



Humpback Whale #441 (Festus)

Life, Death, Necropsy, and Research Findings

Natural Resource Report NPS/GLBA/NRR—2021/2250





ON THIS PAGE

Flukes (2013) and left flank (2006) of humpback whale #441 (Festus). On June 27, 2016, the whale was found floating dead, and towed to shore for examination.

NPS photos taken under authority of scientific research permits issued by NOAA Fisheries.

ON THE COVER

A team of NPS staff, students, and local volunteers assist veterinary pathologist Stephen Raverty in the necropsy of humpback Festus on June 28, 2016.

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April 2021

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

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Please cite this publication as:

Gabriele, C. M., L. F. Taylor, K. B. Huntington, C. L. Buck, K. E. Hunt, K. A. Lefebvre, C. Lockyer, C. Lowe, J. R. Moran, A. Murphy, M. C. Rogers., S. J. Trumble, and S. Raverty. 2021. Humpback whale #441 (Festus): Life, death, necropsy, and research findings. Natural Resource Report NPS/GLBA/NRR—2021/2250. National Park Service, Fort Collins, Colorado.
<https://doi.org/10.36967/nrr-2285345>.

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Executive Summary

In late June 2016, a mature male humpback whale was found dead in Glacier Bay National Park waters just outside the mouth of Glacier Bay, Alaska, coincident with an elevated rate of whale deaths attributed to the “Blob” marine heatwave that dominated the Northeast Pacific Ocean from late 2013–2016. This whale, Southeast Alaska catalog #441 (nicknamed Festus) had been documented over a period of 45 years in Alaska and Hawaii. Sightings of the whale over many years documented a large swelling on the whale’s left flank, likely as a result of having been satellite-tagged in 1999. At the necropsy, the whale had no visible external injuries but was emaciated and his skin was in poor condition and heavily infested with cyamids (whale lice). The whale’s internal organs were moderately decomposed; thus, only limited sampling was possible. Histological analysis of tissue samples revealed a variety of health conditions that we describe in this report and its Appendices. Counts of annual growth layers in the ear plug collected at the necropsy indicate that the whale was about 66–68 years old, indicating that he was born around 1950.

Poor body condition and low oil content in the blubber suggest nutritional stress as the primary proximate cause of death. Multiple infections were detected in tissue samples: *Toxoplasma gondii* and *Sarcocystis spp.* in the heart, and bacteria, yeast and diatom infections in the lymph nodes. The detection of both domoic acid and saxitoxin in the feces confirms that harmful algal bloom toxins (HABs) were present in the environment, but without clinical signs of neurotoxicity, toxin values alone are not sufficient to determine if toxin exposures would cause health impacts.

A five-year stable-isotope (SI) profile in the baleen showing a continued rise of ^{15}N in the final year of life suggests extreme nutritional stress that is consistent with results from the baleen of other humpback whales that died during the marine heatwave (Rogers et al. in review). A five-year endocrine profile from the whale’s baleen shows a long period of elevated levels of the adrenal stress hormones cortisol and corticosterone, and low levels of testosterone until a large spike prior to death, all of which are consistent with poor health.

Analysis of the whale’s ear plug provided lifetime profiles including bulk C and N stable isotopes as well as testosterone and cortisol. The lifetime SI profile revealed relatively stable periods prior to 1970 and from 1980 until death. Variability in bulk C and N signatures could indicate migratory movements and/or trophic shifts whereas the decline in bulk signatures between 1970 and 1980 may represent behavioral (movements), trophic (prey), or anthropogenic inputs (fossil fuel carbon in the ocean). The ear plug lifetime hormone reconstruction revealed typical male mammalian peaks early in life with maximum values at approximately 12 years (i.e. probable puberty). An increase in stress response (cortisol concentrations) in 1999 may be associated with possible negative response to satellite tag attachment, validated with sighting data.

Months after the necropsy, thorough examination of the whale’s bones on the beach revealed two unusual bone pathologies; 1) severe infective spondylo-osteomyelitis and ruptured facets in nine caudal vertebrae and 2) severe ankylosing spondylitis in three thoracic vertebrae. Given the osteological response near the tag site, there is a high probability that microbes introduced during or

after the tagging event resulted in the localized bone infection. Moreover, these bone pathologies likely limited the whale's mobility in ways that contributed to the whale's degenerating health.

This collaborative work represents a remarkably thorough workup of a well-documented individual whale. Validating physiological profiles with life history and pathology results provided powerful insights on the life and death of this individual whale and suggests two significant recommendations. First, the baleen and earplug profiles provided such valuable physiological context for the whale's death that we recommend stable isotope and endocrine profiles, from multiple sample types where possible, as standard samples and analyses in future whale strandings. Second, the bone pathology findings would have been missed in a typical necropsy and thus demonstrate the value of repeated visits to beached whale skeletons. Applying these techniques on a broader scale could greatly advance our knowledge of these remarkable mammals.

Acknowledgments

This report gathers information from a multitude of sources and represents the contribution of many people, including NPS staff, volunteers, NMFS collaborators, and subject matter experts.

A whale carcass is a treasure trove of information and even moreso when the whale was a well-documented individual, so we owe our gratitude to Paul Johnson of Chichagof Charters for promptly reporting the whale carcass that he encountered on his way into Glacier Bay. Aleria Jensen (NMFS) contacted Dr. Stephen Raverty and granted approval for towing and necropsy of the carcass. Dr. Kate Savage of the National Marine Fisheries Service (NMFS) provided guidance and equipment for the necropsy. Sonia Kumar of Alaska Veterinary Pathology Services assisted with histopathology and shipping of samples to diagnostic laboratories. We thank Maryjean Willis and Emily Bowers (NOAA/Wildlife Algal-Toxin Research and Response Network [WARRN-West]) for help processing samples for HAB toxin analyses. Thanks to Anibal Guillermo and Armien Medianero at University of California Davis for poxvirus electron microscopy interpretation. Thanks to Dan DenDanto for assistance with skeletal anatomy. Jim Lesh, Todd Bruno, and Miles Stipek (NPS staff) assisted with towing the whale and securing it onshore. Craig Murdoch, Javan Bailey, and Elisa Weiss performed pre-necropsy reconnaissance of the carcass and site. Janet Neilson, Tania Lewis, Paul Netter, Emily Kirby, Todd Bruno (NPS staff) helped with the necropsy. Students and volunteers Luke Williams, Michele Fournet, Jenny Helm, Anya Maier, Jen Landry, Linnea Lentfer, Christina Senac helped with the necropsy. Sean Mattson (NPS staff) assisted with sectioning bone samples. Janet and Sean Neilson made a heroic effort to collect the baleen for the hormone timeline part of the study. Thanks to Andrew Peacock for the use of the cover photo. Thanks to Scott Baker, Charles Jurasz, Daniel Palacios, Bruce Mate, Dena Matkin, Joe Mobley, Adam Pack, and Jan Straley for sharing sighting data to tell the story of this whale. Peer-review comments from Dr. Frances Gulland and Dr. Kate Savage improved the final product.

Glossary

Ankylosis: Abnormal adhesion of the bones of a joint; Anatomy: the union or consolidation of two or more bones or other hard tissues into one.

Bifid: Bone that develops from two separate centers of ossification. Typical of cervical vertebrae.

Cupped disc: Hollowed like a cup or cup-shaped.

Edema: Swelling caused by excess fluid build-up in tissues.

