active dives, with smaller increases occurring following stationary dives. The effect of dive behaviors on the phagocytic and respiratory burst response to N2 bubbles was variable.

Serum C5a was slightly higher following active dives as compared with baseline, though this response was quite variable among three trials. Following stationary dives there was a greater and more consistent increase in C5a. The C5a response to N2 exposures showed marked differences from baselines following both the active and stationary dives in response to 0.5 ml/min, with an even larger increase, though more variable response, to the higher flow rate of 1 ml/min (Fig 1).

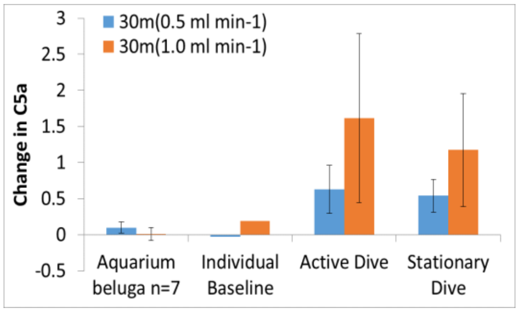

Figure 1: Measured changes in C5a in belugas following the highest flow rates of N2 bubble exposures under baseline conditions and following active and stationary dive.

Aim 4: A signal for C5a was detected in blow, and the calculated % CV (variability) of blow C5a measures was <15% for samples as small as 10 µl. There was a positive correlation between serum and blow C5a (r^2=0.824, p=0.001). Blow samples collected in conjunction with dive behaviors (Aim 3) also display patterns of C5a similar to those seen in serum i.e. changes in blow C5a were minimal following active dives, while slightly larger changes in C5a were observed following stationary dives. It is likely that there is a lag between changes in blood and the corresponding signal in blow and more work is needed to define the physiological timeframe represented by immune markers in blow.

Notes:

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‘PCoD+’: Developing widely-applicable models of the population consequences of disturbance

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Background

Policy makers and managers are increasingly concerned about the effects of disturbance on wildlife populations. However, forecasting population level consequences of changes in individual behavior is difficult. Working groups established by the US National Academy of Sciences and the US Office of Naval Research developed conceptual models of the population consequences of disturbance (PCoD) for marine mammals (NRC 2005). The PCoD framework provides a conceptual framework for developing PCoD models, identifying the transfer functions that should be included either explicitly or implicitly in such models. However, it does not provide any guidance on the costs and benefits of developing a full PCoD model compared with more simplified ones, nor on how the parameters of the transfer functions are best estimated, or how aggregate exposure to disturbance can be assessed.

Objectives

The overall objective of this project is to overcome the most important impediments that have limited the implementation of the PCoD framework. This is being achieved via five project tasks: 1): Develop Methods for Assessing Aggregate Exposure. 2): Develop a PCoD Decision Framework. 3): Benchmark Models to inform PCoD analyses. 4): Develop a Standard Protocol for Expert Elicitation (EE) in a PCoD Context. 5): Identify Priorities for Monitoring PCoD.

Methods

Task 1: We are developing a computationally-efficient model fitting and simulation framework for assessing the distribution of aggregate exposure for individuals in a population over an annual time scale. Our main case study datasets to date have come from telemetry data studies of Blainville’s (*Mesoplodon densirostris*) and Cuvier’s beaked whales (*Ziphius cavirostris*), and fin whales (*Balaenoptera physalus*).

Task 2: We have been developing a decision framework that can be used to prioritize the development of future PCoD models and to identify the most appropriate form of model for a given population, based on likely data availability. A first draft of the decision framework was presented at a workshop to a group of invited marine mammal scientists and regulators and policymakers. Since then the framework has been iteratively refined and finalized.

Task 3: We have developed a range of benchmark models which can be used to support other PCoD+ project tasks and have wider applications outside of this effort. These involve Stochastic Dynamic Programming (SDP) and Dynamic Energy Budget (DEB) models for species that are mixed capital and income breeders.

Task 4: The task is divided into two components: student testing and formal elicitation including professionals from the marine research community. An online e-learning resource has been developed to train experts in elicitations and in making probabilistic judgements. Through student tests and dedicated expert elicitation workshops on beaked whales (*Family Ziphiidae*) and blue whales (*Balaenoptera musculus*) a Protocol for Expert Elicitation for PCoD (PEEP) has been developed and refined.

Task 5: A literature review of grey and published literature was conducted to identify the most appropriate monitoring methods by which to detect early warnings of population change. The outcomes of the review were presented to experts spanning a range of disciplines and, following a ‘lines of evidence’ approach, was used to assess the feasibility and utility of each approach for different marine mammal species. A sensitivity analysis was also conducted using existing PCoD models to identify if changes in population structure demographic parameters could be used to detect provide an early warning of changes in population size.

Results

Task 1: To avoid having to track individual animals at a fine spatial and temporal resolution for an entire year, we are developing both coarse- (using a discrete-space continuous-time two-state hierarchical Markov model) and fine-scale models; the coarse-scale model allows us to determine whether each animal is inside/outside the area where sonar may operate. Only animals that are inside are subjects for the fine-scale model which can operate at 1s time-scales (in continuous space) and is used to track animals once they are inside the area. This fine-scale model also efficiently approximates the animal’s trajectory by considering only the locations of dive starts, dive duration and vertical depth distribution (methods of estimating the movement model parameters from data are in development).

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Task 2: The decision tree consists of a series of five questions which explore the sensitivity and susceptibility of marine mammal populations, with respect to the temporal and spatial extent of the activity being assessed, and the reproductive strategy and the life history stages of the species. In addition, the risk of multiple exposure is assessed. Finally, the kind of PCoD models that could be developed for species identified as high priority is explored using the framework presented in Pirotta et al. (2018).

Task 3: Pirotta, et al (2018) reviewed applications of the PCoD framework to marine mammal populations, highlighted key research gaps and future directions and provided a decision tree to select the most appropriate modelling approach given data availability. Hin et al (in review) describes a DEB model for a medium-sized cetacean, the long-finned pilot whale (*Globicephala melas*) and explores how short periods of severe disturbance can lead to the pre-weaned death of the first one or more calves of young females. Longer periods of severe disturbance levels also affect survival of calves produced later in the life of the female, and may even degrade female survival itself. These results strongly depend on the severity of disturbance and on the available resources in the environment. An SDP equivalent of this DEB model has been in developed, to allow comparison of these two modelling approaches. Pirotta et al (in press) describes an SDP model spanning the lifetime of a female blue whale with environmental and anthropogenic disturbances. The model indicates that juvenile blue whales are more susceptible to disturbance and that mature females prioritize survival over reproduction. In general, lifetime reproductive success is robust to anthropogenic disturbance but environmental variations have a potentially large effect.

Task 4: The e-learning resource is now available online at: http://tiny.cc/EEtraining. This training course has been used in multiple PCoD expert elicitation workshops and found to be extremely valuable. In addition, the PEEP has also been tested and represents a marked improvement for guiding expert elicitation for PCoD. Use of a robust EE protocol and a well-trained facilitator are important to the success of any EE.

Task 5: Using existing PCoD models, we determined that changes in certain demographic variables (e.g. proportion of immatures, etc.) are strongly correlated with changes in abundance or population status, and can therefore provide some early warning of future changes in abundance. We observed that demographic parameters tend to be most commonly estimated from monitoring using established approaches, such as visual surveys and capture-recapture approaches. The continued development of remote tissue sample libraries and analytical approaches to improve our understanding of marine mammal stress response, physiology and –omics fields is critical.

Notes:

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

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Background

The long-term goal of this research is to create a Population Consequences of Acoustic Disturbance (PCAD) model for evaluating and monitoring the health of the Cuvier's beaked whale (*Ziphius cavirostris*, hereafter Zc) population on the U.S. Navy's Southern California Offshore Range (SCORE) in California, with a focus on the possible population-level effect of mid-frequency active sonar (MFAS). The geographic focus within SCORE is the Southern California Anti-submarine warfare Range (SOAR), which includes an array of hydrophones that are used to monitor Zc vocalizations and sonar. This work contributes to the Navy's goal to measure the "health of populations", rather than simply monitoring their size, especially those exposed to MFAS.

Objectives

1. Use passive acoustic monitoring (PAM) data from SOAR hydrophones to estimate long-term abundance of Zc on SOAR.
2. Use PAM data and ship tracks to obtain assess risk of behavioral disruption as a function of sonar exposure.
3. Use sightings data from a photo-ID study to derive indices of population demographics.
3. Use a bioenergetic model to investigate the potential effect of foraging dive disruption on individual survival and breeding and the population-level implications.

Methods

1. Long-term abundance.

Eight years of beaked whale detection data were collected on SOAR on a near-continuous basis. Estimates of mean monthly Zc population abundance were derived from an extended version of the dive counting method of Moretti et al. (2010). Extensions were required to allow for automated processing of the long time series, leading to false positive and false negatives in the count data. The frequencies of these errors were estimated from samples of data that were manually processed, and the error rates were accounted for in the abundance estimation methodology.

2. Risk function.

Acoustic data from 2015 was processed to give the approximate location of beaked whale group dive starts ("Group Vocal Periods", GVPs) and the timing of sonar. Sonar received level at beaked whale group locations was modeled assuming a source level of 215 dB dB re µPa @ 1m and transmission loss due to spherical spreading with no sound attenuation. The presence or absence of GVPs per 30-minute window close to each SOAR hydrophone was modelled as a function of sonar RL using a binary Generalized Additive Model; results were translated into a risk function using the method described by Moretti et al. (2014) which relates predicted probability of a GVP to baseline levels.

3. Population demographics.

Zc photo-ID data were collected on and around SOAR from 2006 to 2017. Females with dependent calves were noted, and an estimate fo the ratio of dependent calves to adult females was derived.

4. Bioenergetic model.

A generalized bioenergetics model was developed, based on one created under the ONR-funded PCoD+ project (N000141612858) for long-finned pilot whales. The sonar and GVP data were used to estimate the probability that an individual Zc on SOAR during a particular 30-minute interval would be disturbed, and the duration of the disturbance. This information was used to simulate disturbance histories for adult female of different ages to investigate how this might affect calf survival and inter-calf interval.

Results

1. Long-term abundance

Average population abundance per month ranged from 10 to 56 animals, with a distinct dip around September (Figure 1).

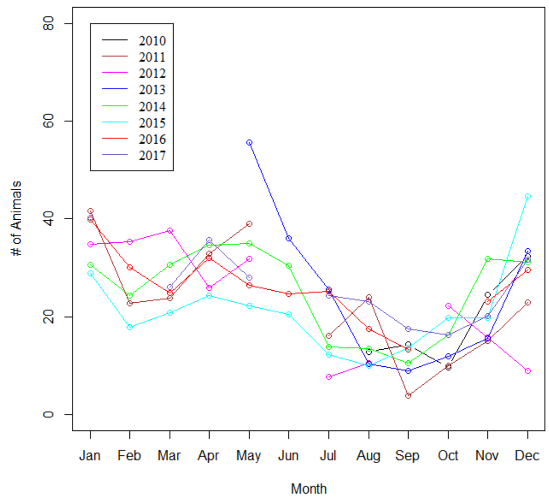

Figure 1. Mean monthly estimated Zc abundance on SOAR for years 2010-2017

2. Risk function.
Data were available for 12,014 30-minute periods, of which GVP starts were detected on 4,775. Probability of GVP start declined as

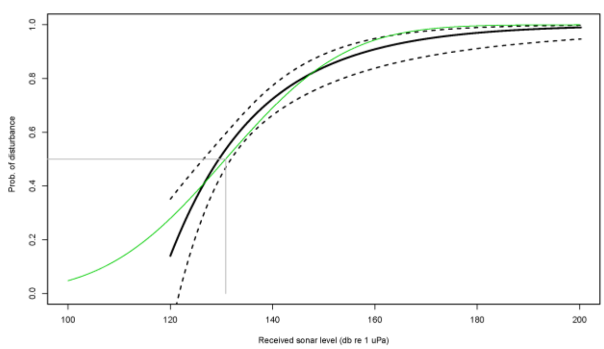

Figure 2. Probability of foraging disturbance as a function of sonar received level (black solid line) with 95% credible intervals (dashed lines). Green line shows a parametric (GLM) fit to the same data.

received sonar level increased, with the resulting risk function showing a 50% probability of disturbance at a received level of 130 db re µPa (Figure 2).

3. Population demographics.
Of 184 individuals assigned to an age class, 56% were classified as adults, 14% subadults, 6% juveniles and 10% as calves.

4. Bioenergetic model.
At the observed levels of behavioral disturbance at SOAR, for females to successfully raise their calves required that either they spend some time in undisturbed habitats, or if resident that the habitat quality at SOAR was higher than elsewhere (Figure 3). Results show how different assumptions about how disturbance affects energy acquisition affect reproduction and survival. Further work is needed to undertake sensitivity analysis on inputs and to generate a population-level model.

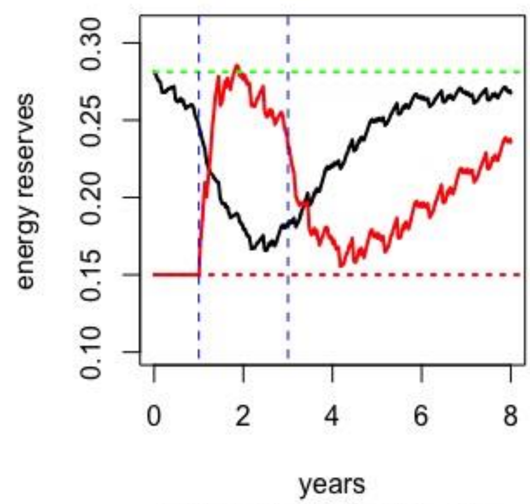

Figure 3. Predicted changes over time in energy reserves of 12 yr old female Zc (black) and her calf (red) over female's pregnancy and lactation, assuming female spent 100% of time on SOAR but with 10% higher productivity than baseline. Green dashed line is energetic threshold for breeding and red dashed line for mortality.

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Development of an Index to Measure Body Condition of Free-Ranging Cetaceans

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Background

Disturbance of marine mammals can induce missed feeding opportunities, lowering energy intake. Over the long-term body condition of individuals could decline, which can have profound population consequences. Photogrammetric techniques have been used to monitor the body condition of free-ranging mysticetes and killer whales, where variations in dorsal body widths were examined and attributed to nutritive condition. Despite the use of this methodology, a validation study had not been done to link changes in body width with changes in the underlying blubber thickness and to identify the region of the body where blubber thickness varies consistently with caloric intake, because in some regions blubber has a structural function rather than an energy reserve function. Moreover, the use of this methodology may be limited with odontocetes because body width variations will be much smaller and body contours could be occluded by waves, water spray, and turbidity.

Objectives

1. Validate underlying assumptions of photogrammetry; body width changes in accordance with changes in underlying blubber thickness in relation to caloric intake.
2. Provide a validated, non-invasive tool to measure body condition of odontocetes to track energetic consequences of disturbance.
3. Provide data on interrelationship between caloric intake and body condition to be incorporated into future PCAD models.

Methods

Investigations of marine mammals in human care provide opportunities to validate body condition metrics. Pilot whales (n = 2 males and 4 females) housed at SeaWorld San Diego and Orlando were studied longitudinally at five intervals for nearly two years. Standard operant training protocols were used. Caloric intake and water temperature were recorded daily. The whales were weighed weekly by beaching on a platform scale. Quarterly morphological (via tape measure) and blubber thickness (via ultrasound) measurements were taken while the whales remained in the water (see Fig. 1). During the same sample intervals, overhead photographs were taken as the whales swam underneath a camera (EOS-1DX Mark II Canon camera with EF50mm f/1.8 STM lens and WFT-E8 wireless file transmitter attachment that enabled remote operation via a laptop) mounted high above the pool. The whales swam straight and upright with their dorsal side up. Wake and water splash were minimized by having the whales swim slowly. Refraction was minimized by having the whales swim at the water's surface.

