**Pre-Proposal Submitted to the Office of Naval Research for FY22/23**

**White Paper**

**Title**: Determining intrinsic characteristics of age class, reproductive state and health status in short-finned pilot whales: Underpinning existing data on individual behavioral response to sound and linking to population dynamics and vital rates within the PCoD Framework.

**Principal Investigator**: 1Nicola Quick, Ph.D., e-mail: [njq@duke.edu](mailto:njq@duke.edu)

**Co-Principal Investigators**:

1Andrew Read, Ph.D., email: [aread@duke.edu](mailto:aread@duke.edu)

2Julian Dale., email: [julian.dale@duke.edu](mailto:julian.dale@duke.edu)

Enrico Pirotta/Cormac Booth??

**Institutions**:

1 Duke University Marine Lab, Beaufort, NC

2 Duke University Marine Robotics and Remote Sensing

**Mailing Address:** Nicola Quick Ph.D.

Duke Marine Lab

135 Duke Marine Lab Road

Beaufort

NC 28516

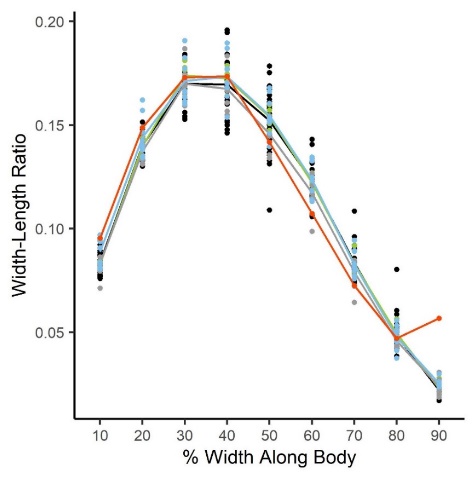
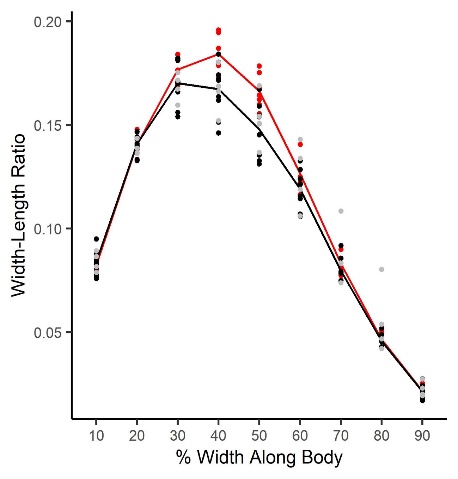
**New Effort/Duration:** 3 years (1 Oct 2022 – 31 Sept 2025)

**Total Budget:** $483,381

**Project Summary:** Assessing the non-lethal effects of anthropogenic noise on marine mammal populations requires extensive baseline knowledge of life-history demographics and behavioral patterns (Booth et al., 2020). However, quantitatively linking disturbance to population dynamics (i.e. size and age composition) is a major objective for modern conservation (Gill et al., 2001), but poses a significant challenge for many populations where this data is lacking (Booth et al., 2020). The development of the PCoD framework to scale up population level effects from individual based studies to assess population consequences of disturbance (Pirotta et al 2018) involves parameterizing a series of transfer functions to link behavioral and physiological changes. Understanding the intrinsic and extrinsic characteristics of populations exposed to anthropogenic stressors is key to populating the PCoD framework and if estimates of basic demographic rates are not available then PCoD modelling is not possible (Pirotta et al., 2018). Short-finned pilot whales off Cape Hatteras have been the subject of significant controlled exposure and tagging effort, with data collected on multiple individuals. However, the plasticity in their behavioral response coupled with their large group sizes and far ranging behavior make data collection on intrinsic characteristics of the population difficult. Data on sex, age class, reproductive state, nutritional state, social aggregation and how patterns of behavior and movement vary with season is not consistently collected and pilot whales do not feature in the summary of PCoD studies of marine mammal populations (Pirotta et al., 2018), despite a wealth of data on individual response during controlled exposure experiments. In order to utilize the existing Behavioral Response data on pilot whales there is an **urgent need** to: Quantify the demographics of pilot whale populations to understanding how changes in individual vital rates may affect population dynamics and inform the PCoD framework for this species.

Our **overall goal** is to gather seasonal metrics on sex, age class, pregnancy status and body condition for a large proportion of the population of short-finned pilot whales off Cape Hatteras. To accomplish this goal, we will systematically collect overhead imagery of large pilot whale groups using a custom built unmanned aerial system and assign age-sex class and pregnancy status with models trained and validated with biopsies of a subset of individuals. We will repeat this approach within and between seasons to maximize the number of individuals we sample. This method has been used successfully to collect demographic information on age, pregnancy and sex for bottlenose dolphins in Scotland (**Figure 1**, from Cheney et al., (In Review)). We will utilize this same approach to build population level sex and life-history curves for pilot whales.

