Narrative Report- Beluga whale, ID 'Kharabali' Report Date: 12/15/23

<u>History</u>

A female beluga whale ID 'Kharabali' was moved from Marineland in Canada to Mystic Aquarium on 15 May 2021 under NMFS research permit #22629. Preshipment diagnostics and transport planning were conducted by Marineland veterinarian Dr. June Mergl and transport was approved by the Canadian regulators, Dr. Mergl, and Mystic's board-certified veterinarians. Preshipment diagnostics included bloodwork indicative of healthy, stable condition, negative for all infectious diseases tested, and gastric sample and respiratory sample testing that were normal. The transport occurred without incident.

The whale acclimated well. Serious medical concerns did not develop until September 2023, more than 2 years after transport.

Narrative

In early 2023, Kharabali developed mild pleural (lung) lesions on ultrasound, which were medically managed, and which waxed and waned. Throughout this time, she remained clinically normal. In September 2023, Kharabali began showing some abnormal swimming and reduced energy, which were progressive. A bronchoscopy was attempted; abnormal redundant tissue was noted around the glottis preventing entry with the endoscope. In the 6 weeks preceding her death, Kharabali's swimming became progressively more abnormal, and she exhibited abnormal body position in the water. Due to the abnormal swimming, she sustained wounds on her pectoral flippers, flukes, jaw, and rostrum, despite extensive efforts including use where feasible and appropriate of padding in the habitat and staff in the water, aimed at preventing her from contacting any surfaces. While Kharabali ate normally through most of her illness, in the month prior to her death her appetite decreased. She demonstrated periodic crunching which was interpreted as lower gastrointestinal discomfort, thicker than normal feces, and she was identified to have abnormal bacteria in her feces. She was given antimicrobial medications, probiotics, and fecal transfaunation to treat, and the abnormal bacterial component resolved. Her general condition became progressive, however, and she stopped eating on her own, resulting in weight loss. Aggressive fluid therapy and nutritional support were provided, but she continued to struggle with motility, limiting the ability to increase the amounts of support she could receive; at this time, her bloodwork demonstrated an increasingly inflammatory profile and her abnormal swimming continued to be progressive.

On 2 December 2023, Kharabali was moved off the beluga habitat to the Aquatic Animal Study Center to continue critical care in order to have the ability to provide her heated water to help with weight gain, and for her protection from trauma associated with abnormal swimming. In the days before her death, gastrointestinal motility was extremely decreased preventing administration of additional food or fluids. The abnormal swimming and posture in the water were also progressive, and she seemed to be frequently disoriented. Additional findings noted in the week prior to death include poor cardiac contractility and dilated hepatic vessels and possibly also biliary tracts on ultrasound, and elevations in Total Bilirubin, GGT, and AST, on bloodwork. Despite aggressive medical management she experienced an emergency on 11 Dec 2023. Emergency treatment was initiated but Kharabali did not respond. Kharabali was transported to the University of Connecticut for necropsy. Pathological results are pending.

A 24-hour monitoring system has been in place for belugas at Mystic Aquarium and was in place for the entirety of this animal's medical issues and continues to the present day.

This animal was currently under a pause in research per NOAA-NMFS request. That pause began August 6, 2021. The death is unrelated to any research activities.

Steps Being Taken & Continuing Efforts

We have ensured and will continue to ensure that medical conditions in one whale do not pose risk to the others. No communicable diseases that could impact other whales have been identified. The prior deaths in transported belugas (Havok and Havana) were found to be due to incurable, likely genetic, conditions that could not be diagnosed antemortem. All other whales at Mystic Aquarium are presently healthy.