Results

A state-space model and a model that provided for a month lag between caloric intake and body mass demonstrated that body mass was influenced by caloric intake but not water temperature [ranging from 15.51 to 25.39 °C at San Diego (average ± SD = 20.29 ± 2.85 °C) and 13.00 to 25.20 °C (average ± SD = 19.2 ± 3.90 °C) at Orlando]. To account for body length variations across whales, body mass, body girth, blubber thickness, and body width indexes were calculated as the residual values of these variables regressed against length. At $p<0.1$, only blubber thickness measured laterally at the anterior pectoral fin, anterior dorsal fin, and posterior dorsal fin insertion sites varied in relation to body mass. This suggests that blubber in these regions serves a metabolic function. Lateral blubber thickness measured at the genital slit and "mid-peduncle" sites did not vary in relation to body mass. This supports previous hypothesis that blubber in the peduncle region primarily serves a structural role. Moreover, body girths at sites #1-3 varied in relation to body mass, while body girths at sites #4-5 did not. Body width from overhead photographs could only be reliably measured at sites #1-3 (Fig. 1). Amongst the three sites analyzed, photogrammetric body width at the posterior dorsal fin site was the only body width

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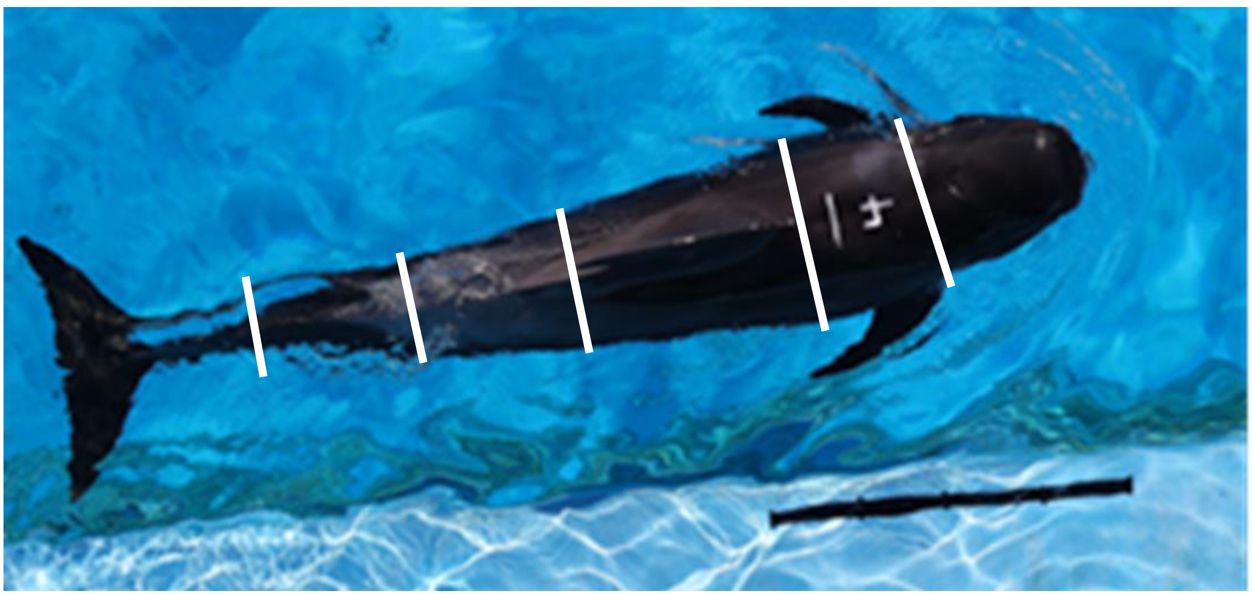
to have a significant positive relationship with underlying blubber thickness and body mass. Only body width body length ratio at this site predicted mass, thus photogrammetric measured body width at approximately 47% of total body length from the rostrum can be used as a reliable metric to monitor body condition of pilot whales. Additional studies are warranted to determine if body width at this site reliably predicts condition across odontocete species with varying body morphologies.

Notes:

Fig. 1. A photograph of pilot whale #4 taken from overhead. The black line in the photograph is a painted line on the pool edge that provided the scale for the photographic measurements. Colored zinc oxide was used to mark the whales with their indentifying number and to draw a line near the anterior insertion of their dorsal fin. Heavy white lines mark the 5 areas of the body that were investigated; going from the rostrum to the tailfluke these were, #1 anterior pectoral fin insertion, #2 anterior dorsal fin insertion, #3 posterior dorsal fin insertion, #4 genital slit, and #5 “mid-peduncle”, halfway between the genital slit and fluke insertion. Body girth and lateral blubber thickness was measured at each of the sites. Body width measurements from overhead photographs were only possible at sites #1-3 because body contours at sites #4 and #5 were occluded by waves.



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Sponsor: ONR
Status: ONGOING

Assessing resilience of beaked whale populations to human impacts: Population structure and genetic diversity in impacted and semi-pristine areas

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Background

Genetic diversity, life history traits, social structure and social cohesion influence the persistence and resilience of cetacean populations (Matkin et al. 2008; Carroll et al. 2011; Wade et al. 2012). Within cetaceans, beaked whales (family Ziphiidae) are especially vulnerable to naval sonar. To provide information for management of beaked whale populations, the goal of this project is to assess if beaked whales inhabiting areas exposed routinely to naval sonar, or areas where several mass strandings related to naval activities have been reported, might have suffered population level effects. For this, we will perform a comparative analysis of genetic diversity and social structure of beaked whales living in neighboring impacted and semi pristine areas with little or no connectivity of beaked whales between these areas. This analysis requires first to investigate general patterns of genetic differentiation in target beaked whale species and their population structure among and within ocean basins.

Objectives

To compare genetic diversity and population structure of Cuvier´s and Blainville´s beaked whales between semi-pristine/low sonar impact beaked whale populations and populations in nearby areas where several mass strandings of beaked whales have been related to sonar activities. The paired-site comparison will use data from El Hierro (western Canary Islands) and the Ligurian Sea (Italy) and their matched disturbed comparators: the eastern Canary Islands and Ionian Sea, respectively, The results will be analyzed in tandem with on-going genetic studies in the Bahamas (Abaco and AUTEC). These three pairs of semi pristine-disturbed areas are inhabited by beaked whales with little apparent connectivity between sub-areas within each general study area. This offers a unique opportunity to perform a comparative analysis of little-connected whales inhabiting similar environments. The results will be placed in the context of a global study of genetic diversity and population structure, pooling samples from a network of collaborators worldwide.

Methods

The first step is to assess global patterns of genetic diversity to frame the interpretation of the results on local diversity. The current beaked whale archive contains 509 samples provided by 78 contributors in 30 countries consisting of the following confirmed species: *Hyperoodon ampullatus, Mesoplodon bidens, Mesoplodon densirostris (Md), Mesoplodon mirus, Mesoplodon traversii, Mesoplodon europaeus,* and *Ziphius cavirostris (Zc)*. A subset of the samples (65) were collected as biopsies from free-ranging animals, mostly from the Canary Islands and the Mediterranean, but the majority are skin, blubber/muscle collected opportunistically from stranded individuals. Many of these samples were already gathered by collaborator Dr. Merel Dalebout and made available to this project with consent of the original donators of the samples. Some biopsy and stranded animal samples were provided directly from NOAA's Southwest Fisheries Science Centre as extracted DNA. We used restriction associated digest tag sequencing (RADseq) to generate high-resolution genomic profiles for Cuvier's and Blainville's beaked whale populations. For broader scale phylogeographic assessment, and to supplement the ddRADseq data, complete mitogenomes were obtained from a subset of the samples by "shotgun" sequencing. These were analysed in a maximum likelihood phylogenetic framework together with previously published data, as well as novel data generated from samples of too poor quality for ddRADseq.

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Results

DNA was extracted from 370 of the 421 samples from *Zc* and *Md*; of these, 226 (*Zc* n=170, *Md* n=56) produced high quality DNA for double-digest Restriction Associated DNA sequencing (ddRADseq). ddRAD reads were analysed and filtered through a bioinformatic pipeline that involved aligning sequences to reference genomes for *M.bidens* and *Zc*. The remaining bioinformatic steps identified and genotyped single nucleotide polymorphisms (SNPs) and filtered out loci and individuals with insufficient data.

After quality control and filtering steps, n=129 *Zc* and n=43 *Md* samples were retained for analysis, providing n=25,059 and n=13,988 SNP loci, respectively. To explore patterns of population structure we used a hierarchical analysis with the R package tess3r (Caye *et al.*, 2018). Figure 1 below plots ancestry coefficients by individual for *Zc* (Fig. 1a) and *Md* (Fig. 1b) where each bar represents an individual and each color corresponds with the likelihood of membership to a specific population.

Zc and *Md* individuals cluster into groups defined by major ocean basins: Atlantic, Indo-Pacific and Mediterranean (*Zc* only). There was significant genetic differentiation between ocean basins within species, based on a commonly used measure (F_{ST}, $p<0.05$). Within the Atlantic, both *Md* and *Zc* showed significant patterns of differentiation between the west and east coasts. In the Indo-Pacific, *Zc* samples are structured based on sampling location, with all pairwise comparisons of populations with n>3 individuals being significantly different. In *Md* populations, the same pattern is seen. In the Mediterranean there is significant structure between the west and eastern sides of the Strait of Sicily, a known barrier to gene flow in many taxa (Mejri *et al.*, 2009; Zitari-Chatti *et al.*, 2009) and this same pattern was observed in *Zc.*

For *Zc*, the mitogenome analysis support the existence of a separate North Atlantic-Mediterranean clade, as well as two Indopacific clades including a few animals from the Caribbean, suggesting a past dispersal corridor through the Panama Seaway before its closure 3 mya. In contrast, *Md* appear less admixed, with a clear separation into a North Atlantic and a Pacific-Southern Ocean clade, respectively. For both species, the base of the phylogenetic tree contains samples from Central America, indicating that this might have been a centre of beaked whale speciation.

These results will be applied now to the interpretation of the paired-site comparative analysis of genetic diversity.

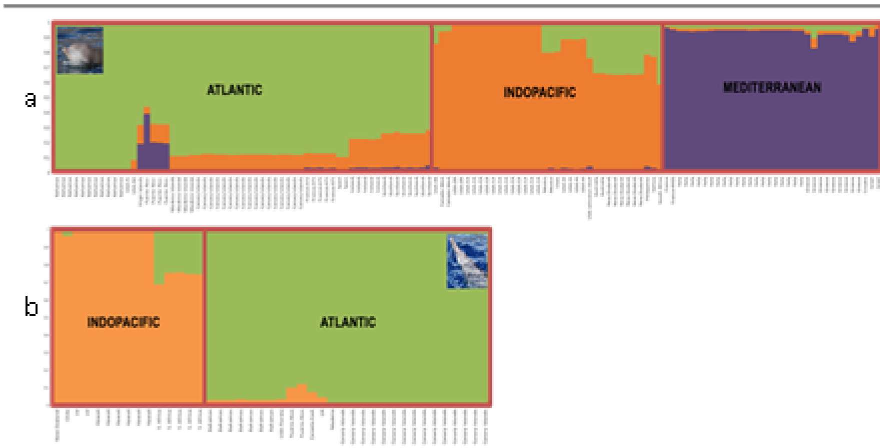

Fig. 1. Population structure illustrated as barplots of coancestry coefficients for three populations of *Z. cavirostris* (a) and *M. densirostris* (b). Each vertical bar represents an individual, and the colour its degree of affinity to one or several populations. Individuals with multiple colours indicate some admixture. Both species show population clustering based on ocean basin (Atlantic and Indopacific) with *Z. cavirostris* also appearing in the Mediterranean.

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Approved, DCN# 43-5191-19

Understanding marine mammal composition in the northern Indian ocean using visual and passive acoustic methods

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The northern Indian ocean (NIO) is a semi-enclosed ocean basin bound by the Asian and African landmasses. This basin is severely affected by strong monsoonal cycles (southwest and northeast monsoons) and reversing surface ocean currents. The southwest coast of India sees significant upwelling during the southwest monsoon season as well as intrusions of fresher waters from Bay of Bengal during the northeast monsoons. We hypothesize these distinct oceanographic processes influence the spatial and temporal variability of regional marine mammal composition here. At least 30 cetacean species including endangered and critically endangered populations reside in the area. NIO low latitudes support resident whale populations that are both genetically and behaviorally distinct. The objectives of this project is to 1) gain a better understanding of marine mammal occurrence over time and space using visual and acoustic methods in the Lakshadweep region, off the southwest coast of India and 2) determine variations in marine mammal composition with respect to oceanographic features such as currents, areas of upwelling and plankton blooms using direct observations and remote sensing data. The project is currently in progress and data through passive acoustic monitoring and visual survey methods is being collected from the Lakshadweep archipelago. Preliminary results on exploratory surveys on marine mammal diversity and distribution will be presented. This research will improve our understanding on relationships between tropical cetaceans and oceanographic conditions in this area of large environmental variability. The northern Indian Ocean is a region of high strategic importance to the United States, and Naval vessels use this area regularly. Continued operational use of sonar in this region requires a better understanding of the risks to marine mammals, and a large part of the assessment of that risk requires characterization of the distribution and abundance of cetaceans and how that distribution changes with changes in the environment.

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QUANTITATIVE GENE EXPRESSION SYSTEM FOR STATE-OF-THE-ART BIOMARKER RESEARCH AND DEVELOPMENT AT AN UNDERGRADUATE INSTITUTION

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Background
Abundance of messenger RNA is a highly sensitive indicator of gene activity in biological systems, which can be altered by physiological and environmental stressors. Measurement of changes in expression of stress-responsive genes in tissues such as adipose is a robust approach for evaluating stress states in wildlife potentially exposed to anthropogenic disturbance, especially in species such as fully aquatic marine mammals from which blubber tissue, but not blood, can be obtained by remote sampling. Assessment of stress states is relevant to the management and conservation of marine mammals potentially exposed to anthropogenic disturbance such as ocean noise, and addresses the requirement of U.S. federal agencies to assess the impacts of their activities in the ocean on the health of marine mammals.

Objectives
This effort seeks to establish a quantitative gene expression platform for measuring molecular stress biomarkers expression in marine mammal tissues. This will advance efforts to evaluate stress states in marine mammal tissues at an undergraduate institution, incorporating ONR-funded research into undergraduate curricula and experiential learning programs.

Methods
Blubber samples were collected from 30 northern elephant seals of variable baseline stress states determined by body condition index and serum corticosteroid levels. Total RNA was isolated from blubber and its integrity was evaluated using microcapillary gel electrophoresis. Abundance of mRNA transcripts of stress-responsive genes that were previously identified by transcriptomics (Deyarmin et al., 2019) was assessed by reverse transcription and quantitative polymerase chain reaction (RT-qPCR). Pairwise correlation analysis was used to identify significant associations between gene expression levels and other stress biomarkers (McCormley et al., 2018), such as corticosteroid and thyroid hormones.

Results
Expression of genes encoding an adipokine (adiponectin), an antioxidant enzyme (GPX3), lipid metabolism enzymes (HMGCS2, ACSL1), lipid droplet proteins (perilipin 1, CIDEA), and regulators of adipogenesis (DKK1, AZGP1) were positively correlated with cortisol and reverse triiotothyroine, rT3 ($p < 0.05$) and negatively correlated with total triiodothyronine (tT3) levels ($p < 0.05$; Fig. 1). HMGCS2 was also negatively correlated with plasma triglyceride levels ($p < 0.05$). Therefore, blubber expression levels of gene biomarkers, which can be easily measured by RT-QPCR at an undergraduate institution, are indicative of stress state in marine mammals.

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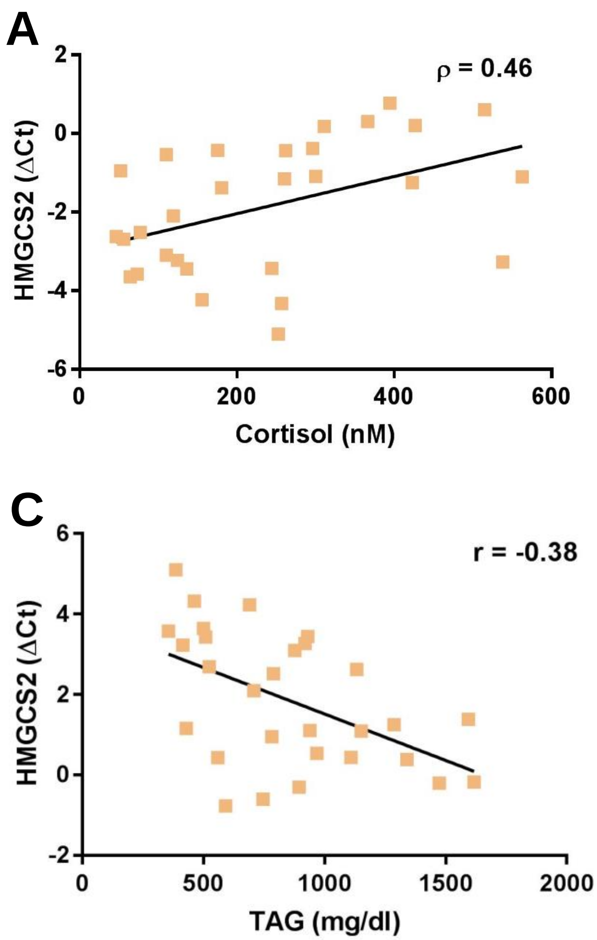

Figure 1. Blubber expression levels of the gene encoding 3-hydroxy-3-methylglutaryl-CoA (HMGCS2), an enzyme involved in fatty acid oxidation, was positively correlated ($p < 0.05$) with cortisol (A) and reverse triiodothyronine (rT3; B) and negatively correlated ($p < 0.05$) with plasma triglycerides (TAG; C) and total triiodothyronine (tT3; D) in 30 juvenile seals of varying baseline stress states. HMGC2 expression, cortisol, and rT3 were elevated and tT3 and TAG were suppressed in response to a repeated stress manipulation experiment in juvenile elephant seals (McCormley et al., 2018; Deyarmin et al., 2019; Champagne et al., in prep). r = Pearson correlation coefficient, ρ= Spearman's rank coefficient correlation (used for variables that were not normally distributed).

