SC/68C/E/07 Rev1

Sub-committees/working group name: E

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INTERNATIONAL WHALING COMMISSION
75 years of science and stewardship 1946 – 2021

**Possible Role of Viral Disease in Gray Whale (*Eschrichtius robustus*)
Unusual Mortality Events (UMEs)**

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ABSTRACT

The ENP gray whale population has undergone two official Unusual Mortality Events (UME) in the past 25 years. While the location and density of gray whale benthic prey has seen changes as the result of oceanographic features and conditions, there is no clear indication of prey limitation. Furthermore, although gray whales are well adapted for benthic feeding, they can also feed on pelagic prey. Thus food availability during periods of high gray whale abundance may not be a limiting factor. Common characteristics in both the 1999-2000 and 2019-2021 UMEs included: 1) increased stranding numbers throughout the species North American range (Mexico to Alaska), 2) apparent emaciation in a proportion of stranded whales, 3) low lipid content of blubber and body tissues in some whales, 4) apparent reduced reproduction (low calf counts) occurred during and following each event, and 5) average to good condition of post-parturient females and their calves observed in the breeding lagoons of Baja California during 2019 to 2021. Emaciation, low lipid content of blubber and body tissues, poor body condition, and reduced fecundity can be associated with a number of infectious diseases of wildlife, in addition to being features of purely nutritionally driven mortalities. This paper explores the possible role of chronic viral infections in gray whale unusual mortality events and highlights the utility of viral discovery work using unbiased metagenomic sequencing for prospective and retrospective investigations of gray whale mortalities.

KEYWORDS: VIRUS, DISEASE, STRANDINGS, HEALTH, BODY CONDITION, NUTRITION, *ESCHRICHTIUS ROBUSTUS,* EASTERN NORTH PACIFIC GRAY WHALES, SPECIES CONSERVATION, UNUSUAL MORTALITY EVENT (UME)

INTRODUCTION

Two populations of gray whales (*Eschrichtius robustus*) are recognized in the North Pacific: The eastern North Pacific (ENP) population is estimated to include 20,000 individuals (Stewart and Weller 2021); and the western North Pacific (WNP) population which is listed by the IUCN as endangered, numbers approximately 300 individuals (Cooke et al. 2018). Some degree of population mixing between the ENP and WNP populations occurs during migration in the ENP, in the wintering areas off the Pacific coast of Baja California Sur, Mexico, and on summer feeding grounds in the WNP off southeast Kamchatka and northeast Sakhalin Island, Russa (Weller *et al.* 2012, Urban et al. *In Press*).

The ENP gray whale population has undergone two official Unusual Mortality Events (UME) in the past 25 years[1]. The first event occurred from 1999-2000 when gray whale strandings and mortalities along the Pacific coast of North America increased significantly (Gulland *et al*. 2005, Moore *et al*. 2001). Between 1995 and 1998 the annual mean gray whale stranding numbers along the Pacific coast of North America was 41; however, in 1999 and 2000 strandings increased to 283 and 368, respectively. Of those whales that were necropsied, poor nutritional state and apparent emaciation was reported in many cases (LeBoeuf *et al.* 2000, Moore et al. 2001, Gulland *et al.* 2005). Surrounding the 1999-2000 UME, the ENP population declined by approximately 23 % between 1997 and 2000 (Laake et al 2009, Stewart and Weller 2020).

The present UME was declared in 2019 and although stranding rates had declined in 2020, the UME is still open in 2021. Gray whale strandings along the entire Pacific coast from Mexico through Alaska were 214 in 2019, 174 in 2020, and 42 as of 10 April 2021. Historically, along the U.S. coastline (California, Oregon, Washington, and Alaska) gray whale strandings averaged ~29 per year from 2001 to 2018, and increased to 122 stranded whales in 2019, 79 in 2020, with 8 standings as of 10 April 2021 (NOAA 2021). Necropsy findings indicated that of 89 stranded whales examined in 2019, 29 were emaciated and 38 were thin with depleted blubber lipid and internal fat deposits. The remaining 22 animals were in average to good nutritional condition and died due to trauma from ship strikes, entanglements or killer whale predation (Raverty *et al.* 2020). The population was estimated to have decreased by approximately 24% from nearly 27,000 whales in 2016 to about 20,000 individuals in 2020 (Stewart and Weller 2021). Gray whale calf counts also declined during the current UME period (Stewart and Weller 2020).