Accredited by the Association of Zoos and Aquariums (AZA), the Alliance of Marine Mammal Parks and Aquariums (AMMPA), the International Marine Animal Trainers Association (IMATA), and certified by American Humane (AH), Mystic Aquarium consistently provides the highest quality medical and husbandry care to our animals. Our veterinary program is led by experienced veterinarians who are board-certified specialists in zoological medicine and our facility has over 4 decades of experience in providing the highest quality husbandry care for belugas. With a strong focus on preventative medicine, our animals are well-monitored during periods of health and receive prompt attention to all medical or behavioral concerns. Husbandry staff are in place 24-7-265 to monitor animals and ensure their wellbeing. Mystic Aquarium has a fully functioning clinical lab and veterinary hospital equipped with portable ultrasound, endoscopy, and radiography for immediate diagnostic results when any condition arises.

Mystic Aquarium is complying fully with informational requests regarding the death of Kharabali.

Connecticut Veterinary Medical Diagnostic Laboratory



Department of Pathobiology and Veterinary Science

College of Agriculture and Natural Resources 61 North Eagleville Road Unit 3089 • Storrs, CT 06269-3089 phone: 860-486-3738 • fax: 860-486-3936 • web: www.CVMDL.uconn.edu

> Accession No: 23_1497 PRELIMINARY REPORT

MYSTIC AQUARIUM 55 COOGAN BLVD MYSTIC CT 06355 Date Collected: 12/11/2023 Date Submitted: 12/11/2023 Date Reported: 12/14/2023

	PH	: 860-572-5955	FAX	: 860	- 572-5972
Animal ID:	Species:	Breed:	Sex:	Age:	Wt:
Kharabali XDL10	Marine	Beluga	F	9Y	338 kg

FINAL DIAGNOSIS: PENDING

MORPHOLOGIC DIAGNOSIS:

BODY (VENTRAL, PROXIMAL REGION): subcutaneous edema, multifocal, moderate SKIN: abrasions, multifocal, moderate NOSTRIL: abrasion with yellow to tan crusting, focally extensive, moderate TRACHEA: mucosal patchy red discoloration, multifocal, moderate PERICARDIAL SAC: hydropericardium, moderate AORTA: fusiform aneurysm, focal, marked LUNG: patchy white discoloration, multifocal to coalescing, moderate LIVER: linear gray discoloration, multifocal, moderate STOMACH (SECOND COMPARTMENT): hyperemia, multifocal, moderate SMALL INTESTINE AND LARGE INTESTINE: dark green discoloration of the mucosa and ingesta, multifocal, severe

COMMENTS:

Gross examination revealed several lesions which require further microscopic examination for characterization. Tissues were submitted for bacteriology, which is pending.

PATHOLOGIC FINDINGS

HISTORY:

Kharabali is a 9.4-year-old female beluga that was born under professional care at Marineland in Canada. She was transported to Mystic Aquarium from Canada in May 2021, and has had no major medical issues since, until recently. Between March 2023 and May 2023, Kharabali exhibited some changes to the quality of her exhalation, including wetter or raspy sounds and changes to the lungs were noted on ultrasound. Throughout this time her behavior and appetite remained normal; she was treated with antimicrobial therapy. In September 2023, Kharabali began showing some abnormal swimming and reduced energy, which were progressive. A bronchoscopy was attempted; abnormal redundant tissue was noted around the glottis preventing entry with the endoscope. Over the past 6 weeks, Kharabali's swimming became progressively more abnormal, and she exhibited abnormal body position in the water. Due to the abnormal swimming, she sustained wounds on her pectoral flippers, flukes, and rostrum. While she ate normally through most of her illness, in the month prior to her death she demonstrated periodic crunching which was interpreted as lower gastrointestinal discomfort, thicker than normal feces, and she was identified to have Clostridium spp. overgrowth in her feces. She was given additional antimicrobial medications, probiotics, and fecal transfaunation to treat, but this became progressive, and she stopped eating on her own, resulting in weight loss. Aggressive fluid therapy and nutritional support were provided, but she continued to struggle with motility, limiting the ability to increase the amounts of support she could receive. In the days before her death, motility was extremely decreased preventing administration of additional food or fluids. The abnormal swimming and posture were also progressive, and she seemed periodically disoriented. Additional findings noted recently include poor cardiac contractility and dilated hepatic vessels and biliary tracts on ultrasound, and elevations in Total Bilirubin, GGT, and,

very recently, AST, on bloodwork.