Epiphysis: The articulating segment of a bone, usually at the proximal and distal poles of the bone.

Exostosis: A spur or bony outgrowth from a bone.

Facet: A smooth, flat surface that forms a joint with another flat bone or another facet, together forming a gliding joint.

HAB: Harmful algal blooms (HAB) occur when colonies of algae rapidly produce toxic or harmful effects in humans, marine mammals, fish, shellfish and/or birds

Herniation: Protrusion of the intervertebral disc from its normal position.

Lesion: A region in an organ or tissue which has suffered damage through injury or disease process.

Lipping: Outgrowth of bone in liplike form at a joint margin.

Osteophyte: Smooth bony growth or deposit that grows slowly over time, associated with the degeneration of cartilage at joints.

Osteolytic lesion: A weakened, "punched-out" area of severe bone loss.

Proliferation: Rapid and repeated production of new parts or of offspring (as in a mass of cells by a rapid succession of cell divisions); a growth so formed; the action, process, or result of increasing by proliferation.

Rugosity: A measure of small-scale variations in the height of a surface; i.e.: roughness.

Spondylitis: Inflammation of one or more of the vertebrae of the spine.

Syndesmophyte: An osseous growth originating in ligaments in intervertebral joints; bridging across intervertebral joints in ankylosing spondylitis.

Stroma: The supportive tissue of an epithelial organ, tumor, gonad, etc., consisting of connective tissues and blood vessels.

Ulceration: A circumscribed inflammatory and often pus-forming lesion on the skin.

Background

In late June 2016, a mature male humpback whale was found dead in Glacier Bay National Park (GBNP) waters just outside the mouth of Glacier Bay, Alaska. This individual, Southeast Alaska catalog #441, also nicknamed Festus, was a well-documented whale of at least 45 years of age based on the length of its sighting history. A necropsy revealed a variety of health conditions. We assembled this report to capture in one place all the data and results related to the life, death and necropsy of this whale and the subsequent bone inventories and laboratory diagnostic test results. The initial objective of the necropsy of this whale was to determine cause of death. Second, because this whale was well known as a long-term resident of GBNP, we hoped to discover his age to provide context to his sighting history. Lastly, by examining his general health, including bacterial, fungal, viral, and parasitic infections, harmful algal bloom (HAB) toxins, and endocrine history, we hoped to gain a deeper understanding of the environmental factors affecting this humpback whale and potentially overall population health.

This whale's death in 2016 coincides with an elevated rate of whale strandings attributed to widespread marine ecosystem disruption from "The Blob" marine heatwave that dominated the Northeast Pacific Ocean from late 2013–2016 (DiLorenzo and Mantua 2019). Ecological impacts throughout the marine food web were documented during the Northeast Pacific marine heatwave (Piatt et al. 2020; Arimitsu et al. 2021; Suryan et al. 2021). NOAA Fisheries declared a large whale "unusual mortality event" (UME) affecting fin and humpback whales in the Gulf of Alaska in 2015–2016 (<https://www.fisheries.noaa.gov/national/marine-life-distress/2015–2016-large-whale-unusual-mortality-event-western-gulf-alaska>). While an average of 13 dead humpback whales per year were documented in the Gulf of Alaska in 2010–2014, during the UME there were much higher numbers: 31 dead humpbacks in 2015, and 19 in 2016. Thirteen of the humpback whale carcasses were examined and sampled (eight in Canada and five in Alaska). Of the 8 humpback whale samples tested for domoic acid and saxitoxin (HAB toxins), four were positive for domoic acid and four were positive for saxitoxin in Alaska, while samples from the British Columbia (BC) whales were positive for HAB toxins in seven of eight whales. In two of the BC whales, neurotoxin levels were in the range of those found in West Coast pinnipeds exhibiting clinical signs of toxicity (NOAA <https://repository.library.noaa.gov/view/noaa/17715>). However, the impact of these levels of HAB toxins on cetacean health is not yet understood. The cause of the UME was not determined, although ecological factors were a contributory cause (i.e., the 2015 El Niño, Warm Water Blob and Pacific Coast Domoic Acid Bloom).

In Southeastern Alaska, the death of Whale #441 occurred during the dramatic humpback whale population decline in Glacier Bay and Icy Strait that began in 2014, documented in the National Park Service (NPS) long term population monitoring program (Neilson et al. 2017). In 2016, observations from this monitoring program revealed poor body condition in 13% of individually identified humpback whales documented in the study area (Neilson et al. 2017). The necropsy of Whale #441 was exceptional because of the extensively documented life history of the whale made possible by monitoring efforts (Gabriele et al. 2017). It is unusual to have this level of detail on the factors contributing to the death of a free-ranging whale because when large cetaceans die, only a small

fraction of carcasses are detected floating or beach cast and few of these animals are available for necropsy (Faerber and Baird 2010, Williams et al. 2011).

In September 2016, NOAA Fisheries removed humpback whales in much of the North Pacific from the list of U.S. Endangered Species, after these populations were judged to have recovered sufficiently from commercial whaling. Based on his sighting history, Whale #441 was a member of the Hawaii Distinct Population Segment (DPS) that was removed from Endangered status in 2016 (NMFS 2016). Although the Hawaii DPS is no longer considered endangered, it remains a population at risk and post-delisting monitoring is a mandate of the Endangered Species Act. Therefore, information related to the health or cause of death of individuals within this population is important for informing conservation and evaluating whether the population continues to recover after the protections of the Endangered Species Act are removed.

Life History

Sighting History

Whale #441 (a.k.a. Festus) was first sighted in 1972 in the Glacier Bay area, according to records of the work of Charles Jurasz. Jurasz, who pioneered the use of fluke photography for individual identification (Jurasz and Palmer 1981), nick-named the whale Festus because it “festooned itself with kelp” in Sitakaday Narrows (C. Jurasz pers. comm. to C. Gabriele in August 2016). Whale #441 was sighted for many consecutive years in a variety of Southeast Alaska locations (Table 1), primarily Glacier Bay and Icy Strait, but also Frederick Sound, Seymour Canal, Lynn Canal, and Sitka Sound, typical of the seasonal movement patterns that humpback whales make within Southeast Alaska to exploit various prey resources (Straley 1994). Whale #441 was sighted off Maui in 1984, 1987, and 1999, and off Kauai during the North Pacific-wide SPLASH study in 2004 (Table 1). His ID number in the SPLASH catalog is # 430675. His 45-year sighting history is the longest duration of any humpback whale (Neilson et al. 2017).

Table 1. Sighting history of Whale #441, Festus.

Year	# Sightings	Sighting Location(s)	Source
1972	unknown	Glacier Bay	Charles Jurasz census data pers. comm. (Gabriele et al. 2017)
1976	8	Glacier Bay	Vequist and Baker 1987, Jurasz census data
1977	4	Glacier Bay	Vequist and Baker 1987, Jurasz census data
1981	1	Glacier Bay	Vequist and Baker 1987
1984	2	Maui	Kewalo Basin Marine Mammal Laboratory (Herman)
1986	4	Glacier Bay	GBNP Humpback Whale Monitoring Program
1987	2	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
1987	1	Maui	Kewalo Basin Marine Mammal Laboratory (Herman)
1989	2	Glacier Bay	GBNP Humpback Whale Monitoring Program
1991	3	Icy Strait	GBNP Humpback Whale Monitoring Program
1994	1	Icy Strait	GBNP Humpback Whale Monitoring Program
1995	3	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
1996	3	Icy Strait	GBNP Humpback Whale Monitoring Program, Dena Matkin
1999	1	Maui	Oregon State University (Mate)
2000	4	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
2001	2	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
2002	2	Icy Strait, Frederick Sound	GBNP Humpback Whale Monitoring Program, University of Alaska Southeast (Straley)
2003	7	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
2004	1	Kauai	SPLASH sighting by Marine Mammal Research Consultants (J. Mobley)

Table 1 (continued). Sighting history of Whale #441, Festus.