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Postnatal development of diving physiology: A review to examine vulnerability of immature beaked whales to hypoxia, hypercarbia, acidosis and decompression sickness (DCS) during their "flight response" associated with exposure to anthropogenic noise

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Under normal circumstances, the exceptionally long and deep dives of beaked whale species do not pose a risk for these animals because of anatomical, physiological, and behavioral adaptations. However, recently there have been multi-species mass strandings of marine mammals that were coincident with naval sonar exercises, which included beaked whale species exhibiting decompression sickness (DCS) like symptoms. The mechanism behind this pathology is still unclear. Moreover, immature beaked whales are undoubtedly the most at risk of suffering from decompression sickness (DCS) because their small body size theoretically results in higher mass-specific oxygen utilization rates compared to larger conspecifics. While at the same time, the physiology that supports breath-hold diving and submerged swimming may require postnatal development, as has been shown in other cetaceans. Because little is known about the magnitude or duration of postnatal development required to achieve mature diving physiology in beaked whale species, a review of the postnatal development in other marine mammal species will be undertaken. Dr. Noren is a recognized expert in the area of the postnatal development of diving physiology. Indeed, she pioneered this work on cetaceans. Dr. Noren will review and collate data from her own published work on this topic, as well as use search engines (i.e. Biosis, Web of Science) to collect information from other publications on this topic. Topics explored will include the postnatal development of every aspect of the oxygen pathway, including lung morphology, blood and muscle oxygen storage capacity and buffering capacity, diving heart rate and metabolism and blood flow patterns. Other topics will be included as needed. Ultimately, the magnitude and duration of the postnatal development of all aspects of the oxygen pathway will be reviewed across species in relation to maternal dependency period, habitat, and diving behavior to predict postnatal development patterns for beaked whale species. This review provides a cornerstone to ascertain the propensity of immature beaked whales to suffer from hypoxia, hypercarbia, acidosis and decompression sickness (DCS) when engaged in a "flight response" associated with exposure to anthropogenic noise. This research will improve the ability to understand biologically-significant effects of sound exposure.

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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. 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 potentially impact survival and reproduction through compromised body condition. This is of particular concern for pregnant and lactating females that have increased energetic demands. Recent findings of low reproductive success of *Md* at AUTEC have led to a hypothesized population consequence of repeated disturbances. However, empirical data on female body condition and growth of calves is lacking, constraining a direct assessment of whether nutritional stress from disturbance is a realistic mechanism for explaining the apparent population responses.

Objectives

We propose to combine photogrammetry, photo-identification and blubber hormone measures to examine nutritional stress and pregnancies in *Md* at AUTEC, with these objectives:

1. Aerial photogrammetry will be used to measure width profiles to directly 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 adult females with (lactating) and without dependent young. Nutritional health of males will also be assessed, and testosterone measurements will be used to determine the likelihood of being sexually mature.
2. Hormone-derived identification of pregnancy (progesterone and testosterone) will be used to ground-truth photogrammetric inference based on body shape.
3. Aerial photogrammetry will be used to compare state-specific size (total length) and body condition (width profiles) of whales at AUTEC to a reference population to investigate size differences as a potential indication of longer-term nutritional stress.

Methods

Photogrammetry measurements and blubber biopsies will be linked to individuals with known life histories, based on the pattern of natural scars discernible from the air and boat platforms. Field teams will operate at AUTEC during good weather windows throughout each year and scheduled as close as possible after Navy exercises to capture any physiological or nutritional stress responses that may be associated with disturbance during these events. Over the 4-year study, we aim to collect photogrammetry data with matching biopsy samples from 70 individuals, focusing on adult females. Multiple visits to the field site will enable follow up re-sightings of females known to be pregnant based on hormone measurements and photogrammetry morphometrics, allowing us to quantify reproductive success and calf survival. Males will be sampled opportunistically

Results

This project has been delayed in its launch due to new limitations for use of drones on Navy ranges. We now have a waiver to use our drone at AUTEC but are still waiting approval for the use of lithium batteries. Despite this delay, we successfully completed a 2-day field trip to AUTEC to collect biopsy samples.

Notes:

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Developing metrics of animal condition and their linkage to vital rates: Further development of the PCoD model

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Background

The PCoD model provides a framework to understand when a disturbance is biologically meaningful. Central to this model is an assessment of how behavioral responses to sound affect life functions, how life functions are linked to vital rates, and how changes in vital rates cause population change through a series of transfer functions. While logistical limitations preclude assessment of these transfer functions and mechanisms for most marine mammals, elephant seals are a robust system, particularly for capital breeders, where the data necessary to parameterize these transfer functions can be obtained. In this effort we are using existing data, along with data from our ongoing elephant seal field work, to further develop and refine a bioenergetics-demographic model, and to quantify the ability of these species to compensate for lost foraging opportunities associated with disturbance. We are detailing the physiological mechanisms that link body condition to reproduction, to better understand how energy status integrates with allostatic load to modulate reproductive effort. Understanding condition thresholds and their relationship to allostatic load is critical to assessing links between condition, health, and survival.

Objectives

1. Determining hormonal metrics to detect implantation, pregnancy, and mediators of gonadal function that link body condition and foraging success to reproduction in northern elephant seals.
2. Identify signatures of pregnant vs. non-pregnant female elephant seals within the diving behaviors.
3. Identify condition indices that indicate thresholds for implantation and termination of pregnancy.
4. Determine maximum and minimum condition thresholds for survival in both adults and pups.

Methods

From hormones to bio-logging.

We are combining measurements of physiological indicators of pregnancy from a suite of tissues with diving behavior data obtained from bio-loggers, to link pregnancy status (non-pregnant, pregnant and loss of a fetus, or successful pregnancy) to the mother's body condition and the environmental conditions experienced during her gestational foraging trip. Progesterone concentrations are being measured in the blubber, urine, blood, fur, and vibrissae collected from adult female elephant seals instrumented with satellite tags and time-depth recorders. This will allow us to determine if individual seals were pregnant at the beginning of their gestational foraging trip and better understand the maintenance of pregnancy during their foraging trip. By combining these approaches, we will gain insight into (1) the timing of the implantation during the molt, (2) the progression of pregnancy throughout the gestational trip, and (3) the behavioral and environmental factors that have an effect on the pregnancy status and maintenance for northern elephant seals. Ultimately, our study will provide us with the tools to empirically determine the body condition thresholds necessary for females to become pregnant and to maintain pregnancy.

Results

We have demonstrated that diving behavior can be used to determine pregnancy status of northern elephant seals: while the dive duration of females increases during the first 3 – 4 months of their post-molt trip, pregnant females experience a plateau and posterior reduction in their dive duration along their trip, whereas the dive duration of seals that do not have a pup continues to increase monotonically along their trip (Figure 1).

We have measured cortisol, progesterone and estradiol in the complete elephant seal sample bank of over 1000 samples. Preliminary analysis

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suggests suppression of sex hormones in high cortisol females at all life history stages. These data suggest that all females ovulate at the end of lactation, irrespective of body condition of stress levels but that implantation after embryonic diapause during the molt is influenced by body condition and stress levels. Females that skip breeding arrive on shore in estrous. We are currently developing and validating an assay for prolactin using a canine antibody. Prolactin has been identified as the luteotropic agent that initiates uterine receptivity for implantation in other wildlife species with obligate delayed implantation including bears and mustelids. We will examine the relationship of prolactin levels to cortisol, progesterone and adipokines to better understand the regulation of implantation.

Adipokines are hormones produced by adipose tissue. We have developed and validated assays using canine antibodies for leptin, resistin and adiponectin. We are currently validating assays for chemerin, NAMPT (visfatin) and kisspeptin. We have targeted these adipokines because of evidence for interactions with immune function and reproduction in biomedical studies. We have developed primers from the elephant seal transcriptome to measure gene expression for a suite of adipokines and their receptors in blubber and have completed a study looking at how expression changes in breeding and molting females. Leptin receptor, adiponectin, and adiponectin receptor gene expression were upregulated during fasting. Adiponectin, leptin receptor, and RBP4 gene expression are negatively associated, while NAMPT is positively associated with body fat). Adiponectin, leptin receptor, RETN, and RBP4 gene expression are positively associated with cortisol. These findings suggest strong variation in adipokine regulation in response to body condition and stress levels. Plasma leptin levels increased with body condition and were highest in breeding females. We will look for associations between plasma adipokine concentrations and reproductive and immune function.

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Notes:

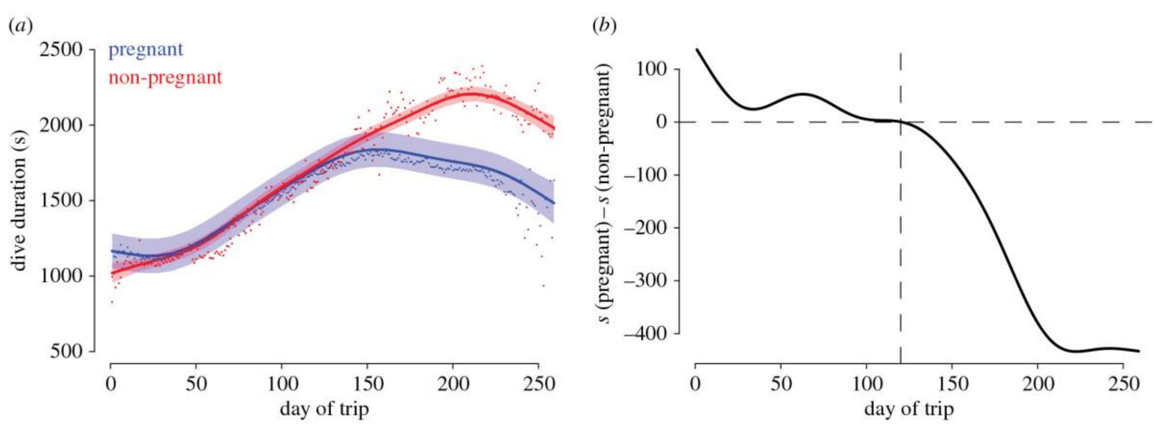

Figure 1. Predicted variation in dive duration along the gestational foraging trip of northern elephant seals. The shaded areas represent the 95% confidence intervals and dots represent daily geometric means. (a) Predicted dive duration for pregnant females (blue, n = 155, n_{dives} = 1,909,136) and non-pregnant females (red, n = 17, n_{dives} = 207,716). (b) Differences in trends between pregnant and non-pregnant females (pregnant – non-pregnant). From: Huckstadt, et al. 2018. The extra burden of motherhood: reduced dive duration associated with pregnancy status in a deep-diving mammal, the northern elephant seal. Biol Lett 14.

On-board calculation and telemetry of the body condition of individual marine mammals

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Background

There are numerous challenges to relate non-lethal disturbance effects to long-term consequences for individuals. Chronic sublethal effects have the potential to affect vital rates via their influence on individual health and the resources they accrue for reproduction. A key component of health for marine mammals is their lipid-store body condition, the amount of lipid they carry. Lipid-store body condition is amenable for study using animal-attached tags because the lipid is less dense than other animal tissues, so net buoyancy of marine mammals is altered as a function of the quantity of fat they carry. These changes in buoyancy lead to changes in hydrodynamic performance of swimming animals that can be recorded in animal-attached tags, enabling longitudinal estimation of body density as a quantitative indicator of the body condition of free ranging marine mammals.

Objectives

The objectives of this project are to develop and validate an animal-attached system to calculate and relay the body condition of a diverse set of free-ranging marine mammal taxa, including pinnipeds and cetacea. Specific tasks are: 1.) to program SMRU SRDL tags to calculate body density of tagged N elephant seals using the glide-performance method, and relay the estimate via the ARGOS satellite system, 2.) to deploy prototype versions of these tags on N Elephant seals, and 3.) to validate body condition estimates obtained by the new tag.

Methods

The tag hardware will be the new version SMRU SRDL tag, which will be equipped with a Silicon Labs EFM32 microprocessor, depth sensor, 3-axis accelerometer, NAND flash archival storage and an Argos transmitter. We will program an onboard real-time algorithm to estimate body density using the glide-performance method which has already been shown to work with a range of marine mammal taxa. A key step is evaluation of the trade-off between simplicity (and lower-power thus greater efficiency) versus accuracy of the body density algorithm. We will produce and deploy two iterative versions of body-condition telemetry tags using SMRU SRDL tag systems during two deployment phases. The project budgets for six seals to be captured and tagged in each phase, with tags being recovered on the recapture of the seals using standard methods. Each tagged animal will be weighed and lipid body store will be measured using stable isotope dilution. These measures will allow us to estimate tissue body density. The telemetered body condition data will be validated against drift rate data also measured by the tag, and the body condition measurements from isotope dilution. Drift rates will be estimated from telemetered dive data via ARGOS using standard methods. Drift rate data and glide data will then be fully re-analysed using the detailed archived data from tags recovered during recaptures. Drift rates will be converted to body density using existing methods

Results

This project is in its first year of performance. We have established our project team, developed the core SMRU-SRDL device to be used in the project, and begun evaluation of the data inputs required to estimate body condition, using archival data made available to the project from partner Dr. Christophe Guinet. We have determined a correction factor to determine body pitch angle from a tag attached to the head, and are currently exploring minimum rates of depth sampling to accurately quantify hydrodynamic performance during glides. Our first set of deployments will take place in October, 2019.

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Web-based Visualization of Marine Mammal Health Across Space and Time with Application Toward Marine Spatial Planning

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Background

Knowing the locations of animals is a crucial first step toward understanding their ecology, and toward making more effective policy and conservation decisions. Armed with information about animal distribution and abundance, we are better able to assess what the impact of anthropogenic disturbance may be on individuals. The impact of a stressor, e.g. exposure to US Navy sonar, or entanglement with fishing gear, may cause a deterioration in animal health. These changes in health can be linked to changes in vital rates, thereby allowing managers and policy makers to make better planning decisions. It is critical with long-lived species that as data are collected, researchers and policy makers are able to assimilate them into existing models in order to make inference on the impacts of stressors on individuals.

Objectives

Here are goals are two-fold. First, we take an existing inferential model and expand it to include interactive web-based graphics that summarize the health of individual right whales. We illustrate the impact of stressors on right whales using entanglement with fishing gear as an example. Second, we use reproducible research techniques in this process to support future applications of this particular model, and also to expose scientists within the ONR/MMB community to such techniques.

Methods

Progress on this project proceeded along two tracks – first, the development of the right whale PCAD model by solidifying, documenting, and packaging the R code-base to estimate right whale health and the impacts to health from anthropogenic stressors; second, by making best-practices for software-engineering known to other researchers in the ONR/MMB community through public workshops and online webinars.

Results

We have conducted 4 workshops on the use of reproducible research techniques, and 3 webinars. The workshops were at: 1) the Ecological Society of America's Annual Meeting in Portland, OR in August 2017; 2) the Society for Marine Mammalogy's Biennial Conference in Halifax, Nova Scotia in December 2017; 3) Duke University Marine Lab in December 2018; and 4) Duke University's main campus in April 2019. The webinars were held in March and April 2019.

We have built an R package for the right whale PCAD model, and for the application of that model to examine the impacts of entanglement on vital rates. The package includes an interactive R Shiny web-app that shows the estimated health and stressor events for each individual right whale. We have structured the intersection of the entanglement data and the estimated health data in a reproducible fashion to ensure that as new data are collected, analysts will be able to produce new estimates of health and the link between health and vital rates.

Notes:

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Sponsor: ONR
Status: ONGOING

Demographics and diving behavior of Cuvier's beaked whales at Guadalupe Island, Mexico: a comparative study to better understand sonar impacts at SOAR

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Background

The effects of repeated exposure to anthropogenic sources, such as Navy Mid-Frequency Active Sonar, need to be measured across both short-term and long-term spectra. To evaluate potential impacts of these sources on a routinely exposed population of whales, a parallel study of a nearby population where they are not used should be conducted to provide a baseline of unexposed behavior and population health.

Objectives

Our objective is to assess Guadalupe Island, Mexico as a comparative study site for the population of Cuvier's beaked whales that inhabit the Southern California Anti-submarine Warfare Range (SOAR).

Methods

Visual surveys for Cuvier's beaked whales at Guadalupe focus on collection of photo-identifications of individual whales, genetic sampling, and deployment of SPASH10-292 LIMPET tags to record diving behavior for periods of up to several months.

Results

One dedicated effort and two additional jointly-funded efforts have been completed. During the seven days of dedicated survey effort, 76 hours resulted in 38 sightings of 101 individuals. Photographic matching identified 27 unique individuals. Individuals identified were matched back to an existing photo-ID catalog from Guadalupe, which includes 73 individuals from previous dedicated an opportunistic research. Recaptures of individuals whales span 11 years, with some individuals having been resighted 19 times suggesting a high degree of site-fidelity. A comparison of whales identified at Guadalupe Island to those photographed in and around SOAR has not documented any individual movements between study areas to date.

Four satellite tags were deployed on four different individuals with transmission durations ranging from 18 to 26 days; they collected 1,256 hours of diving behavior from three of the four tagged whales. The basic pattern observed from Cuvier's beaked whales in other regions was apparent, with deep, presumed foraging dives generally separated by a series of shallow dives. The individual median deep dive durations ranged from 56.6 – 74.0 min (max = 149.2 min). Individual median deep dive depths ranged from 991.5-1135.5 m (max = 2799.5 m). The median Inter-Deep Dive Interval ranged from 101.9 – 160.1 min; the longest time between presumed foraging dives was 485 min (8.1hrs).

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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). The goal of the working group was the development and implementation of analysis methods that made most effective use of the available data. The outputs of the MOCHA project (Thomas and Harris 2016) have 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 that were not addressed explicitly by the MOCHA project. Additionally, there has been an increase in the use of observational studies and of passive acoustic data for inferring behavioral response.