Our **central hypothesis** for this work is that pilot whales form large population aggregations providing an opportunity to sample large proportions of the population within a small time and space window. This will provide extensive data samples with which to build population level sex and life-history curves that will represent the first large scale effort to document population level demographics for this species. The vast aggregations of pilot whales seen off Cape Hatteras provide an unrivalled opportunity to gather these data that could form the basis to parameterize a PCoD model for this species. Furthermore, Booth et al., (2020), rated photogrammetry, including drones, as a high feasibility methodology for monitoring demographic characteristics and health variables to inform PCoD analyses for deep-diving cetaceans. They also outlined that the ratio of calves to mature females and the proportion of immature animals are important demographic characteristics that may provide an early warning of population decline. Due to the large group sizes and offshore inaccessibility of pilot whales, recording these data via any other methods would be extremely challenging. Documenting natural variability in demographic and health variables under undisturbed conditions is vital for assessing non-lethal disturbance effects (Booth et al., 2020). Body condition can vary both seasonally and during different life history stages, so it is important to determine life history stage whilst estimating body condition. Our approach accounts for this through seasonal sampling utilizing three data collection trips each year and by collecting data on mixed age groups through our comprehensive sampling regime of capturing every individual.



**Figure 1:** Width-length ratios at 10% increments along the body of individual dolphins. A. Pregnant (red) versus non-pregnant (black) and unknown individuals (grey). B. Different age classes, adults (black), sub-adults (green), juveniles (grey), calves (blue) and newborn calf (orange)

A

B

Our study will be comprised of the following aims:

**Aim 1: Produce seasonal sex and age structure curves and body condition data for short-finned pilot whales off Cape Hatteras.** Based on the established methodology we have used for bottlenose dolphins (**Figure 1**), we hypothesize that we can produce age-structure plots for pilot whales based on width-length ratio measurements from drone images. This will be possible due to the large volume of images of different individuals (1000+) that will be collected over multiple sampling periods. Females can be identified based on calf association and quantify age and sex classes using length measurements and simultaneous photo-id images where possible. We can also assess if pregnancy status can be determined using the 30-50% width-length ratio increments successfully demonstrated in bottlenose dolphins (**Figure 1**). We will ground truth these measurements using biopsies from a subset of the measured individuals and determining genetic sex, progesterone levels for pregnancy (e.g., Pallin et al., 2018), and age estimates from epigenetic markers (e.g., Bors et al., 2021). This approach will enable us to quantify the age class structure for this population and compare between seasons. Female body condition with and without a calf present compared to calf length may provide metrics on the variation in individual health and potentially the probability of calf survival as well as calving periods for this population. Our approach provides the ability to collect measurable images from hundreds of adults for comparison and the ability to determine male and female cohorts using known (individuals with calves) female lengths and the known differences between male and female lengths. This would provide metrics to determine relationships between reproductive status and vital rates as well as information on the ratio of calves to mature females.

**Aim 2: Analyze water samples for eDNA to** **provide contextual variables of environmental quality and prey resources at each site where imagery is collected**. To help calibrate changes observed during sampling we will collect information on contextual variables to use as covariates in analysis. Collecting water samples in areas where pilot whales forage will provide us with the ability to assess prey types and quantify any seasonal differences in prey availability, that may in turn help us to quantify any observed differences in body condition. The RV Shearwater has the capacity to collect water samples over multiple depths using a CTD cast to assess prey availability using an eDNA approach.

**Aim 3: Use this demographic data to parameterize the transfer functions to inform PCoD Analysis for short-finned pilot whales of Cape Hatteras.** We will provide our demographic metrics for utilization in the PCoD modelling framework. Determining the population level consequences of change in individual fitness is only possible with an understanding about what proportion of the population is affected. The last step of the PCoD framework for understanding how changes in individual vital rates may affect population dynamics is only possible when demographic information about the population of interest is available. For pilot whales off Cape Hatteras, a wealth of data on individual behavior change in response to exposure to noise stressors exists. Similarly, much data exists on the animal residence time and habitat use for high apparent foraging and transit through extensive tagging and photo-identification studies, that show pilot whale distribution along the US East Coast. We will combine our demographic data with these existing data streams.