Year	# Sightings	Sighting Location(s)	Source
2004	3	Glacier Bay, Seymour Canal	GBNP Humpback Whale Monitoring Program, University of Alaska Southeast (Straley)
2005	1	Lynn Canal	University of Alaska Southeast (Straley)
2006	1	Glacier Bay	GBNP Humpback Whale Monitoring Program
2007	1	Lynn Canal	National Marine Fisheries Service (Moran)
2008	2	Sitka Sound, Lynn Canal	University of Alaska Southeast (Straley), National Marine Fisheries Service (Moran)
2010	2	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
2012	2	Glacier Bay, Icy Strait	GBNP Humpback Whale Monitoring Program
2013	1	Glacier Bay	GBNP Humpback Whale Monitoring Program
2014	1	Icy Strait	GBNP Humpback Whale Monitoring Program
2015	1	Glacier Bay	GBNP Humpback Whale Monitoring Program
2016	found dead	Icy Strait	Found floating dead, Paul Johnson of Chichagof Charters

Whale #441 was presumed to be male based on the lack of sightings with a calf, and sightings in Hawaii as an escort to a mother / calf pair, a behavior that is characteristic of males. Sloughed-skin samples (a common source of DNA for sex identification) had not been obtained by the Glacier Bay team during the whale's life. The whale was biopsy darted in 1999 off Maui by Oregon State University, and again in 2004 off Kauai when genetic analysis determined him to be male (SPLASH sample # 41072, MMRC-067-04). Anatomical confirmation of the whale's sex was made at the necropsy.

Behavior and Physical Condition

Whale #441 showed strong site fidelity to Icy Strait and lower Glacier Bay in the summer months. He tended not to show his flukes on all dives, but his distinctive dorsal fin allowed him to be identified on occasions when a fluke photograph was not obtained.

Natural history observations of Whale #441 indicate that he was of typical adult size. One aspect of the whale's physical appearance changed in 2000 when he was noted to have a large dorsolateral subcutaneous swelling on his left side at the level of the cranial dorsal fin insertion (Fig. 1). It was not until 2013 when a Society for Marine Mammalogy workshop was convened by John Calambokidis, Russ Andrews, and Alex Zerbini on the long-term health impacts of cetacean tagging that we learned that the whale had been satellite tagged off Maui in 1999. Whale #441 was not sighted in the Glacier Bay study area in 1999, but the swelling on his left side was notable in 2000 and was consistent in size and anatomic location in all subsequent years of the whale's life. Photos and field notes provided by the tagging team (B. Mate, pers. comm. 2013) indicate that the tag type used was intended to pierce the skin and blubber layers, however the researchers noted that the tag went too deep and they saw blood during the deployment. The whale was also biopsy sampled during the tagging event. The tag did not function. This tag was deployed using a compound crossbow, a

method that was phased out in favor of a pneumatic applicator in 2002 for a more controlled implant (D. Palacios, pers. comm. 2020). In addition, these early implantable tags did not have the delrin ears that have been in use in later tags to prevent tags from implanting too deeply (D. Palacios, pers. comm. 2020). Field notes of the tagging event indicate that about 1.5 feet forward of the tag implant site, observers noted a 2" diameter, $\frac{3}{4}$ " tall nodular swelling on the whale's left side (B. Mate, pers. comm. 2013) which is visible in all Fig. 1 photos. This smaller more cranial nodule was considered incidental to the osteomyelitis and regional lymphadenopathy presumably associated with the tag site (see Gendron et al. 2015).

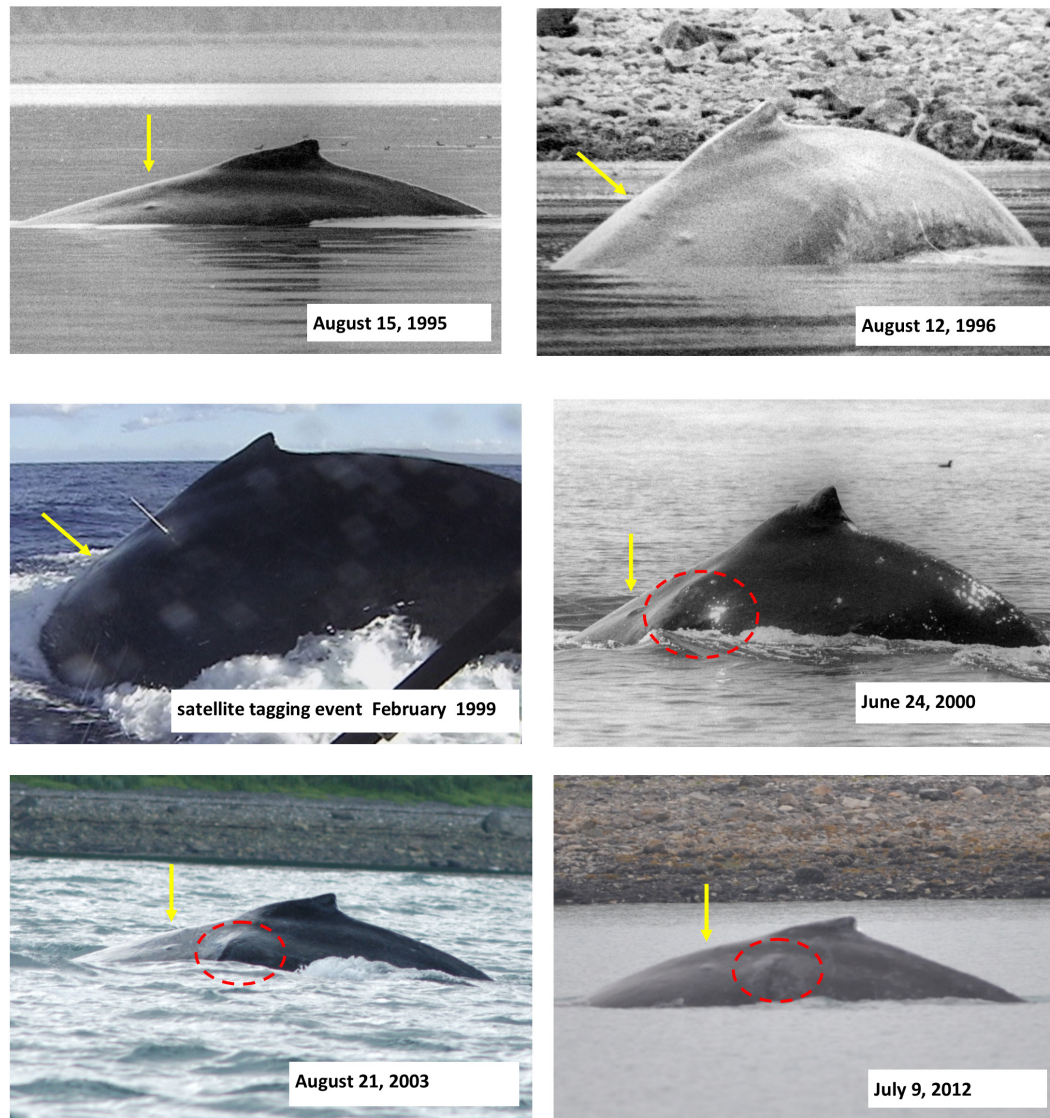


Figure 1. Photographs of the left flank of Whale #441 between 1995 and 2012, including a 1999 satellite tagging event (photo courtesy of Bruce Mate). From 2000 onward, the whale had a persistent subcutaneous swelling near the tag site, indicated here with a red dotted circle (GBNP photos). The yellow arrows here point to the tall nodule observed during the tagging event.