GROSS FINDINGS:

A 9.4-year-old, female Beluga Whale (Delphinapterus leucas), weighing 338.4 kg was submitted for postmortem examination on 12/11/2023. Necropsy was performed on 12/12/2023. There were mild postmortem changes. Externally, the snout had diffuse dark red abrasion with a central area of yellow to tan crusting. Approximately 8 cm caudal to the right eye there was a 0.5 cm in diameter single abrasion with hemorrhage. Bilaterally, proximal surface of the flippers had linear abrasions with erosion and hemorrhage. Nutritional body condition was adequate. There was mild ventral subcutaneous edema proximally. The mucosal surface of trachea had multiple areas of red discoloration. The heart weight was 2.84kg; heart weight to body weight ratio was 0.84%. The aorta had focally marked 2x dilation (fusiform aneurysm). The pericardial sac had approximately 500ml of serous fluid (hydropericardium). The lungs were collapsed, with multiple areas of patchy red to pale discolorations which extended into the parenchyma. The thyroid weight was 340g, and grossly normal. The liver had multiple streaks of gray discoloration. The stomach contained liquid ingesta. The second compartment of the stomach had multiple areas of red discoloration(hyperemia). No evidence of the gastric ulcer was observed. The proximal half of the small intestine contained normal ingesta. The caudal half of the small intestine and the colon had abnormally dark green and thick ingesta; the mucosal surface in these regions were discolored dark green.

Snout to melon: 4cm Snout to angle of mouth: 18cm Snout to blowhole: 30cm Snout to center of eve: 28cm Snout to anterior insertion of dorsal fin: 140cm Snout to tip of dorsal fin: 173cm Snout to fluke notch: 322cm Snout to anterior insertion of flipper: 70cm Snout to caudal end of ventral groove: 45cm Snout to center of genital aperture: 235cm Snout to center of anus: 245cm Flipper length: 37cm Flipper width (maximum): 25cm Fluke width: 71cm Dorsal fin height: 20cm Girth, axillary: 100cm Girth, maximum: 105cm Girth at level of anus: 105cm Blubber thickness, dorsal: 5.0cm Blubber thickness, lateral at mid-length: 4.5cm Blubber thickness, ventral at mid-length: 3.5cm

HISTOPATHOLOGY: pending

A. Nakashima, DVM; Veterinary Pathology Resident N. Mishra, BVSc, MVSc, MS, PhD, DACVP; Veterinary Pathologist

PENDING TESTS:

Culture-Pathology Aerobic

Preliminary Report: 12/14/2023

-----END OF REPORT-----

MARINE MAMMAL DATA SHEET (MMDS)	
for Reporting Captive Births or a Death/Release/Escape	OMB No. 0648-0084
	Expires 11/30/2023 NOAA FORM 89-882
I. Reporting a Captive Birth:	
NOAA Identification No To be assigned by NIMM	
Sex \square Male \square Female \square Unknown	
Species Common Name	
Birth Date	
Birth Date Certainty Actual Estimated	
Owner (Person or other Entity With Custody [i.e., ownership])	
(Infisical location of the animal; if different from Owner)	
Animal Identification No.	
or	а.
Animal Name	
Department of Defense: choose one of the following:	ch and Enhancement
II. This Section is intentionally left blank	
III. Reporting a Disposition (Death or Release/Escape): Note: If you have a previously provided MMDS specific to your marine mammal, please u marine mammal. You may request a MMDS for your marine mammal by contacting us via NOAA Identification No. <u>NOAOOIO671</u> Animal Identification Number or Animal Name <u>XDL10 / kh</u> A. Death Date <u>12/11/2023</u> Cause of Death (Pending Wistopathology)	arabali
B. Release Date	
Permit No OR	release/esser
Geographic Location (as applicable) Lat:	/ Lon:
Tag number or description of other identifying markings:	