**Experimental Approach:**

**Data collection with an unmanned aerial system**: We will conduct three, four-day research cruises to the offshore waters of Cape Hatteras in the Duke Marine Lab RV Shearwater. This vessel will enable us to stay offshore and maximize the data collection period over three different time period over two consecutive years.We will take high resolution aerial photographs using a LemHex-44 UAS (long endurance marine hexacopter), fitted with a Sony A5100 24-megapixel CMOS mirrorless camera, Sony E 50mm F1.8 OSS fixed focal length lens and Lightware SF11/C laser altimeter. Designed and built by Duke University’s Marine Robotics and Remote Sensing (MaRRS) lab, the sensor payload is co-located on a 2 axis gimbal which provides the functionality to tilt the camera to locate and position over the animals, and then hold nadir when taking images to reduce measurement errors. We will launch the UAS from the research vessel and fly at approximately 30m above groups of pilot whales for a maximum of 30 minutes. Due to the fast surfacing sequence of pilot whales, images will be taken at a burst rate of up to 6 frame per second to ensure a useable image of each individual is collected. Flights will be completed multiple times each day when weather allows. The Research platform will allow effective charging of batteries to maximize the number of flights. Once a group has been surveyed we will move to a new group and conduct more flights.

**Biopsy collection**: A subset of individuals which are photographed from the UAS will be approached for biopsy to generate ground truth data sets. A 6-meter SOLAS rigid hulled inflatable boat launched from the R/V Shearwater will be used to collect tissue samples using an established remote biopsy system. Photographs taken from the boat and/or UAS during biopsies will be used to identify individuals. Skin samples for genetic analysis will be stored in RNALater in the field and blubber for hormone analysis will be frozen at -20° C on the R/V Shearwater and transferred to long term storage at -80° C on land.

**DNA extraction and genetic sex determination:** Skin samples will be homogenized, and genomic DNA extracted by silica spin column using the Wizard SV Genomic DNA purification system (catalog no. A2360) and stored at -20°C until ready for PCR amplification. Sex determination will be performed by multiplex PCR of segments on the X and Y chromosomes.

**Progesterone assays:** Progesterone increases by at least an order of magnitude in mammals during pregnancy and has been shown to be a reliable marker of pregnancy in blubber extracts from marine mammals. Longitudinal samples of blubber from biopsy plugs will be homogenized and steroid hormones extracted using standard separation protocols (e.g., Pallin et al., 2018). Progesterone concentrations will be quantified using an enzyme-linked immunoassay kit (Arbor Assays, Ann Arbor, MI, USA, product no. K025-H1).

**Epigenetic age estimation:** Age-associated DNA cytosine-phosphate-guanine (CpG) methylation sites have been discovered across a range of mammals including both mysticetes and odontocetes and proven useful in genetic age assays. We will develop a pilot whale specific tool calibrated using paired samples of teeth and skin. We will produce known ages for our calibration data set by counting growth-layer-groups in sectioned teeth. We will pursue a loan from the Smithsonian Institute of teeth from a mass stranding of pilot whales in 2005 in North Carolina. We have already extracted high quality genomic DNA from 14 of these individuals and will pursue loans of additional samples. Preliminary analysis suggests that these stranded animals are not genetically distinct from the population under long-term study off the coast of Cape Hatteras and therefore should be representative. Candidate odontocete CpG sites will be identified from the literature (e.g., Beal et al., 2019, Bors et al., 2021) and bisulfite sequenced to produce methylation rates across sites and individuals. Candidate sites will be tested for correlation to known ages and a model will be constructed to minimize error in age estimation. This model will be used to produce age estimates from biopsies of individuals of unknown age collected in the field.

**Analysis**

**UAS:**

Good quality UAS photogrammetry images will be selected and the lengths and widths of individual whales in each good quality photograph will be measured using the program MorphMetriX (Torres and Bierlich 2020). We will create age structure and sex curves as well as assessing the ability to determine pregnancy state and body condition. We will investigate differences in body width-length (WL) ratio using beta regression models and explore WL ratio as a predictor of pregnancy. This will be achieved using the body widths expected to change during pregnancy (20-60% widths/increments along the body axis) to determine if pregnancy can be predicted. We will train models and estimate classification error using known females with dependent calves as well as the molecular approaches described above. These morphometric models will be used to determine sex and age class across the full dataset of sampled individuals and build population level curves to determine the underlying demographics of this population.

**eDNA:**

We will analyze the eDNA samples using existing methods????? To provide contextual variables about prey availability in our models.