Objectives

The overall aim of this project is to develop new quantitative models and analytical methods for inferring behavioral response of marine mammal species to Navy sonar. Our focus is on studies estimating the response to mid-frequency active sonar, but the methods developed will be widely applicable. The results will be 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 are being addressed by three project tasks:

Task 1. Develop analytical methods for estimation of behavioral response and subsequent recovery from controlled exposure experiments (CEEs) that allow fusion of multiple input datasets collected across a range of spatial and temporal scales.

Task 2. Develop recommendations for effects analysis of long-term passive acoustic data. The work will support effects analysis of vocal marine mammals by both Atlantic and Pacific fleets, as well as BRS studies that deploy long-term passive acoustic sensors.

Task 3. Develop next-generation models for behavioral response based on our understanding of marine mammal signal detection and the evolutionary drivers of response. This will have longer-term impact on exposure-impact modelling and future BRS design.

Methods

Task 1: This task focusses on the development of analytical methods applicable to multi-scale BRSs. We are investigating methods for inferring behavioral response from long-term low-fidelity data such as from satellite tags, as well as methods for integrating multiple disparate data streams (e.g., satellite, DTag and potentially SMRT tag data) to infer behavioral response and return to baseline behavior. We will investigate methods for including passive acoustic monitoring data from widely-spaced recorders, such as those deployed by the 3S and Atlantic BRS teams. Finally, we will investigate methods for pooling across species and CEEs to borrow strength and improve inference. A series of small workshops will be held with the project team and BRS researchers to ensure good understanding of the different data collection tools and the scientific/analytical questions and challenges.

Task 2: Long term PAM studies have important roles to play in inferring behavioral response. Two statistical approaches have been developed to relate detection parameters such as detection rate to explanatory variables such as presence and type of sonar. One approach was based on a linear modeling framework, using Generalized Estimating Equations (GEEs) while the second approach was based on stochastic temporal modelling, using Hidden Markov Models (HMMs). It is unclear as to which method is better in this context. We will therefore undertake a simulation study to investigate the utility of the two approaches.

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Task 3: This task is focused on synthesizing knowledge generated from studies of animal audition, perception, energetics and population consequence of disturbance with the ultimate goal of creating more biologically-based models for behavioral and physiological response of marine mammals to Navy sonar. First, we will undertake a review of the potential factors ("contextual variables") affecting behavioral and physiological response, and the potential for the statistical distribution of these factors to be predicted in advance so that they can be used in Navy planning. We will then hold a workshop to consider the potential for creation of a quantitative, mechanistic model for behavioral response. The next stage will be to develop mathematical models that build on this framework to enable quantitative exploration of how inputs (contextual variables) relate to outputs (responses) and we will explore the sensitivity of the mathematical model outputs to input parameters, especially considering the contextual variables that can potentially be used in Navy planning. This will enable us to provide guidance regarding priorities for future data collection.

Results

A start-up workshop was held immediately following the ESOMM meeting in the Hague, September 2018. There were 27 participants including BRS researchers, statisticians and navy stakeholders. This provided an early opportunity for the project team to introduce the project goals to the community and to gain input from the community on project direction and priorities.

Specific results for each task are listed below.

Task 1: Two PDRAs have been appointed to this task, one based at Duke University (starting May 2019) and one based at the University of St Andrews (started February 2019). A start-up workshop was held at Duke University in March 2019 in collaboration with contributors from the Atlantic BRS project. Following discussions, it was agreed that Duke University will focus on creating a simulation framework to learn how well we can detect responses under realistic conditions and with different tools (DTAGS, sat tags, etc.) and investigate the fitting of continuous-time movement models to improve on currently available methods for horizontal movement modelling. University of St Andrews will focus on developing continuous-time movement models with changing animal behavior, methods for estimating context-dependent response and time to return to baseline, and pooling inference across individuals and tags using random effects and common response models. In addition, Double MOCHA participants provided input to Atlantic BRS planning and analysis discussions.

Task 2: The same PDRA appointed to work on Task 1 at the University of St Andrews will also be working on Task 2. Work on Task 2 will begin in October 2019.

Task 3: A PDRA has been appointed to this task and work will begin in April 2019 to review the potential factors affecting behavioral and physiological response. It is proposed to hold a workshop at the upcoming World Marine Mammal Conference in December 2019 to obtain input from a wide range of experts from the fields of marine mammal hearing, attention, stress physiology, energetics and behavioral ecology on these factors and to consider the potential for creation of a quantitative, mechanistic model for behavioral response.

Notes:

Harris, C. M., & Thomas, L. (2015). Status and future of research on the behavioural responses of marine mammals to U.S. Navy sonar. (CREEM Technical Report; No. 2015-3). University of St Andrews.
Thomas, L. & Harris, C.M. (2016). MOCHA – Multi-study ocean acoustics human effects analysis. Final report to ONR on award N000141210204.

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FURTHER INVESTIGATION OF BLOW OR EXHALED BREATH CONDENSATE AS A NON-INVASIVE TOOL TO MONITOR THE PHYSIOLOGICAL RESPONSE TO STRESSORS IN CETACEANS

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Background

Through prior ONR funded efforts, our laboratory has conducted studies on the physiological response to stress in a representative cetacean, the beluga (*Delphinapterus leucas*). While blood is the "gold standard" for measuring the physiological response to stressors, it requires capture and restraint to obtain samples from free-ranging cetaceans, which in itself can result in a physiologic response.
Blow collection is feasible from free-ranging cetaceans and offers promise for monitoring and detecting physiologic responses to stressors, such as sound, in order 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 objectives of this project utilize blow samples from aquarium belugas under controlled scenarios and wild belugas for continued validation of utilizing cetacean blow to investigate the physiological response to stressors in cetaceans. Overall objectives include 1) investigating urea and/or total protein as a suitable marker of dilution in order to standardize samples; 2) investigation of other important markers of fitness in blow such as thyroid hormones; 3) investigation of molecular markers of stress and immune system function in blow; and 4) carrying out initial steps for transition of blow collection to small free-ranging toothed whales.

Methods

Blow samples were collected from two Aquarium belugas trained to exhale on signal using petri dishes covered with nitex membrane held 2-3 inches above the blowhole or before and after an Out of water event (OWE). The nitex membrane was transferred to a 50-ml conical tube and spun down at 4000 rpm, for 30 min at 4°C. The supernatant was measured and stored until assay. Cortisol, urea and thyroid hormones were measured using commercial Enzyme Immunoassays (EIAs). DNA and RNA were extracted from blow samples and used either in PCR to amplify sex-related genes or targeted health related gene expression.

Results

The volume of blow collected was higher with multiple exhales as compared to a single breath. The number of exhales collected did not significantly alter the cortisol content, though high variability was observed. There was no apparent relationship detected between sample volume and cortisol content. After being lifted out of the water on a novel hydraulic false bottom, an then a stretcher to obtain a weight (OWE), blow cortisol demonstrated an increase 15-45 min following release back into the water. Correction with urea resulted in a similar pattern of increased values following. Preliminary results of T3/T4 show low levels and indicate further optimization of assays are necessary. Sex determination and gene expression measurements for 4 genes (Cox2, TGFß, Nr3c1, and GAPDH) were successfully carried out in the same blow sample. Additional stress and health related genes are currently being validated in blow.

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Deciphering Mysticete Audiograms using a Prepared Skull

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Background

The geometry of the cranial bones and the ligaments that connect them together, likely determine the characteristics of the bone conduction mechanism for low-frequency sound reception of in baleen whales (Cranford and Krysl, 2015). This project will test that idea. The results of this project will support risk threshold criteria and environmental impact assessment.

Objectives

The specific question we want to address here is: Does the process of removing the soft tissues from a mysticete skull, while preserving the ligaments between the skull bones, disturb the overall geometry of the skull. If so, by how much? This may shed light on the mechanisms of sound reception by bone conduction.

Methods

We propose to CT scan the intact head of a small postmortem gray whale obtained from the Natural History Museum of Los Angeles County.

There are many steps in our procedure. It is essential to maintain the preservation of the specimen throughout the process so that decomposition does not adversely affect its condition. The entire process requires that we conduct two CT scans of the same specimen to test the effectiveness of a specialized treatment to preserve the cranial ligaments.

The procedural steps are as follows: (1) Freeze specimen to -10°F; (2) Prepare a special shipping container and insert frozen head; (3) insert container into subzero freezer for three days; (4) ship it to Michigan; (5) insert container into subzero freezer for three days; (6) transfer and CT scan it; (7) back into freezer for 3 days; (8) ship it back to San Diego freezer; (9) build FEM mesh and run simulations for intact head; (10) thaw surface layers and remove all soft tissue external to the cranium (but do not disturb the ligaments between cranial bones); (11) refreeze the skull-ligament preparation; (12) build a new shipping container and insert skull; (13) ship it to Michigan; (14) insert into freezer; (15) remove and CT scan; (16) inert into freezer again; (17) ship back to San Diego; (18) insert into freezer; (19) build FEM mesh for skull-ligament preparation; (20) conduct comparative FEM modeling analysis between intact head and skull-ligament preparation from CT scans.

The intact head and the skull-ligament preparation will both be contained in plastic bags to reduce the potential for tissue desiccation.

The repeated insertion into and out of the freezing environment should have minimal impact on preservation because we plan to monitor the temperature of the specimen to make sure it is always frozen. We devised a novel means for measuring the temperature of the head within the shipping container.

Once we remove the soft tissues (while preserving the ligaments between skull bones), then we can compare the results of the FEM simulations as a means to understand the elements of sound reception in mysticetes.

The CT scans can also be used to construct low-frequency (**LF**) audiograms and head-related transfer functions (*directional hearing maps*), which can be compared to similar results that we have gathered for two different mysticete species.

We believe that this is best way to acquire accurate skull geometry and ascertain the contribution of soft tissues to the sound reception process.

As an additional check on the process, we can also remove the soft tissues digitally by replacing them with water (uniform loading). Then we can compare the geometry of the skull when the tissues are digitally removed versus the skull when the tissues were physically removed.

We applied a similar technique of digital tissue removal with the fin whale (Cranford and Krysl, 2015). This is a kind of a "digital experiment" that changes only one variable in the simulations. Before and after this manipulation we have the difference due to the absence of the anatomic geometry and elastic loading by the soft tissues.

Results

We are currently waiting for a three day opening the schedule for high-resolution CT scanning of the intact

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head (step 3) in the procedure. The CT scan will be conducted within the next month.

This gray whale specimen required extensive preparation to remedy the some of the misshapen or disturbed geometry of the head caused by the initial positioning in the freezer after the salvage operation. For example, the jaws were not aligned properly because the head had been laid upon its left side in the freezer. The process of correcting the disturbed geometry is necessarily slow and careful so that we do not needlessly add to postmortem time and deterioration out of the freezer.

Once complete, these scans can be used to construct low-frequency (LF) audiograms and head-related transfer functions (*directional hearing maps*) for a gray whale (*Eschrichtius robustus*). The gray whale skull will be CT scanned and analyzed using our Vibroacoustic Toolkit (VATk) in order to calculate basic hearing parameters.

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SeaBASS 2018: A Marine BioAcoustics Summer School

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Background

The goal of the SeaBASS (Marine BioAcoustics Summer School) program was to provide the opportunity for graduate students interested in pursuing careers in the field of marine bioacoustics to develop a strong foundation in both marine animal biology and acoustics, foster technical communication across disciplines, and develop professional relationships within the field. This course gave students an opportunity to learn from experts who discussed topics not often offered at universities due to the relatively small demand at any one institution. Lecturers within the field of marine animal bioacoustics provided half day seminars that described fundamental aspects of underwater sound and marine animal behavior, summarized the present state of the field, identified current obstacles and challenges, and discussed important “hot topics” areas. Each seminar included an introductory lecture followed by group discussions or group projects to gain a more in-depth understanding of the issues. An evening student poster session provided participants the opportunity to obtain feedback on their graduate research from lecturers and peers. Structured social activities allowed for students and research scientists to interact informally to develop lasting professional mentorships and contacts for guiding the next generation of marine bioacoustics scientists.

Notes: SeaBASS website: https://marine.unh.edu/seabass

Objectives

1. To provide fundamental concepts of underwater sound and marine animal biology and behavior to graduate students interested in pursuing careers in marine bioacoustics.
2. To create an environment for the open exchange of ideas related to careers, current hot topics, and challenges facing the field of marine bioacoustics.
3. To foster professional relationships between graduate students and experts in the field.

Day 4: Friday April 26, 2019

2019 ONR
Marine Mammal & Biology
Program Review

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Biomechanical and energetic analyses of whale-borne tag sensor data to assess the population consequences of acoustic disturbance

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Background

Previous studies have demonstrated a link between MFA exposure and behavioral response in cetaceans. Responses have been shown to be dependent on a number of factors including species, received level, behavioral state, and ecological context. Despite these differences, the cessation of foraging in response to sound exposure has been observed in multiple species. Feeding rates in unperturbed states can provide baseline information for the energetic scope of cetacean foraging. The disruption of foraging that occurs at a given level and duration of sound exposure can thus be used to determine energy loss.

Foraging is a key vital rate that has clear implications for cetacean fitness and eventually population health. Baleen whales generally differ from toothed whales in that the former exhibit more of a concentrated summer feeding season on ephemeral prey resources at higher latitudes. These summer feeding months allow cetaceans to dramatically increase their lipids stores that are needed for long distance migration to breeding grounds at lower latitudes. Disruption of feeding from anthropogenic perturbations should decreased lipid stores, which will decrease fetal growth and the viability of offspring. This pathway represents a mechanistic model by which the accumulation of sub-lethal effects in the form of feeding disruption can affect population health. However, what is poorly understood is the degree to which sonar exposure duration corresponds to energy loss. Therefore, analytical framework will enhance our understanding of the population consequences of acoustic disturbance and therefore help inform the Navy's efforts to mitigate the effects of sonar.

Objectives

Our objectives were to better understand the biomechanics and energetics of foraging in cetaceans. First, this required the acquisition of tag data across multiple projects and institutions, including from our own ongoing efforts within the lab. Second, we quantified the fine-scale kinematics of cetacean movements, with a particular emphasize on feeding events. From the kinematics recorded by the tags, we estimated the forces involved in foraging and then calculated the energetic scope and energy budgets across scale from some of the smallest cetaceans (harbor porpoise) to the largest (blue whale). We also analyzed these data within the context of their predicted diving capacities. Lastly, we used these data to estimate the short-term and long-term energetic consequences of acoustic disturbance.

Methods

We calculated the energy budgets of foraging cetaceans by comparing the energy assimilated from ingested prey to that used for swimming, diving, and prey capture. The tag sensors generated a time-series of kinematic signatures and behavioral events, each of which requires power that can be estimated by modeling the known physics. We modeled energy demand and metabolic power output for swimming and feeding using previously developed models. Feeding were modeled as inelastic collisions between whale and prey or prey-laden water. Accordingly, the cost of prey capture in toothed whales is relatively low compared to baleen whales which exhibit high drag feeding mechanisms. However, some toothed whale and baleen whale species accelerate to high speed during pursuit of prey, and we accounted for these dynamic and energetically demanding accelerations in model. Although most of these models for feeding were initially developed for engulfment in large baleen whales, they can be modified and applied to any cetacean species. When available, I will incorporate prey density and distribution data as an input parameter to the energetic models. For some species, like deep diving toothed whales, simultaneous prey data is much more difficult to obtain, so I will use published values for typical prey species consumed by the cetacean species in question.

Results

In general, we found that toothed whales which exhibit the longest and deepest dives during foraging strategies also had the highest number of feeding rates per dive. However, the energy invested in longer dives provided diminishing returns from the most frequently acquired prey, thereby decreasing the energetic efficiency of foraging (energetic efficiency of foraging = the energy from acquired prey divided by the energy expended while obtaining prey including diving costs and post-dive recovery) as their theoretical aerobic dive limit (TADL) is increasingly exceeded (Figure 2c).

Conversely, rorqual whales underperform their TADL because of the high drag and energy required to engulf extremely large volumes of prey laden water, which causes a reduction in available dive time and also fewer possible feeding events per dive.