MARINE MAMMAL	DATA SHEET		
NOAA Animal ID: NOA00106			Form Approved by OMB No. 0648-0084
		(Generated on: 2021-05-17)	Expires 11-30-2023 Page: 1 of 1
I. Animal-Specific: NOAA Identification No. Common Name Birth Date Captivity Date Permit No. Geographic Location Review Date Reviewer Comments/Notes II. Owner/Facility-Spec Owner (Person or other Entity Facility (if different from Owner Custody Date	With Custody of the Marine Mamn	Sex Scientific Name Birth Date Certainty Captive Origin Collector Geographic Name Review Status	FEMALE Delphinapterus leucas ACTUAL BIRTH
Facility Date		05-14-2021 05-14-2021 Mystic, CT 06355 US XDL10 KHARABALI Scientific Research	
Tag number or description of ot	Permit No: at: : ' ' ' / Lon: : ' ' ' her identifying markings:	OR Unauthorized Geographic Name:	d release/escape (reintroduction)
IV. Animal Historical Tr			
Type of Activity: TR	arineland of Canada	Permit Number: 22629 To Facility: Mystic Aquarium Permit Number: 22629	Start Date: 05-14-2021
		To Owner: Mystic Aquarium	Custody Date: 05-14-2021

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Accession No: 23_1497 FINAL REPORT

Date Collected: 12/11/2023 MYSTIC AQUARIUM Date Submitted: 12/11/2023 55 COOGAN BLVD Date Reported: 1/30/2024 MYSTIC CT 06355 PH: 860-572-5955 FAX: 860 - 572-5972 Animal ID: Species: Breed: Sex: Age: Wt: Marine Kharabali XDL10 F 9Y 338 ka Beluga FINAL DIAGNOSIS: NEURONAL VACUOLATION AND SWELLING (INDICATIVE OF STORAGE DISEASE) SUBCUTANEOUS EDEMA MULTIPLE DERMAL ABRASIONS SUPERFICIAL ULCER WITH NECROSIS OF ROSTRUM LYMPHOPLASMACYTIC PERIGLANDULAR TRACHEITIS WITH MUCOSAL HEMORRHAGE HYDROPERICARDIUM AORTIC ANEURYSM WITH DISORGANIZATION OF ELASTIC FIBER PULMONARY FIBROSIS WITH BACKGROUND INTERSTITIAL PNEUMONIA ANDPULMONARY EDEMA HEPATOCELLULAR NECROSIS AND FIBROSIS WITH HEPATOCELLULAR VACUOLATION AND BILE DUCT HYPERPLASIA ACUTE TUBULAR DEGENERATION AND NECROSIS SUPERFICIAL GASTRITIS WITH BACTERIA GRANULOCYTIC COLITIS MORPHOLOGIC DIAGNOSIS: BRAIN: neuronal swelling and vacuolation, multifocal, marked with spheroids and intracytoplasmic PAS positive granules. BODY (VENTRAL THORACIC AREA): subcutaneous edema, multifocal, moderate SKIN: abrasions, multifocal, moderate NOSTRIL: superficial ulcer with necrosis along with suppurative dermatitis, focally extensive, moderate TRACHEA: tracheitis, lymphoplasmacytic, periglandular, diffuse, mild with mucosal hemorrhage PERICARDIAL SAC: hydropericardium, moderate AORTA: aneurysm, focal, severe with disorganization of elastic fiber LUNGS: pulmonary fibrosis with background interstitial pneumonia, multifocal, moderate with pulmonary edema LIVER: Hepatocellular necrosis and fibrosis, random, multifocal, moderate with hepatocellular vacuolation and bile duct hyperplasia, KIDNEYS: tubular degeneration and necrosis, acute, multifocal, mild STOMACH (SECOND COMPARTMENT): gastritis, superficial, with bacteria, multifocal, mild to moderate with autolysis SMALL INTESTINE AND LARGE INTESTINE: [1] colitis, granulocytic, multifocal, moderate

