EFLSG5: Proteomic approach to characterise the functional differences between blubber layers in harbour porpoises

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Project rationale: In cetaceans, blubber can be divided into different layers and each layer is believed to have its own function. The top layer closest to skin is believed to be involved in insulation, the middle in storage and the bottom one closest to the muscle is believed to be involved in metabolism. However, this is an area that is unexplored and is vital in understanding health in cetaceans.

Activities: On average 260 cetaceans strand on the coast of Scotland yearly with the majority being harbour porpoises (*Phocoena phocoena*, ~130). Stranded animals are classified according to a decomposition condition code (DCC) ranging from 0 (live) to 5 (mummified carcass/skeletal remains) (See Figure 1). In this study, samples had to be obtained from those with a DCC of 1 or 2 (extremely fresh, no bloating) to ensure high quality proteins. A lower score would result in denatured proteins and thus not usable for the molecular work described in this project. During the duration of the project, no harbour porpoises stranded with DCC 1 or 2. However, we did obtain samples of 3 beaked whales (2 females and a calf). Post-mortem examination performed by SMASS showed that the female was lactating. These samples are unique as lactating is energetically very costly and thus the mother will engage the stored fat from their blubber to meet those energetic demands. These samples provide the rare opportunity to access the full blubber layer that would normally not be possible from free-ranging animals. As such, this will shed light on how the different blubber layers are used during high energy demands and if we see any difference in the biological mechanism activated in the different blubber layers. We collected samples from 3 different areas: dorsal, ventral and lateral. The dorsal area caudal to the dorsal fin is the area most often sampled in free-ranging cetaceans and thus relevant for

Figure 1. Carcase Decomposition Condition Codes (DCC) used by SMASS and described in document Inf.2.5.a Cetacean Pathology: Necropsy Technique & Tissue Sampling. Pictures: Utrecht University

health marker development. Samples were stored on ice at the stranding site and immediately stored at -80ºC at SMASS, University of Glasgow. These were then shipped on dry ice to the University of Aberdeen.

There, samples were dissected on dry ice using edged razor blades and with liquid nitrogen to avoid defrosting. Approximately 200 mg of each blubber layers was allocated into a new sterile Eppendorf tube. This resulted in a total of 24 samples:

- 3 blubber layers x 3 different positions x 2 adults = 18 samples
- 2 blubber layers x 3 different position x 1 calf = 6 samples

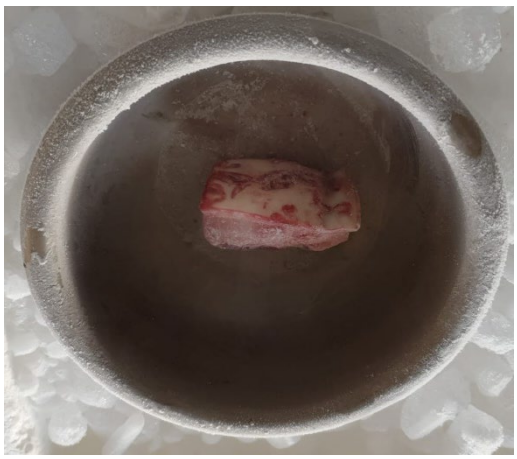

Figure 2. Tissue dissections of blubber samples on dry ice.

Extraction protocols were optimised by the Proteomics facility at the University of Aberdeen and extractions were performed with a RIPA Lysis and Extraction Buffer. Total protein content was determined by BCA Protein Assay Kit. SDS-polyacrylamide gel electrophoresis (PAGE) was performed for purification and enrichment of the proteins in the samples. Extracted protein samples were then ran for 2h on a Q Exactive liquid chromatography–mass spectrometry. This is now being quantified and the proteins (n≈20,000) will then be mapped to biological processes. A metabolic fingerprint will be generated using the R package RforProteomics, and pathway enrichment analysis programs using Gene Ontology datasets or Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. This will tell us which pathways are changed but also how strongly they change per blubber layer. Given that lactation is energetically demanding, we expect to see a strong biological signal.

Future of the project: We collected histology samples of the same animals and we will map the outcome of the proteomics to the size and number of the fat cell (internally funded by the University of Aberdeen). Hormone work will also be performed on the different blubber layers (internally funded by the University of Aberdeen). This will result in a comprehensive analysis of a strong biological signal and what the role is of the different blubber layers. The obtained results will be submitted as a manuscript to a peer-reviewed journal. The data will also be used to target a BBSRC responsive mode in December 2022. This will focus on the characterisation of key metabolic markers to develop novel health markers that are evidence driven. The work is fundamental to understand the impact of human caused stressors on energy stores of cetaceans and can have implications on our current monitoring polices (e.g., sampling top blubber layer). We therefore would like to thank MASTS to provide us with this vital piece of funding that will lead to a wide range of research possibilities.