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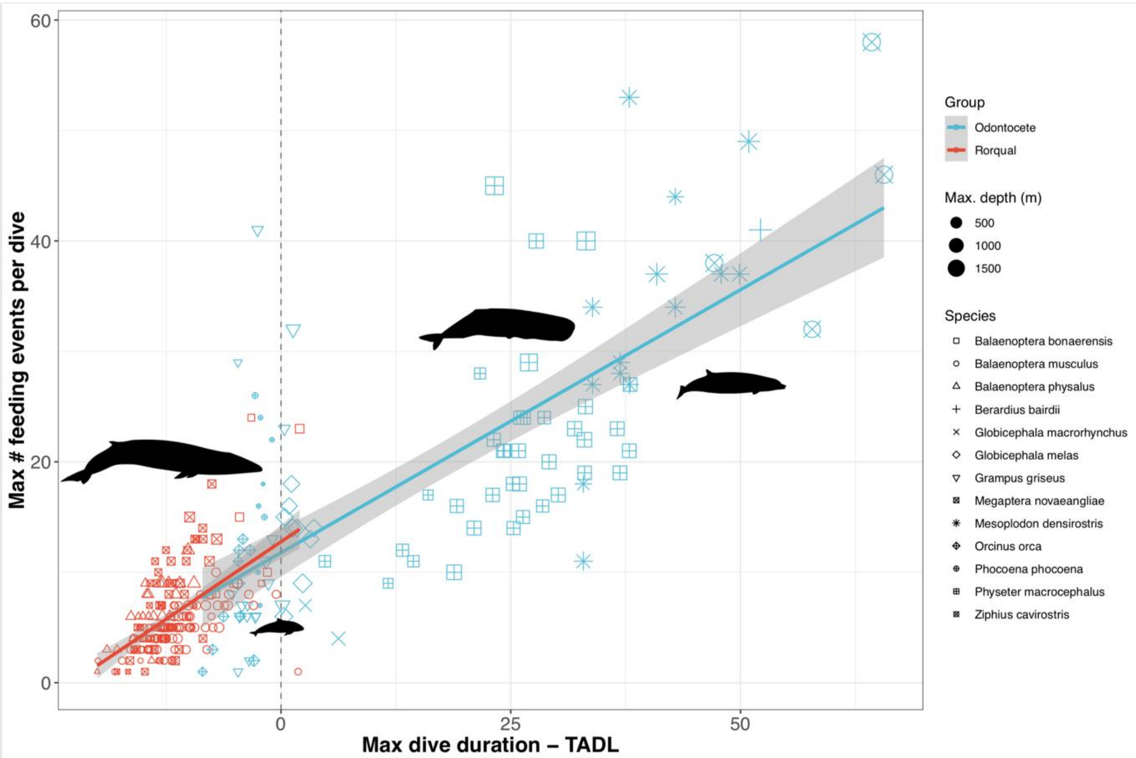
Nevertheless, the investment in high-cost, high-intake feeding events provides considerable energetic returns and the highest efficiencies among all cetacean species. These data suggest that the longest and deepest diving species (i.e. beaked whales) operate on an energetic knife-edge (i.e. very low energetic efficiencies). Such an energy scope, that results from their extreme diving strategy, suggests that they will incur the greatest energy loses if their functional response to sonar consists of cessation of foraging dives.

Our energetics model can be improved with more short-term suction cup tag data as well as tags that record foraging rate data over greater temporal and spatial scales. Further improvements can be made if direct measures of prey data are available for tagged individuals and if drones can be used to measure the size and body condition of the tagged whale. More experimental and observational data on the functional responses to sonar will enable researchers to calculate the energy losses due to foraging cessation.



Foraging performance and efficiency as a function of deviation from theoretical dive time or the theoretical aerobic dive limit (*TADL*). The maximum number of feeding events (Odontocetes: light red; Rorquals: light blue) increases as *TADL* is exceeded and decreases as dives are shorter than expected (Odontocetes: y=0.476+11.78; Rorquals: y=0.56x+12.76). Dives longer or shorter than predicted based on oxygen stores, respectively show positive and negative x-axis values, respectively.

Notes: Biomechanical and energetic analyses of whale-borne tag data to assess the population consequences of acoustic disturbance (N000141612477).

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Iterative PCoD for Oceanic Dolphins

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Background

Oceanic dolphins represent a guild of cetacean species that receive limited management attention due their commonness and lack of research to assess status. Here we explored application of a population consequences of disturbance (PCoD) framework to a sparse, but long-term (26-year) photo-ID data set of Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). The long-term goal of this collaborative research effort is to develop a framework to assess the magnitude of behavioral responses to disturbance that would be necessary to impact vital rates of oceanic dolphins. Our goal was to develop a framework that can predict population consequences from sparse or qualitative data on pelagic delphinids that can be updated as new information becomes available, thus providing iterative estimates of the long-term effects of disturbance on a population. Given the ultimate goal of investigating potential behavioral responses of oceanic dolphins to mid-frequency military sonar, we started by measuring effects of lower-amplitude vessel noise on Pacific white-sided dolphin foraging behavior, as well as the effects of inter-annual variability in prey availability in non-calf survival. The aim was to investigate the utility of the PCoD framework in a data-poor situation and to better understand the effects that anthropogenic activities may have on pelagic dolphins. We assessed how much disturbance it would take to alter vital rates, given sparse and imprecise data.

Objectives

Our objectives were to: 1. Construct a Population Consequences of Disturbance (PCoD) model for a pelagic dolphin popuation using demographic parameters estimated from a long-term photo-identification study, 2. Collect behevioral scan samples of dolphin activity during controlled exposure experiments to boat noise and disturbance, and 3.Model the population consequences of disturbance in the context of kiler whale predation and inter-annual prey variability.

Methods

The iPOD model was constructed through a series of linked tasks: field methods, modeling a prey-demography link, and modeling disturbance in a PCoD framework. Data collection for photo-identification and Pacific white-sided dolphin behavior was conducted in the Broughton Archipelago, British Columbia (BC), Canada from August 25 to September 28, 2016. Scan samples were used to record the predominant dolphin activity state. We modeled interannual variability in non-calf survival using interannual variability in availability of two prey species, Pacific herring and pink salmon (Morton 2000), as candidate covariates. Cormack-Jolly-Seber models were fit to dolphin capture-recapture data with pink salmon and herring as candidate covariates. This selected model gave a relationship between herring abundance and non-calf survival. The goal was to link the potential change in foraging behavior due to disturbance to a population level effect by modifying a prey-demography relationship. Lost foraging time can be thought of as proportional reduction in food availability, so it is possible to link changes in activity budget to effects on survival. Thus, a PCoD-lite approach was taken to investigate the population consequences of disturbance.

Results

The capture-recapture data set provided us with new, empirical estimates of the population’s vital rates, and allowed us to derive a link between interannual variability in adult survival and density of candidate prey species. These results were used in a Population Consequences of Disturbance (PCoD) framework to investigate the potential impact of reduced foraging opportunities on the population’s dynamics. The new demographic analyses lends support to previous studies that Pacific white-sided dolphins are a long-lived, slowly reproducing species that may have a lower intrinsic rate of increase than other delphinids. Apparent survival dropped dramatically during years of low herring abundance, but these models conflated true mortality with temporary

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emigration. The behavioral component to the study provided an opportunity to set behavioral responses in an ecological context. During focal follows, dolphins were exposed to ~126 dB/μPa (broadband) dB received level vessel noise during treatment conditions. During treatments, the altered activity budgets was a mean percentage reduction in foraging of 0.289 (95% bootstrap CI: 0.163, 0.408) between control and treatment conditions. This is in line with previous studies that found an 18% and 25% reduction in time spent feeding in resident killer whales. Given the prey-demography link, modest reductions in long-term accessibility of prey could cause fairly large declines in apparent survival, but additional data are needed to distinguish mortality from temporary emigration. Until then, the iPOD framework allows exploration of demographic consequences of disturbance in a relatively poorly studied pelagic delphinid.

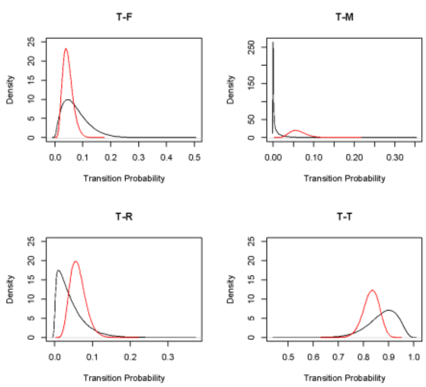

Figure1: The posterior distributions for the probability of remaining in a travelling state (T-T), or transitioning from travelling to foraging (T-F), milling (T-M) or resting (T-R) in the presence (red) and absence (black) of disturbance.

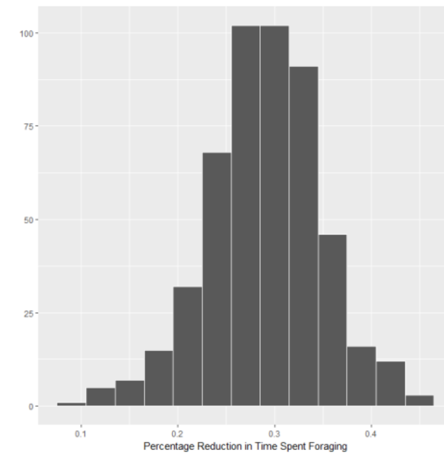

Figure 2: A histogram of the estimated percentage reduction in time spent foraging due to disturbance for each of the 500 simulations.

Notes:

Given behavioral responses of delphinids to playback of vocalizations of mammal-eating killer whales and the dramatic evasive tactics we see in Pacific white-sided dolphins in response to actual predation attempts, we are motivated to continue this line of research. Pacific white-sided dolphins remain a priority species to explore acute and chronic responses of delphinids to predation risk, given the substantial spatio-temporal overlap in habitat use between Pacific white-sided dolphins and mammal-eating killer whales in our study area. Given the increased capacity, we expanded a breath-sampling component to our study to explore prevalence of pathogens in the respiratory microbiome of Pacific white-sided dolphins. Results are emerging, but this experience has built our organizational capacity to explore hormonal stress responses of oceanic dolphins to noise in future research efforts. Previous analyses of young of the year in photographs suggested that inter-calf interval was 4.2 years (Ashe 2015). As the statistical power of our photo-ID study increases, we are keen to explore effects of prey abundance on reproduction, not just adult survival, in order to explore other pathways of effects of human activities on oceanic dolphins.

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Integrating Information on Displacement Caused by Mid-Frequency Active Sonar and Measurements of Prey Field into a Population Consequences of Disturbance Model for Beaked Whales

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Background

Current environmental impact statements require an assessment of the number of behavioral “takes” of animals exposed to Mid-Frequency Active Sonar (MFAS) using the output of simple effects models. Incidental Harassment Authorizations are then requested, based on a finding of “negligible impact”. Population Consequences of Disturbance (PCoD) models provide a potential useful tool for determining “negligible impact” for species that are known to be sensitive to MFAS exposure.

This project will demonstrate how tools developed as part of ONR Award N000141612858 (PCOD+: Developing widely-applicable models of the population consequences of disturbance) can be used to provide detailed PCoD models for two marine mammal species that are known to be sensitive to MFAS exposure, but whose life history is still poorly understood. Model outputs will also provide information on which population characteristics are likely to be affected by MFAS-induced disturbance, and therefore provide input to future developments of the Navy’s Integrated Comprehensive Monitoring Program, which provides an “overarching framework for marine mammal monitoring on ranges, including consistent approaches for reporting and analyzing monitoring data and a review of best practices from the Fleets”.

Objectives

To provide realistic predictions of the long-term effect of disturbance caused by MFAS on the *Mesoplodon densirostris* (*Md*) population at the Atlantic Undersea Test and Evaluation Center (AUTEC) and the *Ziphius cavirostris* (*Zc*) population at the Southern California Offshore Range (SCORE) using the tools developed as part of the PCOD+ project. This will be achieved through four Tasks:

Task 1 Quantification of beaked whale response to MFAS;
Task 2 Development of bioenergetics-based PCoD models for beaked whales;
Task 3 Quantification of beaked whale abundance and exposure as a function of MFAS operations on AUTEC and SCORE;
Task 4 Quantification of beaked whale prey distribution at AUTEC and SCORE;
Task 5 Integration of model results for impact assessment.

Methods

Task1: Passive acoustic data from AUTEC and SCORE will provide start and stop times for MFAS use on both ranges, information on the location of beaked whale foraging groups, and a direct measure of sonar received level for these groups. This information will be combined with existing telemetry data to estimate risk functions for *Md* and *Zc*.

Task 2: An existing Dynamic Energy Budget (DEB) model for pilot whales (Hin et al., in review) will be adapted for both beaked whale species and incorporated into a population model that accounts for the effect of individual energy intake on resource availability. These models will be used to investigate the likely effects of different levels of MFAS exposure on beaked whale health, life history and population structure.

Task 3: Daily estimates of beaked whale abundance within the hydrophone arrays at AUTEC and SCORE will be combined with information on the number, duration and timing of MFAS operations from operational logs and passive acoustic data to quantify levels of MFAS exposure on both ranges. This information will be used to estimate the number of foraging dives lost as a result of MFAS disturbance, and the extent of any MFAS-induced displacement.

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Task 4: Data collected by an autonomous undersea vehicle will be used to estimate the distribution of of potential beaked whale prey on both ranges. Observed prey densities will be related to associated measures of habitat characteristics to estimate prey distributions outside the hydrophone arrays and assess the potential effects of displacement from different parts of the ranges on beaked whale energy intake.

Task 5: Results from the other Tasks will be incorporated into the DEB models and used to predict beaked whale population trajectories and population size structure under different disturbance scenarios and with different prey fields.

Results

Task 1: The existing risk function for *Md* on AUTEC (Moretti et al. 2014) is derived from data collected during multi-ship sonar operations, dominated by the use of ship-based sonars. Additional data have been analysed to provide separate risk functions for the response of *Md* to ship-based sonar alone and small-scale sonar sources (dipping sonars and sonobuoys) alone.

Task 2: A DEB model that describes the effects of energy acquisition on survival and reproduction over the entire life history of individual *Md* has been developed and compared with observations from AUTEC and a nearby undisturbed population at Abaco.

Task 3: Daily data on *Md* Group Vocalization Periods (GVPs) and sonar events on AUTEC in 2012 have been analysed to provide estimates of the changes in the number of animals on the range over time, and estimates of the probability that individuals will be displaced from the range by different sonar events or cease foraging. A similar approach will be applied to data from SCORE.

Task 4: Acoustic surveys of SCORE, AUTEC and their surrounding waters have been conducted. The results are currently being analysed using the approach described in Southall et al. (2019).

Task 5: The DEB model for *Md* has been used to predict the ratio of calves to adult females CA ratio) likely to be observed at Abaco at different levels of environmental quality, and at AUTEC with different assumptions about the effects of MFAS-related disturbance. The CA ratios observed by Claridge (2013) at these two sites (with a CA ratio at Abaco that is 2x that at AUTEC) can be replicated if the environment at AUTEC is assumed to be substantially poorer than the environment at Abaco. They can also be replicated if the environments are similar but displacement results in a 50% reduction in energy assimilation on the day displacement occurs. In both cases, the AUTEC population is predicted to be declining by approximately 2.5-3.0% per annum. A decline on this scale (equivalent to a 50% reduction over approximately 25 years) has not been observed.

Notes:

Claridge, D.E., 2013. Population ecology of Blainville's beaked whales (*Mesoplodon densirostris*) (PhD Dissertation). University of St Andrews.

Hin, V., Harwood, J. and de Roos, A.M. (in review). A model for medium-sized cetaceans shows how resource availability modifies the population consequences of disturbance. Submitted to *Ecological Applications.*

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A Model for Linking Physiological Measures of Individual Health to Population Vital Rates for Cetaceans

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Background

The Population Consequences of Disturbance (PCoD) framework describes how a stressor may impact a population through a series of transfer functions linking stressor exposure, physiological changes, behavioral changes, health, and vital rates[1]. While conceptually straightforward, developing quantitative transfer functions is challenging, especially for linkages involving the health component of the PCoD framework. Some progress has been made in linking observations of body and skin condition to population vital rates in endangered North Atlantic right whales[2], however, such visible indicators of health may not be generalizable to all adverse outcome pathways, and may also have low sensitivity because body condition changes are often not detected until late stage disease.

Bottlenose dolphins (*Tursiops truncatus*) are accessible for hands-on sampling, allowing for collection of a comprehensive suite of health measures. In addition, *inshore* bottlenose dolphin (BND) populations show long-term residency, allowing for longitudinal photographic follow-up to estimate survival and reproductive rates. As such, BNDs make a good model species to investigate linkages between health measures and vital rates. By studying BNDs, we hope to identify priority health indices that are most predictive of vital rates. This information will help prioritize future research for technology development that will allow sampling for these same indices, or suitable surrogate measures, from other less tractable cetaceans that are of greatest concern for the Navy.

Objectives

1. Develop a quantitative model to link population vital rates to a suite of physiological and/or health measures, using comprehensive data from bottlenose dolphin capture-release studies conducted across multiple sites and a range of stressor conditions.
2. Apply the model to identify the indices, or suite of indices, that best serve to predict vital rates.
3. Extend model results to assess feasibility of collecting the priority indices for identified species of concern for the U.S. Navy. Where collection of the priority indices is not feasible, provide recommendations for surrogate measures or potential research approaches to bridge information gaps.

Methods

Working with multiple collaborators, we collated data from bottlenose dolphin studies that have been conducted at 7 sites along the U.S. coast, over a range of stressor conditions. We are using two types of data: 1) physiological and health measures from hands-on veterinary assessments, and 2) sighting histories from longitudinal photo-identification studies on the same populations. The physiological/health measures include stress, thyroid, and reproductive hormones; hematology; serum chemistry; immune indices and cytokine measures; lung disease score; and body condition (mass/length).

We are developing a model, the Veterinary Expert System for Outcome Prediction (VESOP), that uses the collection of health measures to estimate probabilities of 1- and 2-year-ahead survival, as well as estimate probability of successful reproductive outcome for pregnant females. As an initial step, we organized the numerous blood-based parameters into panels indicative of organ status or specific disease condition, and identified abnormal cases for each panel using previously established reference ranges[3]. We then convened an expert Veterinary Advisory Panel (VAP) to review and discuss the classifications, providing guidance for the model development and refinement of the panels.