Death and Necropsy

A dead humpback whale was reported to the National Park Service radio dispatcher by Paul Johnson of Chichagof Charters at 10:22 am, Sunday, June 26, 2016, offshore of Point Carolus in the waters of Glacier Bay National Park. The law enforcement vessel *Arete* was dispatched by Glacier Bay National Park to locate and stand by the floating carcass. At that time, the location of the carcass was 58.301378N, -136.239008W and drifting west towards Dundas Bay. The whale was floating on its right side (left pectoral fin up). Park staff notified National Marine Fisheries Service (NMFS) and obtained permission to tow the carcass. NMFS assisted with identifying a qualified veterinarian available to travel to the site immediately.

In the strong currents of North Passage, two park vessels were needed to tow the whale carcass, the law enforcement vessel *Arete* and the research vessel *Fog Lark* using a single tow line secured around the peduncle. A third boat, whale research vessel *Sand Lance*, assisted from behind, attempting to push the carcass with its bow. While being towed, the whale was floating with its left side up, and the Sand Lance had prolonged contact with the whale's rostrum on its left side, forward of the eye. The carcass was towed approximately one mile to a sandy beach in North Passage between Pt Carolus and Pt Dundas. Red fluid was observed trailing from the carcass during towing and was attributed to serous fluid draining from the mouth or blubber where the skin was incised by the tow rope. On arrival at the beach, the carcass was secured to a large drift-tree by a rope around the peduncle, approximately one half hour after high tide.

At this time, a skin sample was obtained for genetics. Photographs of the left fluke tip led to identification of Whale #441. This whale was well known and had been photo-documented in park waters over three decades. An attempt was made to remove the left eye for age determination by aspartic acid racemization of the eye lens, but the carcass was submerged in deep water and it was not possible. Sex was confirmed (visually) as male and length estimated as 40 ft (12.2 m).

A reconnaissance team was dispatched on Monday, June 27, 2016 to confirm the location of the whale, re-check that it was fastened in place, look for signs of scavenging, take measurements, and collect an eyeball. They observed eagles scavenging on the fluke and pectoral flippers but no sign of bear activity. The beach was shallow sloped and fairly accessible to both researchers and the boating public.

Necropsy

A necropsy of the whale was performed on Tuesday, June 28, 2016 led by veterinary pathologist Dr. Stephen Raverty. The necropsy team consisted of 11 NPS staff and collaborators plus four additional volunteers who stopped by to watch and ended up providing valuable help. At the time of the necropsy, the whale was positioned on its back with its rostrum oriented towards the water and leaning slightly on its right side. The necropsy was conducted between low and high tide cycles and took five and a half hours. The necropsy had four primary objectives: investigate cause of death, obtain fecal samples for HAB toxin analyses, extract an ear plug for age determination and lifetime chemical analysis, and attempt to inspect an unusual lump on the whale's left flank and look for satellite tag fragments.

Under the leadership of Dr. Raverty, the necropsy team worked in several small teams to find and remove the ear wax plug, record morphometrics, and flense the blubber away to expose the internal organs. The blubber thickness was recorded and parasites, skin-tags, and other external features were documented. Other members of the team were dedicated to specific tasks including photography, recording notes, labeling and storing samples, sharpening tools, monitoring the perimeter for bears, and managing human onlookers.

At the conclusion of the necropsy, the carcass was left on the beach un-secured. Samples (preserved in formalin or frozen) were transferred to NMFS personnel on June 29, 2016 accompanied by a chain of custody document. Please refer to the Necropsy Report in Appendix A for additional details.

Gross Findings from Necropsy

Dr. Raverty's complete necropsy report is found in **Appendix A**, but a summary of the more salient gross findings is provided here. The 13.9 meter whale was judged to be in fair nutritional condition although the position of the carcass on the beach made it difficult to assess the overall status.

However, on incision of the blubber, the stroma was prominent and a lack of lipid in the blubber was consistent with an animal in poor condition. There was an unusually large number of whale lice on the skin, along with scattered superficial erosions and ulcers suggestive of a compromised healing response. There were feces in the colon and turbid yellow brown urine in the bladder. The left prescapular lymph nodes were swollen and had abscessation consistent with a localized bacterial infection.

A prominent, transverse depression of the skin and soft tissue was noted about 1 m caudal to the tip of the rostrum and spanning the width of the jaw and may correspond with where the *Sand Lance* pushed on the carcass while it was being towed to the beach. On the left dorsolateral thorax (i.e. the area that we thought corresponded with the past tag implant site), focally extensive subcutaneous and muscular hemorrhage and edema were noted. These changes are more consistent with an acute event, rather than a chronic injury. Although close dissection of the affected area did not reveal any chronic inflammation, scar tissue or retained tag pedicles or detached and retained barbs, it is possible that the position of the animal or hemorrhage and edema may have obscured a more chronic process related to long past tagging (Appendix A). Efforts to identify tags with metal detectors proved unsuccessful.

Limitations of Necropsy

The condition of the carcass and the fact that it was lying on its back hampered gross examination of this animal. The response team was mobilized in less than 48 hours but by the time of the necropsy there was post-mortem decomposition, so some internal tissues and fluids were autolyzed.

One goal of this necropsy was to explore the anatomy surrounding a persistent bulge observed on the left flank of this whale for 16 years (Fig. 1). Unfortunately, the area examined during the necropsy did not coincide with the location of the bulge. Various factors led to this outcome, with the root cause being confusion stemming from the whale's posture (upside down and twisted).

The only bone examined during the necropsy was the posterior of the skull which happened coincidental to extracting the waxy ear plug. No signs of skull fractures or other broken bones were discovered at that time. A lesson learned in this case is that if time allows, additional flensing of the carcass after all priority samples have been taken may have revealed the caudal vertebrae abnormalities that were discovered later on.

Pathology

In an effort to determine factors potentially linked with the whale's death, numerous samples collected during the necropsy were frozen or preserved in formalin and later analyzed by Alaska Veterinary Pathology Services (Eagle River, Alaska). The full pathology report can be found in **Appendix B**, but here we briefly summarize the main findings. Histopathological examination of these tissues supported the gross diagnosis of emaciation with atrophy of fat, dermatitis, inflammation of the lymph node due to bacteria and yeast, chronic osteomyelitis (inflammation of the bone), and subcutaneous hemorrhage consistent with trauma. Additional samples were further analyzed for bacteria, virus, and fungus as directed by histopathologic findings.