COMMENTS:

Thank you for the submission of this interesting case. There were multiple significant lesions in this beluga whale, primarily in the brain, lungs, aorta, liver, and gastrointestinal tract. In multiple sections of the cerebral cortex, there were gray matter areas with neuronal swelling and vacuolation that often-contained PAS and mucopolysaccharide positive granules; this presentation is consistent with a storage disease. This storage disease could serve as an explanation for abnormal swimming patterns and reduced energy described in the clinical history especially in the absence of an infectious agent and absence of ocular lesions. The storage disease can be genetic disorder which could be inherent, which can be correlated with the

<u>Owner:</u> Mystic Aquarium	Pg #2	<u>Accession No:</u> 23-1497
Hysele Advartan		

previous beluga whale case (Per submitter ID "Havana") diagnosed as storage disease in 2022. In other species affected with storage diseases the age of onset, manifestation of disease and speed of progression vary with type of storage disease i.e. enzyme activity. Electron microscopy, enzyme assays, special stains on frozen sections and or sequencing are required to characterize the type of storage disease in this Beluga whale. For these testing additional cost will be required as the samples need to be sent outside of CVMDL for testing.

Multiple skin abrasions were evaluated grossly and microscopically, which revealed there is evidence of tissue necrosis within the rostrum; however, no evidence of bacterial or fungal organisms was observed. The lungs had multifocal pulmonary fibrosis, indicative of background interstitial pneumonia which is consistent with the changes in the lung ultrasounds antemortem. Microscopic examination revealed hepatic necrosis and fibrosis. This is likely a chronic degenerative condition rather than acute. These findings could be related to the storage disease. There were other lesions within the gastrointestinal tract including the second gastric compartment, which could be associated with inappetence and most likely a precursor for gastric ulceration. Aortic aneurysm could be related to degenerative or genetic disease processes. Bacteriology results of lung revealed E. coli(moderate), Enterococcus faecalis (moderate) which is most likely secondary overgrowths as histology did not reveal any evidence of bacteria. Fecal culture yielded Pseudomonas aeruginosa(heavy), Enterococcus faecalis(heavy), Enterococcus faecalis(heavy), Bacillus species(heavy). Significance of fecal culture cannot be established as they are normal inhabitants which could also be implicated in gastrointestinal lesions noted.

LABORATORY FINDINGS BACTERIOLOGY RESULTS: Kharabali XDL10 Lung Culture-Pathology Aerobic -<u>E. coli(moderate), Enterococcus faecalis(moderate)</u>

Kharabali XDL10 Feces Culture-Pathology Aerobic - <u>Pseudomonas aeruginosa(heavy).</u> <u>Enterococcus faecalis(heavy), Enterococcus faecalis(heavy), Bacillus species(heavy)</u>

Robert Polkowski, BS Lead Technician Mastitis and Microbiology Technical Assistant Avian and Regulatory Serology 12/20/2023

PATHOLOGIC FINDINGS

HISTORY:

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<u>Owner:</u> Mystic Aquarium	Pg #:3	<u>Accession No:</u> 23-1497
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HISTOPATHOLOGY:

Histologic sections of the following tissues were evaluated on slides A-KK: BRAIN, EYES including LENS, TRACHEA, LARYNX, ESOPHAGUS, LUNG, HEART, LIVER, KIDNEY, SPLEEN, STOMACH (FIRST, SECOND, and THIRD COMPARTMENT), SMALL INTESTINE, LARGE INTESTINE, OVARY, MESENTERIC LYMPH NODE, DIAPHRAGM, SKELETAL MUSCLE, BLUBBER (DORSAL, LATERAL, and VENTRAL), and NOSTRIL.