Completion of our aims will have the following **positive impacts** for our understanding of the effects of anthropogenic stressors on marine mammal populations

1. The first large scale effort to document population level demographics for this species
2. The ability to use our data with existing Navy funded data on movements and behavioral response for this species
3. The provision of data to advance the development of the PCoD Framework for this species

**Budget Estimate ($):**

**Duke:**

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| --- | --- | --- | --- | --- | --- |
| **Item** | **FY22** | **FY23** | **FY24** |  |  |
| A. Salary | $85,450 | $86,580 | 31,292 |  |  |
| B. Field work | $52,000 | $52,000 | $0 |  |  |
| C. Equipment/supplies/lab | $10,000 | $10,000 | $0 |  |  |
| D. Other costs | $10,000 | $10,000 | $10,000 |  |  |
| E. Indirect costs | $52,979 | $53,679 | $19,401 |  |  |
| Annual total | $210,429 | $212,259 | $60,693 |  |  |
| **Total Duke costs =** | $483,381 |  |  |  |  |

**SEA:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Item** | **FY22** | **FY23** | **FY24** |
| A. Salary and fringe | $7,184 | $7,292 | $0 |
| E. Indirect costs | $3,341 | $3,391 | $0 |
| Annual total | $10,525 | $10,683 | $0 |
| **Total SEA costs =** | $21,208 |  |  |
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**SEA budget information:**

Southall Environmental Associates (SEA), Incorporated is a small business that conducts basic and applied research. We have worked under both grants and contracts from many federal and state agencies in the 12 years of our existence, all proposed and conducted in accordance with all relevant CFR requirements. Our employees are biologists, acousticians, physiologists, and other research disciplines, all of whom are supported on research and consulting external funds.

For all federal awards, including this proposal, we follow a consistent and federally accepted procedure for determining billing rates. This has been successfully proposed, billed, and accepted by six different agencies, including our cognizant government agency (the Office of Naval Research (ONR)) with whom we currently have active grants utilizing this accepted procedure. We have developed this accepted procedure through review and feedback from the Defense Contract Audit Agency. Indirect costs are estimated based on actual audited costs from previous or existing federal awards averaged over a five-year period. We then apply actual costs during the period of performance to determine an actual-cost-based indirect rate structure and adjust relative to proposed indirect rates accordingly. Where cost-based indirect rates are lower than that proposed, the government is reimbursed. If cost-based rates exceed those proposed, no adjustment is made and there is no additional cost to the government.

SEA will use an identical structure and amount from negotiated rates with many previous and current federal funders, including our cognizant government agency (ONR) with which this current approach is applied. Our cost-based indirect rates included in this proposal are identical to those used in multiple currently funded projects, including three current grants from ONR. Fringe rates are calculated at a rate of 38.25% and indirect (overhead) costs are calculated at a rate of 46.5%. An overall total budget of $21,208 is proposed, which includes one (1) fully burdened month of Dr. Cioffi’s time in each of FY22 and FY23 ($10,525 and $10,683

Respectively).

**SEA Budget justification:**

Senior Scientist III (post-doc) (1 mos. each in FY22 and FY23). Dr. William Cioffi will lead the molecular lab work including coordinating the effort to generate a pilot whale specific epigenetic aging tool, determining sex from biopsies and establishing pregnancy from progesterone in blubber. He will lead the procurement of loans for tissue samples, conduct the lab work and lead the statistical analyses. He will also participate in field work.

**Hubbs:**

**Total Project Cost: $XX**

**Is this project a change in research direction?** No. This is a collaborative effort that builds on prior collaborations among the investigators with experience in behavioral response studies, drone imagery, PCoD Modelling and effects of stressors.

**Scientific significance:** This proposal represents a major advance in understanding the baseline demographics of a short-finned pilot whale population that has been exposed to anthropogenic noise effects. The work provides a comprehensive approach to gather data to underpin a modelling approach that has so far been a challenge due to data gaps. This work will use custom equipment to identify ways to collect data on demographics for cetacean populations that are far ranging and occur in large offshore aggregations.

**Naval significance:** This research will provide the first set of comprehensive demographic and body condition data for a short finned pilot whale population of Navy interest. This population off Cape Hatteras has already been the subject of Navy funded Behavioral Response and tagging studies. In **the near term**, our work will fill an important gap in our knowledge of seasonal age structure, sex composition, pregnancy status and body condition for this population. This is of direct relevance for computing PCoD models for this population, with demographic information representing the first branch in the decision tree to guide PCoD assessment as outlined in Figure 3 of Pirotta et al., (2018). Our **long-term goal** is to provide baseline variation and seasonal change to help monitor this population and address the non-lethal effects and population-level consequences for anthropogenic noise disturbance.

**Navy requirement addressed:** This project directly addresses the Research Concentration Area: *Effects of Sound on Marine Life*. Within this area we address the Population Consequences of Acoustic Disturbance by providing baseline demographic data to help parameterize the link functions between demographic information and vital rates for short-finned pilot whales. We will also leverage the large tag and behavioral response datasets for this population to help with understanding the population level effects of exposure to sound for this population.

**References**:

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