Concurrent with the 2019-2021 UME, the proportion of live single gray whales photographically determined to be in "poor body condition" in the winter breeding/calving lagoon at San Ignacio in Baja California Sur, Mexico increased from 8.2% in 2018, to 23.6% in 2019, 30% in 2020 (Ronzón-Contreras *et al.* 2020, Christiansen *et al.* 2021), and 23.9% in 2021 (Ronzón-Contreras, *pers. comm*.). Additionally, counts of female-calf pairs in the breeding lagoon decreased significantly from typically 100-120 pairs during the years 2011 to 2017, to less than 20 pairs during the period from 2018 to 2021 (Urbán *et al*. 2019, 2020, and Ronzón-Contreras, *pers. comm*.). Interestingly, in all winters from 2018 to 2020, females with calves of the year appeared to be in good to average body condition, not showing any evidence of poor body condition (Urban *et al.* 2019, 2020).

The growth trajectory of the ENP population as derived from a data time series of abundance estimates between 1967 and 2020 suggests that large-scale fluctuations of abundance are relatively common, occurring approximately every 20-years, and that not all of the historical declines were associated with increased numbers of strandings (Stewart and Weller 2021). It is possible that gray whale UMEs occur or are triggered during the years following elevated population densities when the number of whales nears or exceeds carrying capacity (K) of the environment. However, it is clear from the abundance time series carrying capacity for this population is dynamic and therefore may not be an underlying driver of these UMEs. While the location and density of gray whale benthic prey changes as the result of oceanographic features and conditions, there is no clear indication of prey limitation (Moore et al. 2001, 2003, 2008). In addition, although gray whales are well adapted for benthic feeding, they can also feed on pelagic prey like krill, mysid shrimp, cumaceans, fish roe and roe deposited on marine plants, and small schooling fish, etc. (Rice and Wolman 1971, Nerini 1984, Sumich 2014), This feeding flexibility suggests that even during periods of high gray whale abundance food availability may not be a limiting factor.

POSSIBLE ROLE OF PATHOGENS

Common characteristics in both the 1999-2000 and 2019-2021 UMEs included: 1) increased stranding numbers throughout the species North American range (Mexico to Alaska), 2) apparent emaciation in a proportion of stranded whales, 3) low lipid content of blubber and body tissues in some whales, 4) apparent reduced reproduction (low calf counts) occurred during and following each event, and 5) average to good condition of post-parturient females and their calves observed in the breeding lagoons of Baja California during 2019 to 2021. Emaciation, low lipid content of blubber and body tissues, poor body condition, and reduced fecundity can be associated with a

[1] Under the Marine Mammal Protection Act (MMPA), an unusual mortality event (UME) is defined as "*a stranding that is unexpected; involves a significant die-off of any marine mammal population; and demands immediate response*"

number of infectious diseases of wildlife, in addition to being features of purely nutritionally driven mortalities (Hudson et al. 2002).

For example, in sheep there are a number of viral and bacterial diseases with wasting as the presenting clinical sign ("thin ewe syndrome", *e.g.* Johne's disease, maedi-visna, caseous lymphadenitis). Prion diseases (*i.e.* mad cow disease, scrapie, chronic wasting disease), caused by infectious proteins are also associated with chronic wasting syndrome in cattle, sheep, goats and some cervids (Terry & Wadsworth 2019, Myers et al. 2020). Chronic emaciation is also observed with endoparasitic infections (*e.g.,* stomach and liver flukes, hemoparasites). Common non-infectious wasting conditions in animals and humans include cancer and organ failure due to chronic disease (*e.g.,* chronic heart failure, chronic kidney disease, maldigestion, malabsorption). Pathophysiological mechanisms underlying emaciation (severe and diffuse loss of fat and skeletal muscle) caused by endogenous diseases (infectious diseases, cancer, chronic diseases) differ from nutritionally driven emaciation due to starvation in key aspects, including accelerated protein/fat metabolism and anorexia (voluntary food intake reduction). As an immune mediated type of wasting (Yishida and Delafontaine 2015) it is not responsive to nutritional cues, unlike starvation which responds to increased food availability. Overall, emaciation or poor body condition alone are often not diagnostic of a specific etiology but an outcome of chronic conditions and end stage of disease.