Emaciation

Blubber depth measurements and observations made during necropsy (see Gross Findings, above) indicated poor body condition (Appendix B, page 20). Histological examination of fat cells in blubber tissue revealed they were markedly atrophic with little lipid. This whale was in poor nutritional condition that may indicate starvation but could also be attributed to chronic disease or other pathologic entities.

Dermatitis

Moderate erosive and ulcerative dermatitis was observed associated with environmental bacteria, with subcutaneous nodules of inflammatory infiltrate and some proliferative lesions. One proliferative lesion had intracellular inclusions consistent with poxvirus, but electron microscopy revealed no viral replication in the epidermal keratin cells (A. Medianero, pers. comm.). However, similar changes have been associated with cytoplasmic condensation of cytoskeletal elements associated with advanced autolysis. The heavy parasite load of cyamids (whale lice) is consistent with debilitation (e.g., reduced swimming speed).

Trauma

A large area on the left dorsolateral thorax contained hemorrhage in the subcutaneous tissue and blubber suggestive of blunt force trauma. No manipulations of the thorax were noted during towing and beaching of the carcass, but there was notable blood in the water as the whale was being towed to shore. Histological examination of tissue from this site revealed small, focal areas of acute hemorrhage. Examination of the skeleton conducted months after the necropsy did not reveal any fractured bones. It is possible this area of hemorrhage was incidental to the mortality and occurred immediately before death. Based on the bone evaluation and lack of a discrete "impact site" at the time of necropsy (at least for the exposed portion of the carcass), it is unlikely that ship-strike was involved with the loss of this animal; however, in light of the observed hemorrhage, the possibility of ship-strike cannot be entirely discounted.

Lymphadenitis

The left prescapular lymph node was swollen and pale green. Histopathology of this lymph node revealed inflammation with multifocal abscessation and abundant bacteria and yeasts. Frozen samples sent to the University of Illinois Veterinary Diagnostic Laboratory for consensus polymerase chain reaction (PCR) identified *Malessezia restricta* and *Navicula vara* (Appendix B). *N. vara* is a

diatom and has not previously been associated with pathology in cetaceans and *M. restricta* is a common yeast on human skin and has been associated with dermatitis and dandruff in humans (Morand et al. 2019, Byrd et al. 2018, Gaitanis et al. 2012) and rare systemic disease in immunocompromised individuals. Samples submitted to Athens Veterinary Diagnostic Laboratory (AVDL) yielded bacteria *Granulicatella balaenoptera* and *Edwardsiella tarda*. *G. balaenoptera* was originally reported in a minke whale stranded in Scotland and has been linked to human cases of vertebral osteomyelitis, valvular endocarditis, septicemia and other systemic issues (Christensen et al. 2001, Collins et al. 2000, Rosenthal et al. 2002). This isolate may be the first reported isolate from a cetacean stranded in the Pacific Ocean. Conversely, *E. tarda* is a common environmental and intestinal commensal of cetaceans and infections have been associated with localized abscessation as well as septicemia. The lymph node tested negative for *Brucella* and morbillivirus by polymerase chain reaction (PCR) at the Athens Veterinary Diagnostic Laboratory (AVDL), University of Georgia, GA, and the Animal Health Center (AHC), Abbotsford, BC, respectively.

Due to the anatomic location of the prescapular lymph nodes, inflammatory infiltrate and infection may be attributed to the subcutaneous nodule along the flank, the vertebral osteomyelitis or other localized source of inflammation. Unfortunately, bone samples were not obtained until months after the postmortem, when autolysis and desiccation significantly diminished the ability to identify infectious agents. PCR analysis of tissue samples near the infected bone collected September 19, 2016 (three months after the necropsy) tested negative for *Brucella* sp. at AVDL and fungal PCR was deemed non diagnostic due to postmortem overgrowth at University of Illinois (Appendix B).

Internal Parasites

Heart tissue collected from this animal tested positive by PCR for *Toxoplasma gondii* and a novel *Sarcocystis* protozoan, *Sarcocystis monodontidis* (Appendix B). Two of seven humpback whales tested in Alaska exhibited this co-infection (Burek Huntington Feb 2019, pers comm). Unfortunately, the heart was not collected for histopathology, so the significance of this finding is unknown. These protozoal agents are typically cystic in skeletal and heart muscle or may become clinically significant when fast-growing, invasive tachyzoites cause inflammation and necrosis.

Harmful Algal Bloom Toxins

Urine and fecal samples collected at the necropsy were tested for the presence of domoic acid (DA) and saxitoxin (STX) at NOAA's Wildlife Algal-toxin Research and Response Network (WARRN-west) laboratory in Seattle, Washington, with the results shown in Table 2. Both HAB toxins were detected in the fecal sample, but only DA was detected in the urine. The fecal STX concentration of 100.18 ng/g was higher than the maximum concentration of 62 ng/g reported for Alaska humpback whales in a large study of HAB toxins in stranded and subsistence harvested Alaskan marine mammals (Lefebvre et al. 2016). The fecal DA concentration of 356 ng/g was 7 times higher than the maximum reported level in the previous survey described above (51 ng/g, Lefebvre et al. 2016), but is still not considered an exceptionally high concentration. How these toxins may have affected the behavior and mortality of this animal is unknown as subsistence-killed bowheads that appeared healthy at harvest, had similar and up to 10 times higher concentrations of DA (WARRN-West unpublished data), while subsistence caught walruses also had much higher levels of STX (Lefebvre

et al. 2016). Although the concentrations of DA detected in the humpback feces and urine are within the range of those quantified in California sea lions exhibiting clinical signs of toxicity (Lefebvre et al. 2016), those values are not sufficient for diagnosing DA toxicosis in dead animals because they are not an accurate indicator of total toxin dose consumed by the animal. As such, multiple factors are used for diagnosing DA toxicosis in sea lions including clinical signs of neurotoxicity (i.e. ataxia, head weaving, seizures) in addition to toxin presence. Therefore, in the absence of clinical signs of neurotoxicity and toxin levels below seafood safety regulatory limits, the interpretation of health impacts due to HAB toxin exposure is challenging.

Table 2. Levels of domoic acid (DA) and saxitoxin (STX) in fecal samples from Whale #441 quantified by Enzyme-Linked Immunosorbent Assays (ELISA).

Test Type	Sample Tested	Agents	Positive or Negative	Measured Result
Biosense ELISA for domoic acid	Feces	domoic acid	POS	356 ng/g
	Urine	domoic acid	POS	2.7 ng/g
Abraxis ELISA for saxitoxin	Feces	saxitoxin	POS	100.18 ng/g
	Urine	saxitoxin	NEG	(BDL) Below detectable limit

Ear Plug and Age

Given the long sighting history of this whale, we made a concerted effort to collect an ear plug at the necropsy to use for age estimation (see Gabriele et al. 2010 for background). Chris Gabriele and volunteer Anya Maier, M.D., focused on removing the ear plug and accomplished this based on prior experience and hands-on orientation using the whale skull on the Snow Exhibit at Bartlett Cove prior to the necropsy (Fig. 2). The ear plug was preserved intact in 10% neutral buffered formalin. In late 2016, we shipped this ear plug to our colleague Dr. Christina Lockyer at Age Dynamics in Tromsø, Norway, with three other ear plugs. The samples were shipped under a CITES permit issued to the Marine Mammal Health and Stranding Response Program (MMHSRP) by Alaska Veterinary Pathology Services, Eagle River, AK. After age estimation was completed, the ear plug was shipped to Dr. Stephen Trumble at Baylor University for further analysis of bulk carbon and nitrogen isotopes, hormone and contaminant levels over the course of the whale's life.