SPECIAL STAINS: TWORT'S GRAM STAIN. SLIDE O. SECOND COMPARTMENT OF THE STOMACH: Few bacteria within the gastric mucosa are predominantly gram-negative short rods with few gram positive cocci. SLIDE V. LUNGS: No bacteria were identified with representative sections. SLIDE D. NOSTRIL: No bacteria were identified with representative sections.

GROCOTT METHENAMINE SILVER STAIN. SLIDE EE. NOSTRIL: No fungal hyphae or conidial spores were identified with representative sections.

MASSON'S TRICHROME STAIN. SLIDE K. AORTA: There is disorientation of elastic fiber, which is replaced by blue-staining collagen, consistent with aortic aneurysm.

SLIDE V. LUNGS: There are occasional variably broad bands of blue-staining fibers across multiple lobes, and increased amounts of blue-staining fibers expanding alveolar septa, consistent with pulmonary fibrosis.

SLIDE AA. LIVER: Within centrilobular and periportal regions and between regions of degenerating and necrotic hepatocytes are robust, anastomosing bands of blue-staining fibers, consistent with hepatic fibrosis.

VERHOEFF VAN GIESON STAIN SLIDE K. AORTA: There is evidence of elastin fiber disarray, which is consistent with aortic aneurysm.

PRUSSIAN BLUE HISTOCHEMICAL REACTION FOR IRON.

SLIDE V. LUNGS: Macrophages within the lumen of alveoli, bronchioles, and bronchi do not have blue-staining granules within their cytoplasm. (negative for hemosiderin) SLIDE AA. LIVER: Brown pigments within the liver have blue-staining granules, consistent with hemosiderin.

HALL'S BILIRUBIN STAIN. SLIDE AA. LIVER: Brown granules within the hepatic cytoplasm stained negatively. LUXOL FAST BLUE STAIN.

SLIDES C, D, E. BRAIN: Axons were not demyelinated in representative sections.

PERIODIC ACID SCHIFF STAIN. SLIDE C, D, E, HH, II, JJ, KK. BRAIN: Neurons have PAS positive granules within the cytoplasm.

ALCIAN BLUE MUCOPOLYSACCHARIDE STAIN. SLIDE C, D, E, HH, II, JJ, KK. BRAIN: Neurons have mucopolysaccharides positive granules within the cytoplasm, consistent with storage disease.

A. Nakashima, DVM; Veterinary Pathology Resident N. Mishra, BVSc, MVSc, MS, PhD, DACVP; Veterinary Pathologist

Preliminary Report: 12/14/2023 Final Report: 1/30/2024

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MARINE MAMMAL DATA SHEET (MMDS)	OMB No. 0648-0084		
for Reporting Captive Births or a Death/Release/Escape	Expires 11/30/2023 NOAA FORM 89-882		
I. Reporting a Captive Birth: NOAA Identification No To be assigned by NIMM Sex Male Female Unknown Species Common Name Birth Date Birth Date Certainty Actual Estimated Owner (Person or other Entity With Custody [i.e., ownership]) Facility (Physical location of the animal; if different from Owner) Animal Identification No. Animal Name			
Purpose Dublic Display Discientific Research Defenses are of the following: Description Defenses are of the following: Defense are of the foll			
□ Department of Defense; choose one of the following: □ Active □ Retired □ Permit No(as applicable)	Breeding Loan		
II. This Section is intentionally left blank			
III. Reporting a Disposition (Death or Release/Escape): Note: If you have a previously provided MMDS specific to your marine mammal, please use that MMDS to report the disposition of your marine mammal. You may request a MMDS for your marine mammal by contacting us via any of the contact options provided on page 2. NOAA Identification No. NOAOOIO67/ Animal Identification Number or Animal Name XDLIO / Kharabali A. Death Date 12/11/2023 Cause of Death S-forage disease			
U			
B. Release Date	zad valanza/azanza		
Permit No OR Unauthoriz Geographic Location (as applicable) L	zed release/escape at: / Lon:		
Tag number or description of other identifying markings:			