To fit a preliminary model, we used only observed outcomes. For survival, outcome was failure (0) if a carcass was found, or success (1) if the dolphin was

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sighted after the end of the study period. For reproduction, pregnancy outcome was based on sighting of the female after her expected due date with or without a calf (success or failure, respectively).

We are simultaneously developing capture-mark-recapture (CMR) models using the photo-identification data to estimate survival rates for each population, as well as derive estimates of survival probability for each dolphin that was given a health evaluation. The factors affecting capture probability and survival vary among sites, so we are using variable selection techniques to select the most appropriate model for each site.

Results

We compiled records from 797 dolphin health evaluations collected across the seven sites during 2000-2018. Using refined panels as recommended by the VAP, we calculated proportion of cases for each panel for both survival outcome classes (Fig. 1A) and estimated relative risk (Fig. 1B). We found the highest relative risk associated with cases of anemia and/or inflammation, with lesser but still significant increased risk for cases of abnormal electrolytes, and lung disease. We fit a logistic regression model and found the best model fit by including anemia ($p<0.0001$), inflammation ($p<0.0001$), and abnormal electrolytes ($p=0.06$). Similar logistic regression analysis for the reproductive outcomes found that elevated white blood cell count ($p<0.01$) and low cortisol ($p=0.06$) were most associated with a negative reproductive outcome.

Two factors have hindered our analyses to date: 1) censored survival observations (i.e. the dolphin was not observed again after the relevant time point, but a carcass was never found), and 2) missing data for one or more health measures. While the suite of hematology, serum chemistry, and hormone measures has been consistently collected and analyzed by the same laboratories across the nearly 20-year period, other parameters such as immune measures and pulmonary disease classifications have only been collected more recently (beginning in 2010). We are evaluating whether multiple imputation can be used to address the missing values for some of these newer health measures. To address the censoring, we will integrate individual survival probabilities from the appropriate CMR model once they are completed.

Notes:

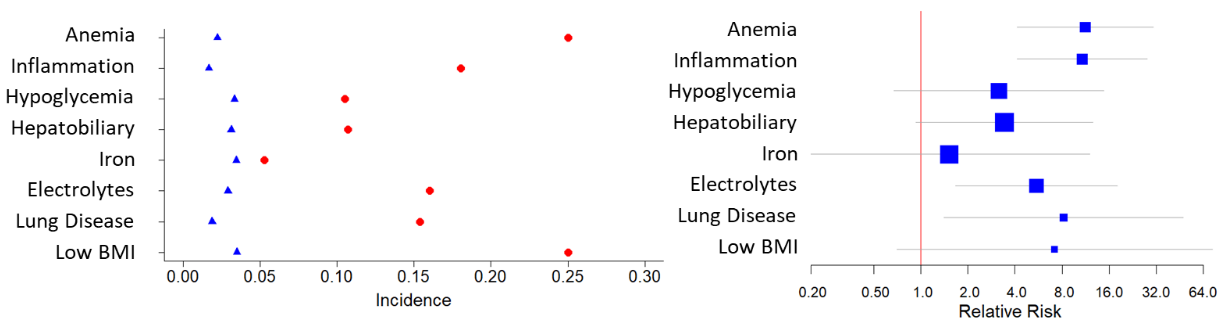

Figure 1. (A) Incidence of death within 2 yrs for dolphins with normal panel (blue triangle) and with abnormality (red circle), and (B) estimated relative risk with 95% confidence intervals. Red line represents no change in risk.

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Investigation into the Gross and Microscopic Anatomy of the Endotracheal Plexus of Cetaceans

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Background

A robust submucosal venous plexus has been described in bottlenose dolphins, striped dolphins and pygmy sperm whales (Costidis & Rommel, 2012; Cozzi et al, 2005; Davenport et al., 2013). Aside from cursory gross anatomical references and histological evaluations, nothing is known about the plexus, it's anatomical connections, extent or function(s). Engorgement of the plexus may affect the dynamics of pulmonary compression and alveolar collapse, and therefore either alter the depth at which diving nitrogen gas absorption and elimination take place by emulating a compliant trachea (Bostrom et al., 2008). This is turn could have significant influence on diving gas kinetics and modeling of the risk of decompression-related pathologies.

Objectives

The main goal of this project was to describe the morphology, anatomic connections and extent of the submucosal tracheobronchial venous plexus in the bottlenose dolphin. A secondary goal was to compare the histologic appearance and extent of the plexus in deep and shallow-diving cetaceans.

Methods

To examine the morphology of the submucosal plexus and identify its anatomic connections to systemic circulation, a combination of gross dissection and computed tomographic (CT) angiography were used. In-tact specimens were perfused with anatomical latex or a mixture of anatomic latex and barium sulfate. Once connections to the systemic venous circulation were identified through CT angiography and dissection, dissections and targetted latex injections were performed in order to ensure adequate engorgment of the venous plexus. Injected specimens were dissected to reveal the gross anatomic structure of the venous plexus. Two attempts to perform targetted injections of radio-opaque latex mixtures failed due to the small size of the lung specimens. An arterial contrast injection was performed on a bottlenose dolphin to examine possible arterial connections to the venous plexus. Histological samples were obtained from airways of a sperm whale, Cuvier's beaked whale, and pygmy sperm whale. Samples will be processed using H&E and special stains to compare the microscopic structure. Cross sectional images of the airways of the different species will be compared in order to compare the luminal surface area occupied by the venous plexus. Decreased availability of appropriate specimens resulted in a no-cost extension of the research to facilitate acquisition of additional specimens to complete the proposed research. Tasks remaining include 1) pre- and post-injection computed tomographic angiography of the submucosal plexus to estimate volumetric changes due to plexus engorgement, 2) vascular corrosion casting to verify lack of arterial communication of the submucosal plexus, and 3) completion of histologic evaluation of plexus in species with different diving abilities.

Results

This study identified the main connections between the submucosal tracheobronchial venous plexus and systemic venous circulation in the bottlenose dolphin and found them to be similar in the common dolphin. While several small venous emissaries exist between the laryngeal portion of the tracheobronchial plexus and the surrounding pharygeal plexus and jugular system, the largest emmissary emerges from the bifurcation of the accessory and main right bronchi. Sizable secondary branches connect to this emmissary after exiting the proximal portions of the accessory and main right bronchus. On dissection, the excised plexus is dense enough to form a cast of the tracheobronchial walls. No arterial connections to the venous plexus have been confirmed, however the laryngeal emissary veins presented as paired veins surrounding a structure likely composing an arterivenous triad.

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From a computed tomographic (CT) evaluation of a short bronchial section of the common dolphin, the engorged venous plexus accounted for approximately 38% of total luminal volume, suggesting it can occupy considerable space within the airways. As this venous system is valveless, retrograde venous flow is possible and may be likely during diving-related thoracic redistribution of blood. Such diving-related engorgement would likely occur concurrent to pulmonary compression and may, therefore, act in opposition to the forces driving alveolar collapse. Venous engorgement within the airways may in turn delay alveolar collapse and increase the depth of nitrogen absorption ablation, thereby increasing nitrogen absorption. Conversely, upon ascent, alveolar expansion may occur at greater depth, facilitating earlier nitrogen elimination from the blood. Due to its potential to prolong diving-related nitrogen absorption, the submucosal plexus may prove to be less extensive in deeper or slower diving cetacean species. Preliminary gross evaluation of tracheobronchial cross-sections suggests that the submucosal plexus of shallow-diving delphinids may occupy more volume than that of beaked whales and sperm whales.

An unexpected finding was a direct venous connection between the left gastric vein and the caudal vena cava. This connection appears to create an exhtrahepatic portosystemic shunt that bypasses hepatic filtration and provides a direct communication between gastrointestinal venous return and systemic venous circulation similar to portosystemic shunts in dogs (Nelson and Nelson, 2011). A connection between the left gastric vein and a robust esophageal plexus was also found, similar to gastroesophageal varices in humans (Hilzenrat and Sherker, 2012). While the implications of these connections are not clear, a persistent portosystemic shunt would provide an unobstructed pathway for gastrointestinal gas emboli into systemic circulation.

Summarize the results from your work effort. Insert tables and figures as separate image files that include notes or captions. Your abstract should be no more than two pages, including the designated notes section at the bottom of the page. Use the horizontal lines as your guide.

Notes:

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REPRODUCTIVE HORMONES AS RELATED TO AGE, SEX, SEASON AND LEVELS OF STRESS HORMONES IN THE BOTTLENOSE DOLPHIN

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Background

A critical issue in determining the impact of Navy activities on marine mammals is the potential to affect important life history functions, in particular those related to reproductive effort and success. Reproductive hormones (e.g. androgens, estrogens, and progestogens) are responsible for regulating the complex physiological and behavioral processes associated with reproductive behavior and are known to vary in many species as a function of age, sex, and season. In addition, the action of reproductive hormones are themselves affected by other hormones, including those related to the stress response (e.g. cortisol/corticosterone). Within toothed whales (odontocetes), little information exists on baseline levels of stress and reproductive hormones, how they influence one another, and how they vary as a function of season, age, sex, and reproductive status. Understanding these relationships increases our knowledge of how stressors experienced by marine mammals, including anthropogenic noise, might translate into alterations in reproductive hormone secretion and action and should facilitate predictions of how anthropogenic stressors might indirectly affect both reproductive effort and success.

Objectives

The objective of the proposed study is to increase our knowledge of sex hormone variability and the interrelationship between stress hormones and reproductive hormones in a representative delphinid, the bottlenose dolphin (*Tursiops truncatus*).

Methods

Serum samples from a prior ONR-sponsored effort to characterize age, sex, and seasonal variation in dolphin stress hormones were utilized. These samples were collected from bottlenose dolphins of the United States Navy Marine Mammal Program (MMP). Voluntary blood samples were collected biweekly from dolphins over the course of a year. Serum was separated from the blood and stored at -80°C. More than 500 samples were available for analysis for each of the sex hormones to be tested.

Serum samples were processed via radioimmunoassay (RIA) for testosterone, estradiol and progesterone. All kits were validated for the bottlenose dolphin for parallelism and accuracy. Linear mixed models (LMMs) were used to determine relationships between sex hormones and main effects consisting of sex, age, and season. In each LMM, the subject was treated as a random effect. Similarly, relationships between stress and metabolic hormones and sex hormones were analyzed with LMMs to determine how each exerts a relative influence on the other class of hormones. Where relationships between hormones and continuous predictors (e.g., age) were indicated, the relationships were further explored with regression analyses. Variation in sex hormones as a function of reproductive status was also explored (i.e. several females became or were pregnant during the sample collection).

Results

Both season ($F_{3,\ 174.4}$=12.7, p<0.0001) and age ($F_{1,\ 6.0}$=36.2, p<0.001) had a significant effect on circulating testosterone levels in male bottlenose dolphins. Testosterone was highest in the spring (defined as March 20-June 20; Tukey's HSD, p<0.05) and increased with the age of the male; immature males (<10 yrs age) produced the lowest levels of testosterone. Testosterone was significantly related to levels of free thyroxine (fT4; $F_{1,\ 124.6}$=14.8, p<0.001) and reverse triiodothyronine (rT3; $F_{1,\ 124.8}$=23.5, p<0.0001), as well as a significant interaction between the two thyroid hormones ($F_{1,\ 120.8}$=6.6, p<0.05). The relationship was also observed with fecal T3 metabolites ($F_{1,\ 146.6}$=7.9, p<0.01), further supporting a dynamic relationship between testosterone and thyroid hormones. The inclusion of age in the LMM relating serum thyroid hormone to testosterone yielded a whole model R^2=0.74, suggesting that elevations in testosterone are coupled with changes in the metabolic rate, presumably in preparation for breeding and particularly during the spring.

For purposes of analyzing progesterone and estradiol, the females that were known to be pregnant at the time of sampling, or that became pregnant during that time, were removed from the analysis (n=3). In addition, one female that appeared to have a pseudopregnancy was also removed. Estradiol was significantly higher in the fall than in the spring ($F_{3,\ 180.9}$=6.3, p<0.001; Tukey's HSD, p<0.05) and was influenced by age ($F_{3,\ 180.5}$=4.6, p<0.01), which appeared to be related to a greater variability in estradiol between seasons for the youngest female dolphins (<12 yrs age). Estradiol was also significantly related to total T3 ($F_{1,\ 182.5}$=6.9, p<0.01) and total T4 ($F_{1,\ 183.7}$=8.1, p<0.01), and as with testosterone in the males, significantly varied with fecal T3 metabolites ($F_{1,\ 160.6}$=4.0, p<0.05). There was a significant effect of season ($F_{3,\ 182.5}$=3.6, p<0.05) and age ($F_{1,\ 6.7}$=5.7, p<0.05) on progesterone in non-pregnant females; females had higher progesterone levels in the spring than in the fall (Tukey's HSD, p<0.05), which was opposite the trend in estradiol, and showed higher levels and greater variability in older females. Progesterone levels were unrelated to all other measured hormones, except for tT4 ($F_{1,\ 133.2}$=5.8, p<0.05). Occasional spikes in progesterone were observed in several older females, which could potentially be misinterpreted as a sing of pregnancy if based only on a point measure.

Pregnant females demonstrated an increase in serum progesterone across the gestation period, although the increase was not consistent and potentially showed pulsatile spikes in levels. In two animals sampled until just before parturition, a drop in serum progesterone and a spike in fecal glucocorticoid (GC) metabolites was observed. Relationships with other hormones measured during pregnancy were found only with norepinephrine ($F_{1,\ 78.9}$=7.9, p<0.01). One female demonstrated a pseudopregnancy (verified through ultrasound inspection) that lasted over five months, a condition which might not be uncommon in the species.

Bottlenose dolphins did not show relationships between the corticosteroids associated with stress, i.e. cortisol and aldosterone, but showed a number of relationships with thyroid hormones. The interplay of thyroid hormones and sex hormones reflect dynamic changes in energy partitioning around periods of known breeding activity, which has potential implications for understanding energy balance as a function of reproductive state. The lack of relationships between corticosteroids and sex hormones may not be surprising given that all animals voluntarily participated in blood draws. However, recent "stress tests" performed with bottlenose dolphins suggest that, at least in males, acute stress related increases in corticosteroids are accompanied by reductions in testosterone.

The finding of periodic spikes in progesterone in non-pregnant females, as well as a pseudopregnancy that spanned a period of five months, suggests that caution should be exercised in the interpretation of progesterone measures obtained from wild dolphins (e.g. as measured in blubber biopsies). The rate of reproductive failure for surveyed populations based on progesterone levels could be overestimated if pseudopregnancy is common in this species (or other delphinid species).

Notes:

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Molecular Indicators of Chronic Stress in a Model Pinniped - the northern elephant seal

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Background

Glucocorticoid release following acute stress causes metabolic alterations that facilitate the response to, and recovery from, immediate stress. Repeated or chronic stress, however, can result in persistent activation of the stress response with maladaptive consequences.

Objectives

The aim of this research effort is to establish biomarkers capable of distinguishing acute and chronic stress states in marine mammals. The specific objective of this study was to characterize the responses to acute and repeated stress in a free-ranging marine mammal at different physiological levels: hormone release, alterations in gene expression, and impacts on metabolic pathways—using immunoassay, transcriptomics, and metabolomics, respectively—to evaluate the consequences of stress on animal function.

Methods

We simulated stress responses in study subjects (seven juvenile seals) by administering adrenocorticotropic hormone (ACTH) each day for four consecutive days. Samples were collected for eight hours following ACTH administration on days 1 and 4 to characterize the acute and chronic responses, respectively. Samples were collected prior to the initial ACTH administration to define the initial baseline state, and 24 h after each administration to monitor stress recovery. The hormone response was measured from blood samples using immunoassays (McCormley et al, 2018); influences on gene expression were evaluated from blubber biopsies using transcriptomics (RNAseq, Deyarmin et al, 2019) and proteomics; and metabolic effects were assessed using a global metabolomics platform (Metabolon, Inc.).

Results

Repeated ACTH administrations elicited stress responses in elephant seals for at least four consecutive days. Both cortisol and aldosterone responded to ACTH, but their patterns differed based on stress state (Figure 1). Within 24 hours of each ACTH administration, cortisol returned to baseline level, whereas aldosterone concentration remained elevated, and total triiodothyronine remained at lower concentrations than the baseline condition.

We identified over 370k transcripts in blubber tissue with homology to known vertebrate genes; and 8k proteins were sequenced using LC-MS/MS. We detected altered gene and protein expression in response to stress (Figure 2): 61 genes and 31 proteins in the acute response, 12 genes and 22 proteins in the chronic response, with at least 12 genes and 45 proteins that responded differently to acute and chronic stress. An important consequence of the altered gene and protein expression was on metabolic pathways—evidenced by substantial alterations in circulating metabolites. We identified 388 circulating metabolites in seal serum, most of which (281) were altered during the stress response. These effects were distributed across several metabolic pathways including amino acid, carbohydrate, and lipid metabolism (Figure 3). Differences in gene and protein expression, and metabolites were detected despite similarities in glucocorticoid concentration.