Figure 2. Whale #441's ear plug just after removal from the carcass. Ear plugs are keratin and lipid structures found in the ear canal of baleen whales that biologists have used since the mid-twentieth century to estimate the whale's age.

In 2017, Lockyer prepared the ear plug by carefully paring the ear plug along its long axis to expose the core at the midline using a razor and then polishing the cut surface with a whetstone. The exposed surface, covered by water, was examined under low power magnification (5x – 40x) using a binocular dissecting microscope and/or a magnifying headset. The annual GLGs (Growth Layer Groups) were counted many times on 9 different occasions. Lockyer evaluated the ear plug and

provided a marked image as well as a tabular record of the nine counts of GLGs. The counts ranged from 61 to 66 years of age, with notes indicating that the “higher age is favoured” (Fig. 3). Notes on the count were as follows: *“Intact dark coloured ear plug core. Closely packed adult GLGs, almost impossible to distinguish. The GLGs near the core base are the most unreliably counted, and hence two potential total counts (higher age is favoured). Early GLGs are clear; a likely transition at 10.”* (Lockyer, pers. comm. 2017). The transition zone of a baleen whale ear plug denotes where widely spaced GLGs abruptly become much closer together and is believed to be linked with changes in the skull’s growth rate after sexual maturity (Lockyer 1972, Kato 1983). Lockyer concluded that Whale #441 had 66 GLGs, indicating 66 years of age, indicating that he was born in 1950, or as late as 1955 if the true count was 61 GLGs. Either way, this animal was alive during the commercial whaling era in the North Pacific.

M. Novaeangliae no 2016094
ear plug age 66 yr

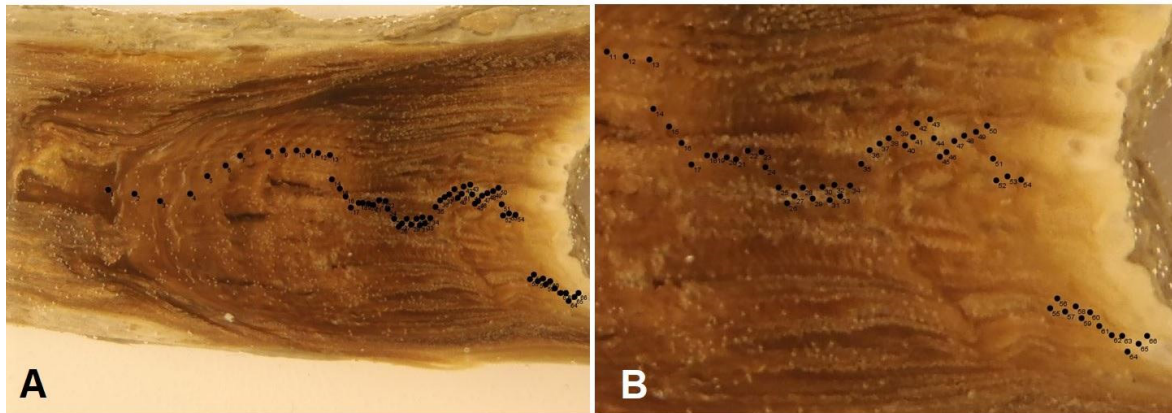


Figure 3. Growth-layer-groups (GLGs) marked on image of #441’s ear plug, by Christina Lockyer. Inset A shows GLGs 1-66 and inset B shows GLGs 11-66 in higher magnification.

Hormone and Stable Isotope Profiles

Baleen-derived profiles of hormones and stable isotopes

Janet Neilson, with her volunteer field assistant Sean Neilson, collected a baleen sample in September 2016, several weeks after the necropsy. We provided this baleen plate, along with others from different stranded whales, to research groups studying hormone levels and nutrition. A summary of these results follows, courtesy of the principal investigators.

Hormones in Baleen

Mysticete baleen grows slowly and continuously from a highly vascularized "root" area in the upper palate, simultaneously eroding steadily at the distal tip (Werth et al. 2020). A large body of evidence derived from stable isotope (SI) analysis in other mysticetes suggests that in most if not all mature mysticetes, this baleen growth process has a relatively steady growth rate, even in species with annual feeding-fasting cycles. Steroid and thyroid hormone levels can be derived from these keratinaceous tissues in mysticete whales; hormones are deposited from circulating blood into new baleen in the growth zone. Thus, a single plate of baleen contains a linear time series of hormones spanning the entire period of baleen growth (Hunt et al. 2014, 2017). Researchers at Northern Arizona University (Carley Lowe, Kathleen Hunt, C. Loren Buck) analyzed one baleen plate from #441 to construct a multi-year profile of hormone levels, as part of a research program studying hormones in baleen from a variety of mysticetes (Fig. 4). In particular, they focused on two glucocorticoids (cortisol and corticosterone), adrenal hormones that are indicative of physiological stress, which can elevate in concentration due to a variety of conditions such as nutritional stress, pregnancy, lactation, entanglement, and disease. Both cortisol and corticosterone were analyzed due to the fact it is not yet clear which is the predominant glucocorticoid in mysticete baleen. Since #441 was a mature male, they also analyzed baleen testosterone to explore the possible occurrence of seasonal testosterone peaks (i.e., reproductive cycling). In other mysticetes (blue whale, North Atlantic right whale, bowhead whale), testosterone from baleen of adult males typically shows pronounced annual cycles, with regularly repeating intervals of high and low testosterone, with a cycle length matching annual baleen growth rate (Hunt et al. 2018). Analysis of annual oscillations in SI ratios (described later) established that the timeline represented by the plate most likely spans mid-2012 (oldest baleen at distal tip of plate) to mid-2016 (time at death, newest baleen from the proximal root of the plate).



Figure 4. Baleen from Whale #441 showing tape affixed to labial edge of plate with drill marks every 2 centimeters. Bottom of photo shows the powder that is collected from each drill site that is later assayed for various hormone concentrations.

Laboratory methodology for baleen hormone analyses followed previously published methods for humpback and other mysticete baleen (Hunt et al. 2017, 2018). In short, baleen was sampled every 2 cm from base to tip, using a rotary hand-held drill (Dremel with tungsten ball tip) to drill a shallow transverse groove at each sampling location, collecting powder on weigh paper. Hormones were extracted with 4.00 mL 100% methanol added to 75.0 mg of well-mixed baleen powder, followed by 2 h of vortexing, 15 min centrifugation at 3000 g, collection of supernatant, dry-down of supernatant (containing hormones) in a rotary evaporator (overnight dry-down at 45° C), and reconstitution in 0.50 mL assay buffer (buffer #X065, Arbor Assays, Ann Arbor, MI, USA). Extracts were diluted as appropriate to keep results near 50% on the assay binding curve, area of greatest precision, with final results corrected accordingly. All hormones were assayed with enzyme-linked immunosorbent assay (ELISA) using assay kits that had previously been validated for humpback whale baleen (Arbor Assay kits K003, K014 and K032, for cortisol, corticosterone, and testosterone, respectively; Ann Arbor, MI, USA; Hunt et al. 2017). Final results are expressed as ng of hormone per g of baleen powder.

