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## MULTIDISCIPLINARY STUDIES ON A SPERM WHALES' MASS STRANDING

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Sperm whales' (*Physeter macrocephalus*) mass strandings remain peculiar and rather unexplained events. Even if several natural factors and human activities have been proposed as possible causative factors, well-defined causes of similar dramatic events are rarely identified. In September 2014, 7 whales were found stranded along the Italian coastline of the central Adriatic (Abruzzo Region) and while 4 animals were extraordinarily refloated, 3 animals died on the shore.

The 3 dead sperm whales were examined in order to collect biological and pathological information. During necropsies, a complete set of tissues were collected and preserved fresh (for microbiology), frozen (for genetics, virology, parasitology, stomach content and stable isotopes analysis, toxicology) and 10% buffered formalin-fixed (for microscopic examination, immunohistochemistry and inner ear electron microscopy). A nested RT-PCR was used to investigate evidences of dolphin morbillivirus (DMV)2 along with immunohistochemistry (IHC) using an antibody targeting canine distemper virus nucleoprotein antigen. Finally, gas and fat embolic syndrome was assessed using dedicated approaches.

The 3 dead individuals were females (SW1, pregnant, total length 8.95 m; SW1b, male, fetus; SW2, total length 8.38 m; SW3 total length 7.33 m), all belonging to the same Mediterranean population and social unit; the wind and marine currents pushed get them towards the beach where they died. Postmortem analyses revealed that SW1, the older pregnant female and likely the stranded pod's leader, exhibited a prominent hydronephrosis secondary to a large kidney stone likely causing renal impairment. Molecular evidence of DMV infection was achieved in all the examined animals with an immunopositive reaction of circulating monocytes and follicular dendritic cells confirming the infection. Scant and highly digested food remains and the parasitic burden support the hypothesis of a non recent feeding as confirmed also by stable isotope analyses. Plastic debris were found only in SW1's stomach. Substantial levels of heavy metals and organic pollutants were detected being not associated to any change. Gas and fat embolic syndrome was excluded as well as ongoing military exercises and seismic surveys.

The results of postmortem analyses revealed that the 7 sperm whales entered the Adriatic Sea encountering adverse conditions and followed northward the ill and pregnant leader of the pod toward the stranding site. DMV infection could have played a crucial role in impairing their health condition and in recognizing the way towards the high seas. In fact, molecular and IHC analyses support the hypothesis of an infection at a very initial phase, characterized by active viral replication in myeloid cells. During this period, even if no severe clinical signs were expected, it seems

plausible that a "general discomfort condition", secondary to the viral circulation could have developed in the 3 stranded animals.

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