Notes:

McCormley MC, Champagne CD, Deyarmin JS, Stephan AP, Crocker DE, Houser DS, Khudyakov JI (2018) *Repeated adrenocorticotropic hormone administration alters adrenal and thyroid hormones in free-ranging elephant seals*. Conservation Physiology vol 6, 10.1093/conphys/coy040

Deyarmin JS, McCormley MC, Champagne CD, Stephan AP, Busqueta LP, Crocker DE, Houser DS, Khudyakov JI (2019) *Blubber transcriptome responses to repeated ACTH administration in a marine mammal*. Scientific Reports 9:2718. /doi.org/10.1038/s41598-019-39089-2

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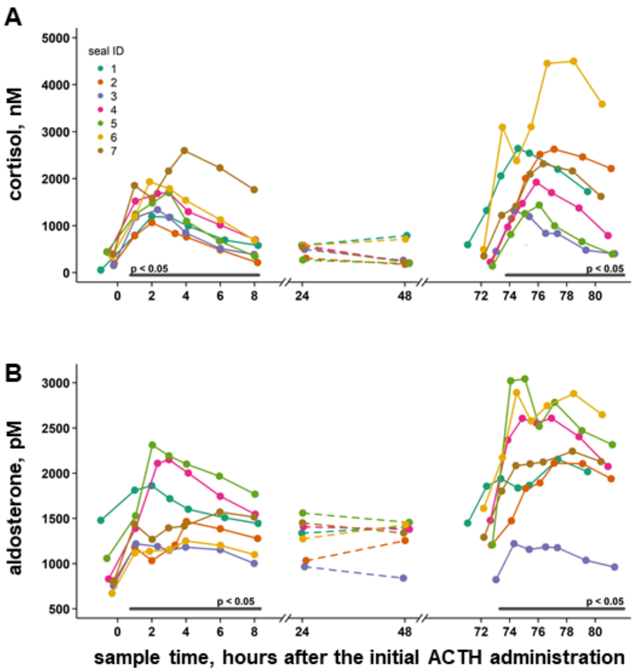


Figure 1. Both cortisol (A) and aldosterone (B) were increased in response to acute (day 1) and repeated (day 4) stress.

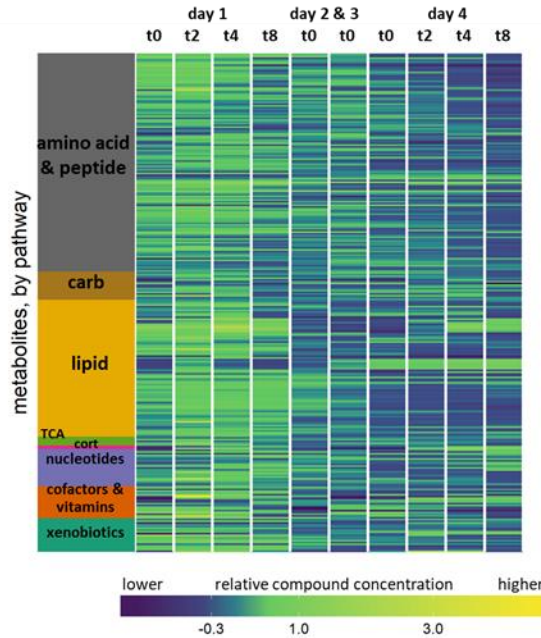
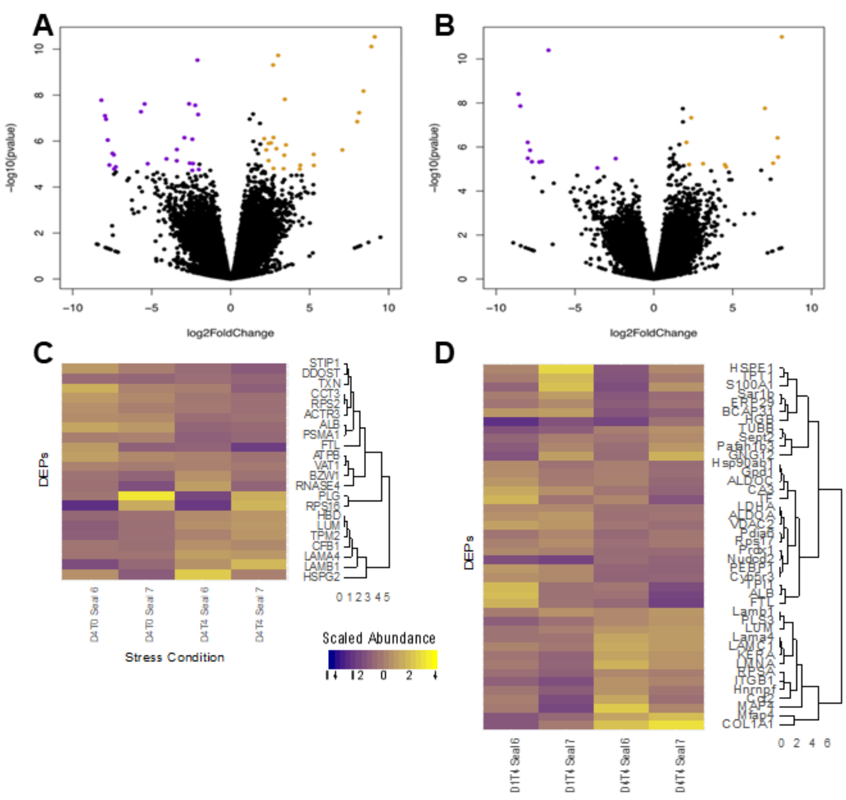


Figure 3. Heatmap showing changes in circulating metabolites during stress responses. Each column represents a sample time; each row represents a metabolite, grouped by major metabolic pathway. Relative metabolite concentrations are shown according to the color scale. Significant differences in metabolite concentrations were assessed by linear mixed model (LMM).

Figure 2. Changes in expression of blubber genes (volcano plots A, B) and proteins (heat maps C, D) in response to repeated ACTH administration. Transcript and protein abundance was compared between baseline (0 h) samples from days 1 and 4 (A, C) and between ACTH response samples (4 h) from days 1 and 4 (B, D).

Volcano plots show fold change plotted against statistical significance for all sequenced transcripts. Significantly upregulated and downregulated genes (adj. $p < 0.05$) are shown in yellow and purple, respectively.

Heat maps show differences in scaled normalized abundance of sequenced proteins (rows) between samples (columns) from two animals. Significantly upregulated and downregulated proteins (adj. $p < 0.05$) are shown in yellow and purple, respectively.



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Reconstructing Stress and Stressor Profiles in Baleen Whale Earplugs

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Background

The Office of Naval Research's Marine Mammal Physiology Program seeks to develop an understanding of the natural variation of stress markers; better understand and characterize the relationships among stress and stressors; define and compare the quantitative and temporal relationships of hormones across the different matrices; and evaluate/ characterize the relationship between the physiological stress response in marine mammals and acoustic exposure and 'biologically significant' disturbances. However, reconstructing complete lifetime patterns is elusive for most, if not all, long-lived terrestrial or aquatic vertebrate species.

Reconstructing lifetime chemical profiles in baleen whales (*Mysticeti*) was unsolved until recently when whale earplugs were found to sequester chemicals in discrete lamina throughout the life of the animal (Trumble et al. 2009).

Objectives

1. Expand earplug dataset to include additional species/populations to assess variation in stress at the individual and population level.
2. To determine population level variability in lifetime stress-related hormones profiles of large whales and to normalize baseline among individuals and species.
3. To assess and validate the accuracy and precision of measurements of hormones and other biomarkers in earplugs with other tissues collected from same individuals.
4. To expand the list of extractable biomarkers from each lamina within each earplug and determine its potential relationship to lifetime stress profiles (e.g. DNA and stable isotopes).

Methods

Objective 1: Several earplugs have been acquired, both from historical archives as well as from recently stranded whales. Since the beginning of this current study, 109 historical samples from fin (n = 86), humpback (n = 14) and (for the first time) Sei whales (n = 9) have been collected (<1972). Also, recent earplugs (c. 2000+; n = 15; N = 124) samples have been collected/analyzed.

Objective 2: Baseline hormone concentration was determined by averaging the lowest three cortisol concentrations within each specific earplug (N = 30). Percent change from baseline value was calculated for each time point (6-month intervals) within each earplug over the entire whale lifespan. To determine significant changes in hormone levels from baseline, we employed quantiles and z-scores. Regression techniques were used to assess the relationships of species, age and time with stress hormones (ng/g lipid) among all earplugs sampled.

Objective 3: Whale earplug samples sampled with corresponding baleen samples from recent strandings are part of a sub-project to determine within whale tissue validation, including earplug-earplug and earplug-baleen comparisons. To date, right-left earplugs (n = 4) and earplugs-baleen (n = 5) have been compared (6 month means from each matrix). Aging and delamination and hormone assays followed Trumble et al. (2013). Hormones were extracted

Objective 4: SI/AA: Bulk stable isotope signature from delaminated whale earplugs were measured using GC-C-IRMS. DNA: Earplug from a female Gray whale (28yrs). Earwax from three layers (early, mid, late) were used for DNA extraction. A deparaffination protocol was used to release epithelia cells (Zymo Research, Irving, CA, USA). DNA was extracted and isolated using Qiagen QIAamp DNA FFPE Tissue Kit (Qiagen Inc, Valencia, CA, USA) and Zymo Quick-DNA Miniprep Plus Kit (Zymo Research, Irving, CA, USA) following the FFPE protocol. Samples of baleen were obtained from the same individual. DNA was extracted and isolated using Qiagen QIAamp Fast DNA Tissue Kit (Qiagen Inc, Valencia, CA, USA)

Results

Objective 2: A 146-year cortisol dataset of stress in baleen whales presents an opportunity to model the potential influence of a wide range of abiotic, biogenic, and anthropogenic factors. Baseline-corrected cortisol significantly differed between the sexes as age progressed (Figure 1). A model indicated that females experienced less stress than did males over their lifetime. Specifically, mean baseline-corrected cortisol was approximately 12% less for females over a lifetime. Adult males had significantly elevated baseline-corrected cortisol levels (P value = 0.004; linear mixed-effects model).

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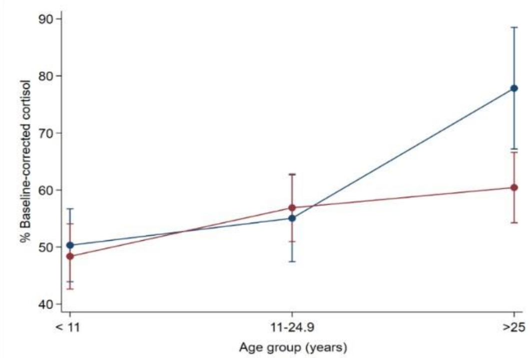

Figure 1. Baseline and age-corrected cortisol in baleen whales

Objective 3: Baseline-correcting cortisol resulted in a consistent baseline to compare across individuals (Figure 1), but did not alter the relative data trends over the lifetime of individual whales (Figure 2). Comparing baseline-corrected cortisol between the right and left earplugs from the same whale revealed no significant difference (p>>0.05, paired Wilcoxon signed-rank test, Figure 2).

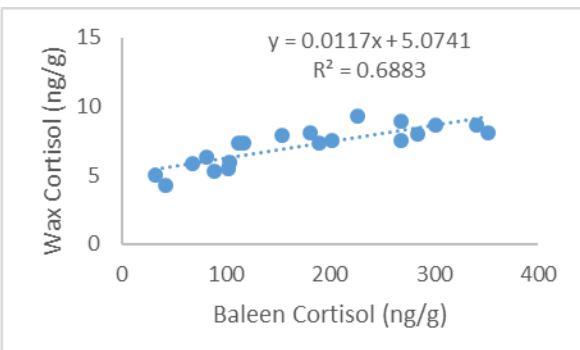

Figure 2. Least-linear regression of cortisol in baleen and earplugs at 6 month intervals from baleen whales

Objective 4: SI/AA: The results showed periods of stability, variability, and decadal trends. Compound specific stable isotope analysis of amino acid has recently proven as a powerful tool to investigate temporal variation at the primary producer (baseline) and those higher in trophic level (Figure 3). To determine possible mechanisms driving spatial and temporal changes of long term bulk isotope variation, carbon and nitrogen stable isotope of amino acid achieved in earplugs were analyzed using GC-IRMS.

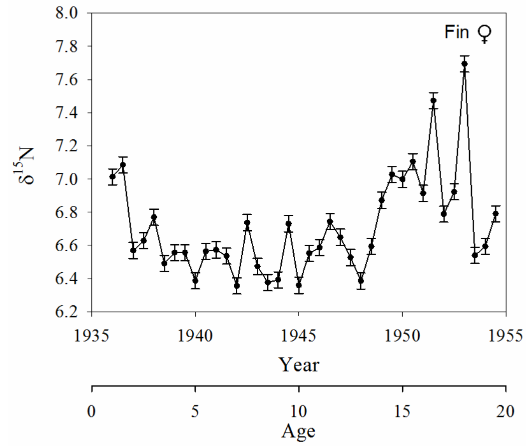

Figure 3. Age-baseline corrected stable isotope values for female fin whale

DNA: For the first time, DNA was extracted from baleen and earplugs as a function of time. DNA methylation drift from grey whale earwax was analyzed (Figure 4).

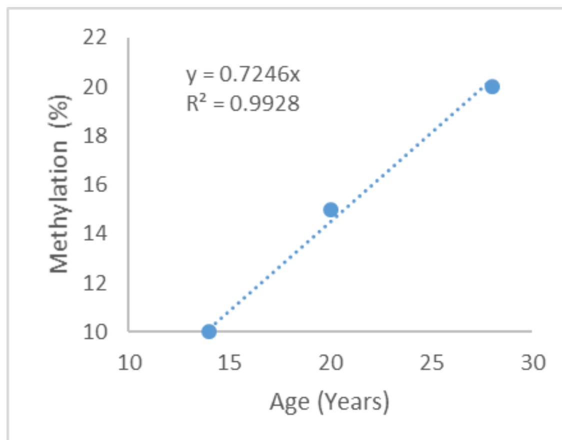

Figure 4. DNA methylation drift in a grey whale as a function of age

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Posters

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Developing tag and prey mapping technologies to measure cetacean physiology, ecology, and the energetic consequences of acoustic disturbance.

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Background

High resolution biologging and prey mapping devices can enhance our understanding of wild cetaceans, allowing for studies of not only natural behaviors and vital rates but also how those processes respond to acoustic disturbance. These commercially available devices have improved dramatically in sampling ability over the last 5 years, allowing for long duration, non-invasive sampling for several days with 400 Hz accelerometry and integrated audio and video recorders that can record for up to 8 hours of video and 24 hours of audio. Integrating these data streams can characterize not just the behavior of the tagged whale, but also of nearby conspecifics or other species in multi-species feeding aggregations, thereby informing calling rate calibrations from passive acoustics. Prey-mapping studies have shown that environmental context is critical to interpreting changes in behavior, and these integrated tags provide another window into this context. Our work over the last 5 years has resulted in over 850 hours of on-animal video footage synchronized with accelerometer-based behavioral descriptions. In addition to these tags, our work has increased the spatial resolution of standard acoustic prey-mappers by mounting ek80 transducers on 4-6 m rigid-hulled inflatable boat (RHIB) and zodiacs, allowing for directed focal follows that determine prey concentrations at the location of the tagged whale. Together, if properly processed, these two technologies will allow for increased ability to determine the behaviors of animals in response to stimuli and environmental drivers.

Objectives

Our objectives were to: 1) advance and develop the technology of commercially available cetacean tags that can measure feeding rates, calling rates, and high-sample rate acoustics, and 2) implement a mobile, ruggedized active hydroacoustic system to measure prey fields for coordinated use with behavioral response studies.

Methods

Since 2014, we have collaborated with Customized Animal Tracking Solutions to develop and advance cetacean tag technology. Tags were equipped with a set of inertial sensors including tri-axial accelerometers, magnetometers, and gyroscopes. In modern tag designs, we have also included rapid-acquisition GPS, hydrophones, and HD video with low light sensitivity. For prey mapping equipment, we collaborated with Cascadia Research Collective to adapt an ek80 system for use on a relatively small RHIB. Demonstration of this system was performed with simultaneous large baleen whale tagging studies over the past several years in Monterey Bay and Southern California.

Results

We acquired and installed three frequency EK80 Simrad echosounders on the R/V Musculus, 7.3 m rigid hulled inflatable boat (RHIB) with twin outboard Yamaha engines, that is owned and operated by the Cascadia Research Collective in Olympia, WA. The fabrication involved 2 major components: 1) Pelican Cases to house the Wide Band Transeivers (WBTs), 2) Mounting plate for the split beam transducers and attachment to a RHIB for operation.