The resulting hormone data (Fig. 5) suggest that #441 experienced a prolonged episode of chronic stress that began in 2013. Both adrenal stress hormones (cortisol and corticosterone) were relatively low through 2012 but began a gradual and prolonged multi-year rise in 2013, peaking in late 2015 and early 2016. Testosterone showed the reverse pattern; baleen testosterone showed a sharp elevation in 2012 suggestive of a regular seasonal breeding peak (though note we do not have comparison data from healthy males for this species), followed by a progressive decline to lower levels in 2013–2015 with no further peaks during that period. Thus, it appears that #441 may have experienced his last seasonal testosterone peak in 2012 (estimated 62 years old), with testosterone cycling ceasing in 2013 coincident with the onset of the chronic stress episode. Starting in the beginning of 2016 and continuing until death in mid-2016, however, testosterone increased to more than double the peak seen in 2012. We consider it likely that this massive increase is not due to reproductive activity but rather the result of a stressor (e.g. severe illness or critical injury), since failing adrenal glands (which produce numerous other steroids besides the glucocorticoids, including androgens) may dump various steroids into peripheral blood.

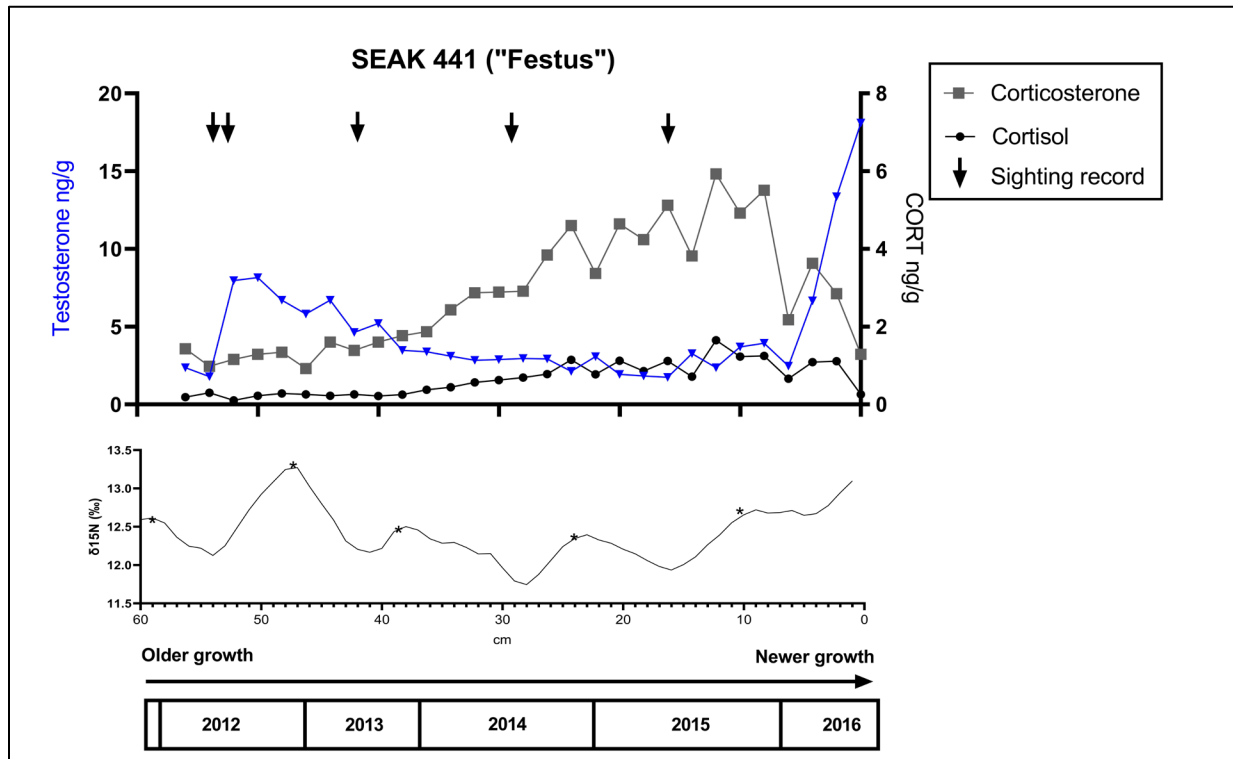


Figure 5. Three hormone profiles for Whale #441 along the growth of a single baleen plate: testosterone (blue), cortisol (black with round points) and corticosterone (gray with square points). Date was estimated using stable isotope analysis of ^{15}N (Rogers et al., in prep.). All hormones are reported in nanograms of immunoreactive hormone per gram of baleen powder.

In the last few months before death, both glucocorticoids declined sharply (Fig. 5). A similar rise-then-fall profile of baleen glucocorticoids has been documented in southern right whale calves, with the collapse in glucocorticoids also occurring shortly before death (Fernandez Ajo et al. 2018, 2020).

Our interpretation is that #441 was at that point near to death and was perhaps experiencing adrenal failure. In sum, the hormone data suggest a prolonged period of chronic stress and cessation of reproductive cycling, with the chronic stress episode beginning in 2013, increasing in severity over several years, and culminating in adrenal failure shortly before death.

The potential impact of the 1999 tagging on stress hormone levels could not be assessed from baleen, because the 2012–2016 baleen timeline for Whale #441 does not span the pre-tagging time period. At present there are no published values for stress hormone levels in humpback whale baleen to use as a basis of comparison, and absolute levels cannot be compared across species.

Stable Isotopes in Baleen

NOAA Fisheries Auke Bay Lab scientists Matt Rogers and John Moran used a sample of Whale #441's baleen as part of a larger study of how nutrition is revealed in stable isotope patterns in Alaska humpback whales. The concentration of ^{15}N was increasing and ^{13}C was decreasing at the time of Whale #441's death (Fig. 6). This is most likely indicative of extreme nutritional stress and potentially end-stage starvation when the whale is using its own proteinaceous tissues for energy and tissue maintenance (Hatch 2012). This continued rising $\delta^{15}\text{N}$ signal prior to death has been observed in most humpback whale baleen plates sampled during the recent large whale Unusual Mortality Event of 2015–2016 (Rogers et al. in review).

