

## Unravelling the intestinal microbiome of killer whale Reveil stranded in De Panne, Belgium: insights into cause of death and anthropogenic pollution impact

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Infectious diseases stand out as the predominant cause of death among stranded cetaceans in the Southern North Sea. Although most infections are caused by host-associated pathogens, some studies reported on infections with human pathogens through contamination from land and river runoff. The exceptional depth of current sequencing technologies allows for an accurate and high-resolution identification of pathogens from human origin and a thorough profiling of whole microbial communities providing valuable insights into cetacean health and disease. However, investigations into the intestinal microbiota of stranded cetaceans, specifically for studying the cause of death and assessing anthropogenic impact, remain limited. This study represents a pioneering effort to explore the intestinal microbiome of a stranded killer whale (*Orcinus orca*) using a comprehensive whole metagenome sequencing approach. The goal was to understand if the intestinal microbiome of a diseased killer whale - strayed in the contaminated coastal waters of Belgium - could yield insights into cause of death and serve as an indicator of anthropogenic pollution. Samples of gut content were collected from the stranded specimen to identify bacterial pathogens with human origin, antibiotic resistance genes (ARGs) and pathways reflecting exposure to anthropogenic pollution. Contaminant DNA was removed and bacterial DNA was isolated for Nextera XT library preparation. The subsequent whole metagenome sequencing on the Illumina NovaSeq platform generated 22 million bacterial reads. Rarefaction curves demonstrated that the entire bacterial diversity was captured. Sequences were taxonomically classified using Kraken, aligned against genomes of both marine, as well as human pathogens and annotated using the comprehensive antibiotic resistance database (CARD) and using a custom database for microbial biodegradation of pollutants. This research establishes baseline knowledge regarding the killer whale intestinal microbiome and pathogens, and proves that the microbiome can give essential clues into cause of death and anthropogenic impact in stranded cetaceans.

### Keywords

Microbiome; Killer Whale; Stranding; Cetaceans; Pollution; Pathogen; Antibiotic Resistance Gene