From 2014 to present, we have designed and developed new tag technologies in collaboration with Customized Animal Tracking Solutions. Through an iterative design process, we have shown that a commercially available multi-sensor (acoustic, video, movement) tag can be both robust and reliable for use on large cetaceans. We have demonstrated the full capabilities of this tag on a wide range of cetacean species, from minke whales to blue whales. We can detect low frequency calls using tag-based accelerometers, as well as feeding events. Therefore, the tags provide an essential tool for quantifying vital rates. Such an approach is key for assessing the consequences of acoustic disturbance

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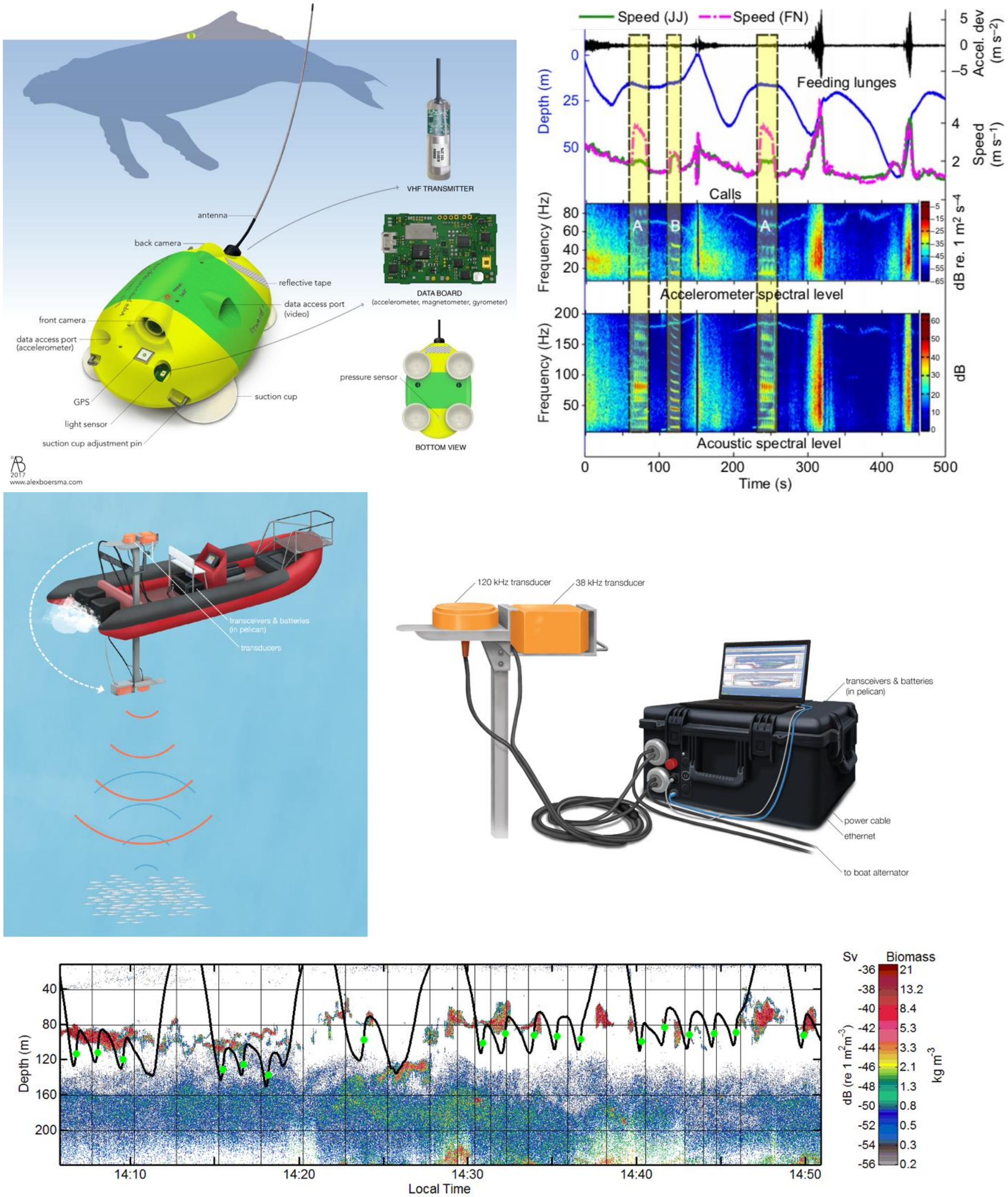

Tag and prey mapping technology. CATS tag schematic (upper left), example tag data showing vital rates for calling and feeding in a blue whale (upper right), prey mapping system schematic (middle panels), integrated tag data with prey mapping data for a blue whale in Monterey Bay (lower panels).

Notes: A mobile active acoustic system for measuring baleen whale prey fields to distinguish behavioral responses to military sonar from natural ecological dynamics (N00014-16-1-2546). Biomechanical and energetic analyses of whale-borne tag data to assess the population consequences of acoustic disturbance (N000141612477).

The ecological importance of glacial habitats to high Arctic odontocetes

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Background

This four-year project investigates the importance of glacial fjord ocean and ice conditions to high Arctic odontocetes. The project couples to the NASA-funded Oceans Melting Greenland (OMG) project, where data sharing and collaborations will improve data products and ecological inference, meeting both NASA physical oceanography and ONR marine mammal program priorities.

Objectives

The objectives of the project are to a) use previously collected data from tagged diving narwhals (*Monodon monoceros*) to fill data gaps on the one-time OMG multi-beam echo sounding survey of sea floor bathymetry in Melville Bay, Greenland, b) combine year-round passive acoustic monitoring (PAM) and remote visual detections of narwhals from land-based cameras to understand how physical properties of glacial fjords (e.g., subsurface Atlantic Water temperature and salinity, surface ice cover) influence narwhal occurrence, relative abundance, and acoustic behavior, c) collaborate with OMG scientists to add oceanographic instrumentation to narwhal moorings (CTDs and standalone temperature sensors) to allow for quantification of the variability of fjord temperature and salinity, and d) use remote camera imagery to quantify glacial ice mélange, glacial velocity, and frontal advance and retreat at three sites in Melville Bay.

Methods

We are using year-round passive acoustic monitoring (PAM) and remote visual detections of narwhals from land-based cameras in ice-covered waters of Melville Bay, Greenland. Two types of instruments are used to detect narwhals: Aurals (MTE-Electronique) and DSGs (Loggerhead Instruments). Moorings including temperature loggers and conductivity-temperature (C-T) sensors that allow quantification of deep fjord temperature and salinity variance driven by external (e.g., propagating coastally-trapped waves) and internal factors (e.g., variability in fresh subglacial meltwater discharge through). Additionally, full water column CTD transects are made across the continental shelf, fjords, and glacier fronts. Data from remote cameras are used to quantify narwhal presence, glacial iceberg discharge, and sea ice freeze-up.

Results

The first field season was conducted from 21-28 August 2018 (Figure 1). Three moorings and four cameras were deployed at three glacial front sites within Melville Bay, Greenland, including 1) Tuttulipaluk/Sverdrup glacier, 2) Nuussuaq/Kong Oscar glacier, and 3) Rink glacier near the Fisher Islands (Figures 2-4). Sightings of all marine mammal and seabird species were made during transects into and out of the glacier fronts. An echosounder EK80 was used to collect acoustic data on prey fields at the glacier fronts and on transects into and out of the study sites. Moorings and cameras will be turned around during a cruise in August 2019 to begin the second year of the project.

Notes:

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Using context to improve marine mammal classification

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ONR Project number N00014-17-1-2867, Prof. Marie Roch. Clustering the calls produced by the same animal or group of animals can improve the performance of acoustic classifiers and provide behavioral insights. Source location and call timing are the most common criteria for clustering calls into groups or tracks. However, in many studies, wide array spacing and/or internal clock drift render a large proportion of animal calls non-localizable. In this research we used spatial information from imperfect detection systems to improve classifier performance. We developed an algorithm for clustering calls that likely are from the same set of animals on the basis of time differences of arrival (TDOA). We measured performance of the algorithm against three sets of data: call timings from an agent-based Monte-Carlo simulation model in which the answers were known; detections of Minke whales from the US Pacific Missile Range Facility (PMRF), in which conditions for localization are very good; and mutli-species data collected from Stellwagen Bank National Marine Sanctuary, where clock drift of acoustic recorders was uncorrected. In our tests of the algorithm against the simulated data, we used the adjusted Rand index to measure the agreement between the model-projected groups and the known groups. The average adjusted Rand indexes of initial simulations from a pair and triplet of hydrophones were 0.56 and 0.76, respectively. The performance of the clustering algorithm showed little variation in performance when up to 2min/day of asynchronous hydrophone drift was added to the simulation. These results will improve the ability to accurately classify acoustically active species.

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Single-hydrophone automated passive acoustic fin whale ranging at Station ALOHA

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Background
Single hydrophone ranging techniques have potential in both naval operational and research environments for a number of reasons. First, researchers in the marine bioacoustics community typically do not have access to large, time-synchronized arrays, so data processing techniques that only require data from a single sensor can be more appropriate for their applications. Secondly, even on large hydrophone ranges operated by the world's navies, it can be difficult to associate the same call in recordings from multiple hydrophones, and in some cases a call may only be detectable on a single receiver. Being able to extract biological insights from single hydrophone recordings alleviates some of these challenges.

Objectives
In this work, a technique for performing passive underwater acoustic ranging with data from a single hydrophone is developed, and builds upon earlier localization approaches which estimate the sound source position using times of arrival of acoustic energy traveling along direct and/or interface-reflecting paths between source and receiver.

Methods
In this work, measured time differences between interface-reflecting and direct path arrival times are compared with a set of model-predicted time differences calculated over a set of candidate source ranges in a way that does not require measured arrival paths to be labeled (e.g., direct, surface bounce, bottom bounce, etc.). The modeled set with the best match to the measured data indicates the best estimate of source range. To enable the processing of multi-year data sets, the detection and localization steps are automated and, where possible, multi-threaded to improve computational efficiency on multi-core computer processors.

Results
This localization approach is demonstrated using 20-Hz fin whale (*Balaenoptera physalus*) calls and seismic airguns recorded by the ALOHA Cabled Observatory (ACO), 100 km N of Oahu (Hawaii) in 4728 m of water. Preliminary results showing seasonal trends in fin whale presence at ACO will also be presented.

Notes:

Integrating marine mammal presence into ASGARD: Arctic Shelf Growth, Advection, Respiration and Deposition rate experiments

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Background

The Arctic is experiencing rapid and extreme changes. The Pacific Arctic Region (PAR), which includes the Bering Strait region, and Chukchi and Beaufort Seas, is a bellwether for these changes with sea ice extent and thickness decreasing and freshwater and heat content increasing. The biological responses to these extreme physical changes are complex but may result in a shift in the northern Bering Sea and Bering Strait from an Arctic-type ecosystem to a subarctic-type ecosystem. One way to monitor changes in, or impacts on, an ecosystem is to observe the response of a suite of upper trophic level species such as sea birds and marine mammals via changes in occurrence and/or distribution. The overarching goal of the project "Integrating marine mammal presence into ASGARD: Arctic Shelf Growth, Advection, Respiration and Deposition Rate Experiments" is to provide data on the occurrence of vocal marine mammals as upper trophic level consumers at the top of a complex Arctic ecosystem and to document ambient noise contributions in this region over two years. This project is jointly funded by the North Pacific Research Board's Arctic Integrated Ecosystem Research Program and is focused on the Bering Strait region from the northern Bering Sea to the southern Chukchi Sea. This region has been identified in the *US Navy Arctic Roadmap 2014-2030* as of strategic importance, particularly as it represents a choke-point between the Pacific and the Arctic and is the region through which all traffic from the Northwest Passage and the Northern Sea Route must pass. Documentation of the inter-seasonal and inter-annual presence of vocal marine mammals (Arctic and sub-Arctic) in the Bering Strait region and integrating their presence with co-located oceanographic data will provide information on how the physical environment influences the biological inhabitants of that environment. Data on ambient noise levels in the region can be used to assess the impact of commercial shipping. Report to local communities on the health of the ecosystem including information on new species, and residency times of Arctic species. The combination of passive acoustic and physical and biological oceanographic data sets will provide urgently required information on how species presence varies seasonally with changes in benthic and water column prey, sea ice, currents, ocean water temperatures and freshwater flow.

Objectives

1.Deploy hydrophones for two years (from 2017-2019) on 3 moorings in the northern Bering and Chukchi Seas.
2. Document the inter-seasonal and inter-annual presence of vocal marine mammals in the Pacific Arctic Region from the passive acoustic data and compare acoustic detections in the eastern, western, and central PAR.
3. Integrate oceanographic drivers with acoustic detections to better understand how the physical environment influences the biological inhabitants of that environment.
4. Collaborate with other ASGARD PIs to develop an integrated understanding of the ecosystem components of the Pacific Arctic Region from physical forcing through to upper trophic level consumers.

Methods

Passive acoustic hydrophone packages (Aural-M2, Multi-electronique.com) were deployed on three moorings (N1, N2 and N3) in the northern Bering Sea in June 2017 (Fig 1). Oceanographic instruments on each mooring included a fluorometer, an acoustic Doppler current profiler, temperature and conductivity sensors and nitrate sensors. The moorings were recovered and redeployed in July 2018. The final recovery will occur in July 2019. All 3 were programmed to record acoustic data for the first 25 min each hour at a sample rate of 16384 Hz for a bandwidth of 10 Hz-8192 Hz. Upon recovery in July 2018, acoustic data were downloaded and converted to long-term spectral averages (LTSA). Spectrograms of each acoustic file will be examined to determine the species present and identify sources of noise from the LTSA and compare ship passages between the eastern and western regions of the northern Bering Sea. Detections will provide inter-annual, -seasonal, and –geographic comparisons of acoustically active species from the regions monitored.

Results

Preliminary results from the three moorings recovered in July 2018 suggest that subarctic species including killer, humpback and fin whales occur well into November in the western Bering Sea. Because the instruments were all deployed on heavily-instrument moorings in shallow water, there is significant noise on many of the recordings, including self-noise and surface wave noise. Arctic species, including

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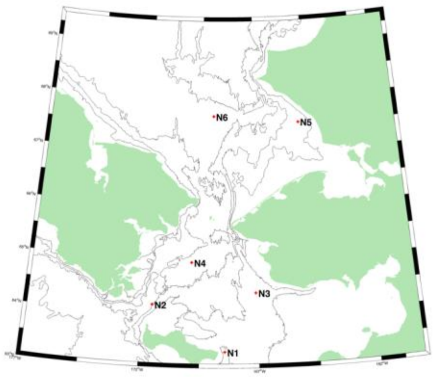
bowhead whales and bearded seals provide the overall greatest contribution to ambient noise levels the winter and spring (Fig 2). 2017 Ship passage data from the Marine Exchange, Alaska, archived by Axiom Data Sciences, show geographic differences in the numbers and types of vessels tracked by Vessel Automated Information Systems passing to the east and west of St Lawrence Island. The contributions of different vessel types to soundscapes local to each mooring location will be further explored.

Notes:



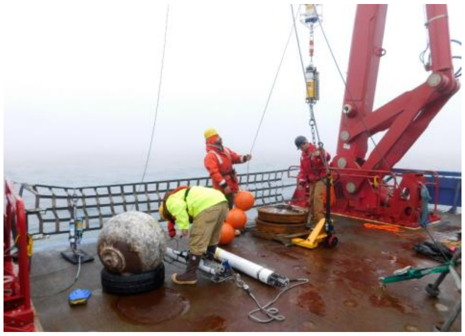


Figure 6.Left: Location of all moorings deployed for the ASGARD project. Hydrophone packages were deployed on N1, N2 and N3. Right: Mooring N1 being prepared for deployment.

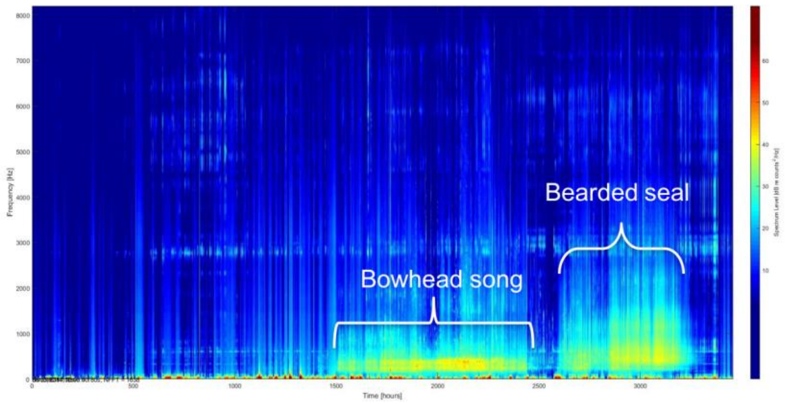


Figure 2. Long-term spectral average from July 2017-June 2018 from mooring N4 (300 s time and 20 Hz frequency resolution). Y-axis is 0-8000 Hz and x-axis is time in hours.

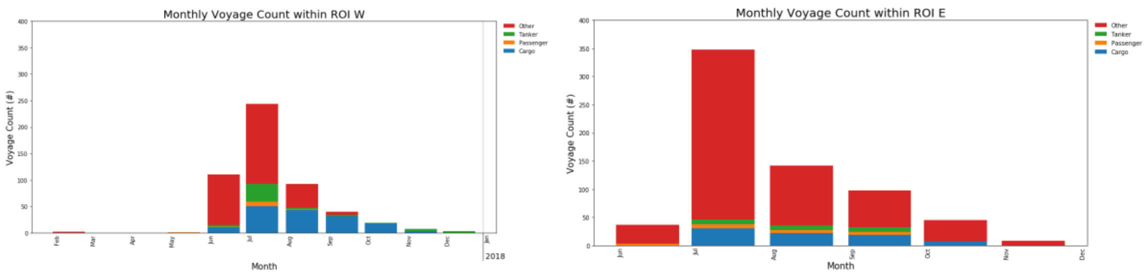


Figure 3. Left: number and type of ship passages west of St. Lawrence Island in 2017. Right: number and type of ship passages east of St. Lawrence Island in 2017.

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