Transmission of directly contagious pathogens tend to increase at high host population sizes, seasonal increased densities and interactions that occur during feeding and reproduction, such as those that occur in gray whales during breeding aggregations in coastal areas and lagoons off Baja California (Hudson *et al.* 2002). The occurrence of high mortality events in the ENP population separated by years of low mortality is reminiscent of the epidemiology of morbillivirus epizootics in marine mammals, such as recurrent phocine distemper virus (PDV) epidemics in seals in the North Sea and dolphin morbillivirus (DMV) epidemics in bottlenose dolphins along the eastern US seaboard (Van Bressem *et al*. 2014). However, the limited number of whales examined by histopathology in the gray whale UMEs have not had classic histopathologic lesions compatible with morbillivirus infection.

Infectious agents can also be endemic, rather than epidemic, causing more continual low-level morbidity and mortality. A range of viruses detected in cetaceans to date, including morbilliviruses, herpesviruses, picornaviruses, arboviruses, astroviruses, anneloviruses and retroviruses (reviewed in Bossart and Duignan 2018) could cause chronic weight loss or mortality, although the effects of many of these emerging infections on cetaceans is unclear.

Samples from internal organs collected during the current 2019-2021UME are limited due to carcass decomposition. A limited number of samples are available for histopathology and PCR analyses to rule common marine mammal viral pathogens such a cetacean morbillivirus and avian influenza, but analyses are pending due to delays in testing associated with the COVID-19 pandemic. However, limited histology on fresh dead whales during both UMEs has revealed lesions suggestive of viral encephalitis in one case, and skin lesions with possible viral inclusions in a few animals (for review Stimmelmayr & Gulland 2020). To date, the decomposed state of most stranded gray whales and limited resources for viral discovery have hampered investigations into the potential role of viral infections in gray whale mortality. Future investigation for viral discovery using new metagenomics techniques could improve understanding of the contribution viral disease may be having on gray whale health and mortality events.

INVESTIGATIVE APPROACH

Viral discovery work using unbiased metagenomic sequencing can be used to identify the presence of viruses in gray whales and will most likely detect a large number of viruses, some pathogenic and some that may be incidental and not related to mortality or health decline. Therefore, ideally animals to be sampled would include both healthy and unhealthy whales to understand which viruses detected may be impacting health. Samples can be collected from both live whales (blow samples) and fresh dead whales from blood and tissue samples

Suitable samples (plasma, lymph node, spleen, liver, lung, kidney, brain) for this metagenomics approach could be collected from fresh dead whales (even though those are extremely rare) and preserved with RNAzol or RNAlater to prevent degradation of RNA (but also preserve DNA) (Kohl et al. 2017). Ideally, fresh dead whales would include both robust healthy whales (acute vessel struck, entangled, or harvested) and stranded whales in poor body condition. Duplicate tissues should also be collected for histologic examination to interpret the sequencing results in the context of the presence or absence of lesions associated with infection. Additionally, blow samples from live whales (both healthy and in poor condition) could also be collected and preserved in RNAzol or RNAlater.

The Vaccine Research Center (VRC) at the U.S. National Institutes of Health (NIH) has considerable experience with large-scale metagenomic sequencing projects for pathogen identification, and is an enthusiastic collaborator for this research. The VRC is part of a large surveillance effort in which plasma from healthy donors around the world (human and animal samples) is being collected to identify the presence of infections and antibodies to infectious disease to understand their potential to cause future outbreaks (Mina *et al.* 2020). The VRC is offering its expertise to advise researchers investigating gray whale health on sample collection and storage, and will perform the metagenomic sequencing and bioinformatic analyses of gray whale tissue samples. If available, the VRC is capable of processing hundreds of samples for this effort.

Possible sources of gray whale tissue samples:

- Existing archived tissue samples.
- Emaciated, stranded dead or dying gray whales.
- Aboriginal subsistence whaling, e.g., healthy gray whales taken off Chukotka, Russia.
- "Ship struck" or acutely entangled stranded whales.
- Blow sampling– expiratory samples from live, approachable healthy and poor condition whales.
- Collection of duplicate tissue samples for standard histology

ACKNOWLEDGMENTS

I wish to express my sincere thanks and appreciation for my co-authors who generously shared their knowledge, insight, enthusiasm, and interest in exploring the little understood relationship between viral disease and the life history of gray whales, and other marine mammals. We are indebted to their ongoing curiosity and their desire to understand the interrelationships between viral microbes, the environment, and health of marine life. We are all hopeful that this document will stimulate discussion of new research approaches to study and to understand the role of viral disease in the natural history of gray whales.

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