

A family affair: from neutral markers to the bacterial microbiomes of a short-finned pilot whale (*Globicephala macrorhynchus*) mass stranding event

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1. MATERNAL RELATIONSHIP: MITOCHONDRIAL DNA

- non-coding control region in mtDNA, d-loop
- relatively high mutation rate
- lacks resolution on contemporary timescales
- maternally inherited; informs on maternal kinship and patterns of dispersal

METHODS:

- samples extracted with a standard NaCl method
- amplified a 596bp fragment of the control region
- amplicons sequenced on 3130xl Genetic Analyzer
- analyzed sequences in SeqScape Analysis
- trimmed sequences to 400bp for comparison to published haplotypes on GenBank

RESULTS:

- a single haplotype was identified for all $n=18$ individuals sequenced
- the haplotype matched Accession #U20921 published on GenBank, which was typed from an Atlantic short-finned pilot whale

DISCUSSION:

- these findings are consistent with previously published research, particularly in samples from the east coast of the United States
- additionally, there is low haplotypic diversity globally for short-finned pilot whales; therefore it lacks the resolving power to determine the degree of matrilineage among social groups

CONCLUSIONS AND FUTURE WORK:

This study highlights the value of collecting and archiving samples that may be used in multiple molecular genetic studies, including future work where new techniques and methods can be applied to historical samples. We demonstrated that frozen tissue samples from over a decade ago can still provide interesting and important information about an individual's exposure to external pathogens and the potential for disease resistance at a genetic level. Applying next generation sequencing techniques revealed pathogenic bacteria that can be classified to species level, and in some cases, to known isolates. Additionally, this multipronged molecular study revealed that a single pod of short-finned pilot whales comprised extremely neutral and non neutral variation. This reflects a far more complex pattern of individual differences in a species that has been generally thought to be closely related, with a matrilineal social structure. Future work should include further investigation into the fine scale structure of this species to better understand how group dynamics can influence the health, fitness, and viability of a social species.

2. KINSHIP: MICROSATELLITES

- short tandem repeats (STRs) found in non-coding regions of nuclear DNA
- hyper-variable
- can have recombination error
- bi-parentally inherited; provides information on breeding patterns and male mediated gene flow
- limited historical signal

METHODS:

- selected and individually optimized 13 loci
- fragments screened on 3130xl Genetic Analyzer
- scored alleles in GeneMapper
- genotypes compared for relatedness in ML Relate and ConCrys software. Network created using EDNetwork

- as predicted, a number of individuals were found to be closely related
- contrary to expectations, a significant proportion (>50%) of individuals were not related to most group members
- central individuals in the network were a mix of different sexes and ages

- from our literature review, the discovery of close kin with unrelated, (or distantly related), individuals within a mass stranding is unusual
- this highlights the need for a multi locus approach when trying to determine relatedness among and between individuals
- these findings underscore the importance of continuing to investigate patterns of behavior and relationships within social species

3. ADAPTIVE IMMUNE FUNCTION: MHC GENES

- The Major Histocompatibility Complex is a family of genes that determines an individual's ability to produce an immune response post antigenic exposure
- class II genes respond to external pathogens by recognizing non-self peptides, initiating the immune cascade
 - determine disease susceptibility and/or resistance at a genetic level

METHODS:

- amplified a 52bp fragment, capturing the entire 246bp exon 2 and peptide binding region in DQA
- amplicons sequenced on 3130xl Genetic Analyzer
- analyzed sequences in SeqScape Analysis
- aligned sequences for comparison of variant calls between samples in BioEdit and MEGA

- we detected variation in nonanonymous sites, including the critical binding pockets of the peptide binding region
- the variable sites detected in these samples have been previously observed in other cetacean species

- these patterns of variation indicate likely differences in susceptibility/resistance to external pathogens among individuals in a single pod
- meaningful genetic variation within social groups may influence group dynamics and survival in the evolutionary arms race

4. BACTERIAL MICROBIOMES: 16S rRNA GENE

- genes coding for the RNA component of the 30S subunit of a prokaryotic ribosome, commonly referred to as 16S rRNA gene
- highly conserved primer binding sites
 - 9 hypervariable regions (VI-IV)
 - highly conserved between bacteria and archaea; useful in phylogenetic studies
 - sequencing the complete 16S rRNA gene allows for species-specific identification

METHODS:

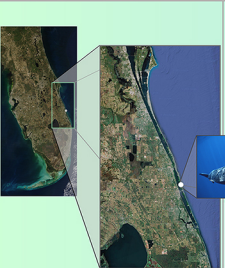
- samples extracted again with DNeasy Blood and Tissue kit for fresh total DNA
- barcoded and amplified using jack SQK-16S204
- ~1500bp amplicon sequenced on OXT MinION
- data trimmed and analyzed in EP2IME pipeline

- a variety of bacterial species were detected, including known dermal and epidermal infecting pathogens (*Acetivibrio rhipidactylus*, *Acinetobacter wofflii*, *Culticoccus acnes* etc.)
- in addition to waterborne species, airborne species were also detected (*Sphingomonas faeni*, *Aerococcus viridans*)
- to our knowledge, many of these have not been documented in cetaceans thus far

- this study demonstrates the utility of microbiome analysis from archived historical samples
- we highlight the power of long read MinION sequencing to identify potential pathogens at a species level
- skin microbiomes may be a useful tool in understanding the unique immune function and health of an air breathing marine species

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ABSTRACT:

In species that form close-knit social groups, an individual's ecology and behavior tends to be similar to other group members. Presumably, external threats to survival are similar, and in highly related or familial groups, all may be genetically predisposed to the same health risks or diseases. Short-finned pilot whales (*Globicephala macrorhynchus*), found world wide from tropical to temperate waters, are highly gregarious cetaceans that form kin-based matrilineal societies comprised of multiple generations of related individuals. Species that form long-term social bonds are prone to mass stranding, and short-finned pilot whales are notorious for these events. In this study, we used genetic and genomic techniques to investigate kinship, immune function, and skin microbiome composition from individuals in a mass-stranding event on Florida's east coast in 2012. We extracted tissue samples from $n=18$ individuals using DNeasy Blood and Tissue kit (QIAAGEN), and a single haplotype was identified from a 400bp sequence of mitochondrial DNA (mtDNA) d-loop. Samples genotyped at $n=13$ microsatellite loci revealed some close kin. To investigate adaptive immune function, we sequenced $n=12$ samples for exon 2 DQA, a class II gene of the Major Histocompatibility Complex (MHC) and found polymorphisms in nonanonymous sites previously detected in other cetacean species. To evaluate individual skin microbiome composition, the 1500bp 16S rRNA gene was sequenced on Oxford Nanopore minION platform for $n=8$ samples. A few bacteria phyla dominated but increased individual variation can be seen at the species level, likely reflecting different responses to the environment. In this study, we demonstrate the utility of retrospective extracting archived frozen tissue samples to investigate adaptive genetic diversity intertwined with bacterial microbiome detection in a closely related pod to understand how individuals interact with conspecifics and the environment, exploring the roles of group similarities and individual differences in the health, fitness, and viability of a social species.

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