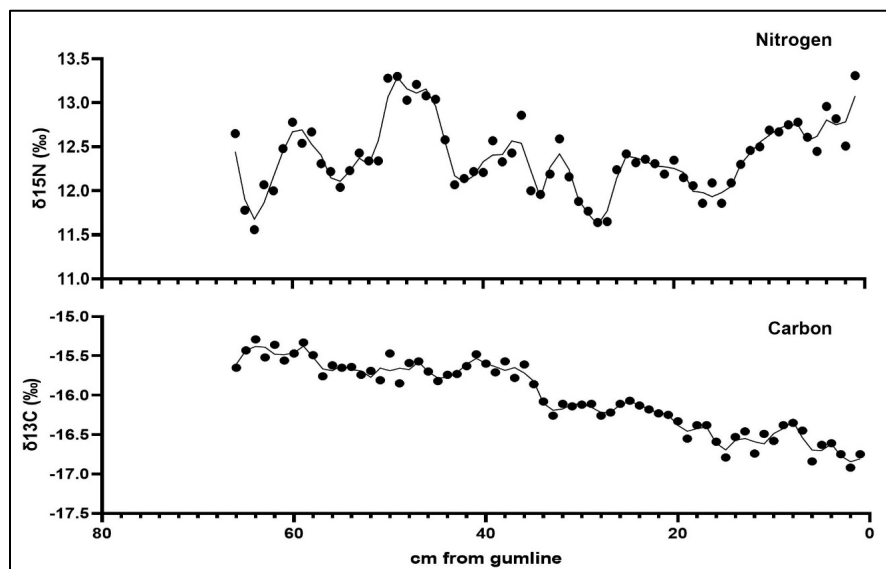


Figure 6. Chart depicting baleen stable isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) from 1 cm subsamples taken along the axis of baleen growth, from near the base of the baleen plate (0 cm) to the distal end of the baleen plate (~66 cm). On the X-axis, the gum line (0 cm, right) corresponds with the whale's death in summer 2016, while the distal end (66 cm, left) corresponds with approximately 2011. Oscillations in the $\delta^{15}\text{N}$ value are due to changes in nutritional status during annual migrations from feeding in Alaska (falling $\delta^{15}\text{N}$) to fasting while at calving grounds in Hawaii (rising $\delta^{15}\text{N}$). Each cycle of $\delta^{15}\text{N}$ from trough to trough represents approximately one year of baleen growth (Eisenmann et al. 2016). The $\delta^{15}\text{N}$ signal shows a sharp, continued rise near the gumline when the signal should typically be falling upon the whale's return to foraging in Alaska.

The $\delta^{15}\text{N}$ minima can be used to infer forage preferences during feeding. Whale #441 shows evidence of mixed foraging, possibly feeding on both forage fish and krill with relatively more krill in years with lower $\delta^{15}\text{N}$ minima of ~ 11.5 ‰. The carbon isotope data is less well understood and may reflect nearshore vs. offshore foraging preferences with Whale #441 potentially moving steadily from more nearshore foraging (fewer negative $\delta^{13}\text{C}$ values near -15 ‰) in the older baleen to more offshore foraging in the more recently formed baleen (more negative $\delta^{13}\text{C}$ values near -17 ‰). This finding may corroborate the hypothesis that the sharp decline in the number of whales in Southeast Alaska starting in 2015 indicated that they moved offshore to feed (Wong et al. 2019). Alternatively, declining $\delta^{13}\text{C}$ values in marine ecosystems are known to reflect increasing anthropogenic carbon dioxide in the oceans (e.g. de la Vega et al. 2019), known as the Suess effect. However, the Suess effect is thought to be quite small on decadal timescales (approximately -0.15 to -0.18 ‰ per decade; Sonnerup et al. 2007) and thus is unlikely to fully explain the decline in $\delta^{13}\text{C}$ noted across just ~ 5 years of baleen growth.

Ear plug Stable Isotope and Hormone Profiles

After Dr. Christina Lockyer completed her examination of Whale #441's ear plug to estimate the whale's age (Fig. 3) she sent it to Dr. Stephen Trumble's lab at Baylor University. There, researchers dissected each growth layer of #441's ear plug to analyze the hormone and stable isotope profile over the whale's lifetime. Trumble concluded that Whale #441 had 68 GLGs and, therefore, an estimated birth year of 1948.

Whale's #441 ear plug had been bisected previously (Fig. 3) and upon arrival at Baylor, it was stored at -20°C until processing. After removal from the freezer, sanding and polishing were performed with a fine grit sandpaper to show clear demarcation between the layers. The ear plug was weighed, measured and photographed with a high-resolution single-lens-reflex (SLR) camera (Canon, 25-megapixel resolution). The photographs were enlarged and used to count GLGs by counting the bands of light and dark lamina; it has been previously shown that one light and one dark layer equals one year of life (Gabriele *et al.*, 2010). Counting light and dark layers is initially performed by three individual readers independently before a consensus is reached for a definitive age. Once an age was determined, each ear plug lamina was painstakingly delaminated using a medical grade zirconia ceramic scalpel blade. Each lamina was homogenized and weighed to the nearest 0.001 g, stored in amber glass vials filled with nitrogen and placed at -20°C until further analysis. Lipids from each lamina were extracted (Soxhlet 2043; FOSS) using 2:1 chloroform to methanol solution heated to 130°C for 60 min. Extractions were then dried under nitrogen and stored at -80°C . Previously extracted lipids from each lamina were aliquoted for hormone assays. Hormones were measured using enzyme-linked immunoassays (ELISA; Enzo Life Sciences, cortisol: ADI-901-071, testosterone: ADI-900-065) which have previously been validated for linearity and accuracy (Trumble et al. 2013). Final results are expressed in concentrations of ng of hormone per g lipid.

Results from the ear plug corroborate and expand upon the baleen hormone and stable isotope profiles shown in Figs 5 and 6, including elevated cortisol in the last years of Whale #441's life. The overall decreasing trend in bulk $\delta^{13}\text{C}$ in the ear plug matched the trend observed in the baleen, which as mentioned previously may be in part indicative of Suess effect (anthropogenic carbon) and/or may

indicate a switch in foraging location. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles both showed a period of stability between 1950 to 1980 then a pronounced drop suggesting that the whale underwent a trophic shift, or the marine ecosystem experienced a change in anthropogenic inputs. The $\delta^{15}\text{N}$ profile is cyclic, potentially reflecting migrations between Alaska feeding season and fasting in the breeding season in Hawaii. The final two decades of the ear plug SI profile is characterized by about 20 years of trophic stability until just prior to death, potentially due to either nutritional stress or acute trauma.

Generally, ear plug testosterone data do not typically show the sharp seasonal testosterone peaks characteristic of baleen data, and this was the case with #441's ear plug testosterone profile. This difference is likely due to differing temporal resolution of the two sample matrices; each point on baleen thought to represent approximately 2 weeks of endocrine activity, while each point on the ear plug can be thought of as a 6–12 month average. Thus, the two sample types offer complementary information, with baleen offering good temporal resolution of monthly seasonal patterns, while ear plugs provide roughly bi-annual or annual averages across many more years. In #441's case, ear plug testosterone showed a general declining trend throughout the whale's life which may signal progressive reproductive senescence. Testosterone levels showed clear peaks after birth, weaning, and at 6, 8, 11 years of age, with the largest peak at age 12 which could indicate onset of sexual maturity or, alternatively the peak of reproductive competence (which in many species can trail the onset of spermatogenesis by multiple years). This corroborates Lockyer's interpretation of the ear plug transition period at 10 years.

Ear plug cortisol in 1999–2000 reached a level that had not been exceeded since 1964, suggesting that the 1999 tagging event may have caused physiological stress. Testosterone levels generally correlate well with cortisol levels, and both may correspond to competitive breeding season interactions between males or may indicate stressors unrelated to reproduction. The lowest cortisol values occurred in summer 2008, followed by a return to fluctuating levels typical since about 1976.

The final 5 years of the hormone profile generally match the results from the baleen (Fig. 6) although the baleen shows a general increase with more variability in 2014–15 where the ear plug shows a decline likely attributable to differences in the temporal resolution of the matrix. In 2016, at the time of the whale's death, both baleen cortisol and ear plug cortisol levels had been rising since 2013–2014. Overall, in combination with the relative stability of the environment reflected in the isotopic signatures (Fig. 7), and the typical hormone patterns (aside from 1999–2000), it is difficult to determine the immediate proximate cause of Whale #441's death (e.g. starvation or acute trauma), but both sample types indicate a multi-year increase in cortisol and relatively low testosterone in the 2–3 years prior to death.

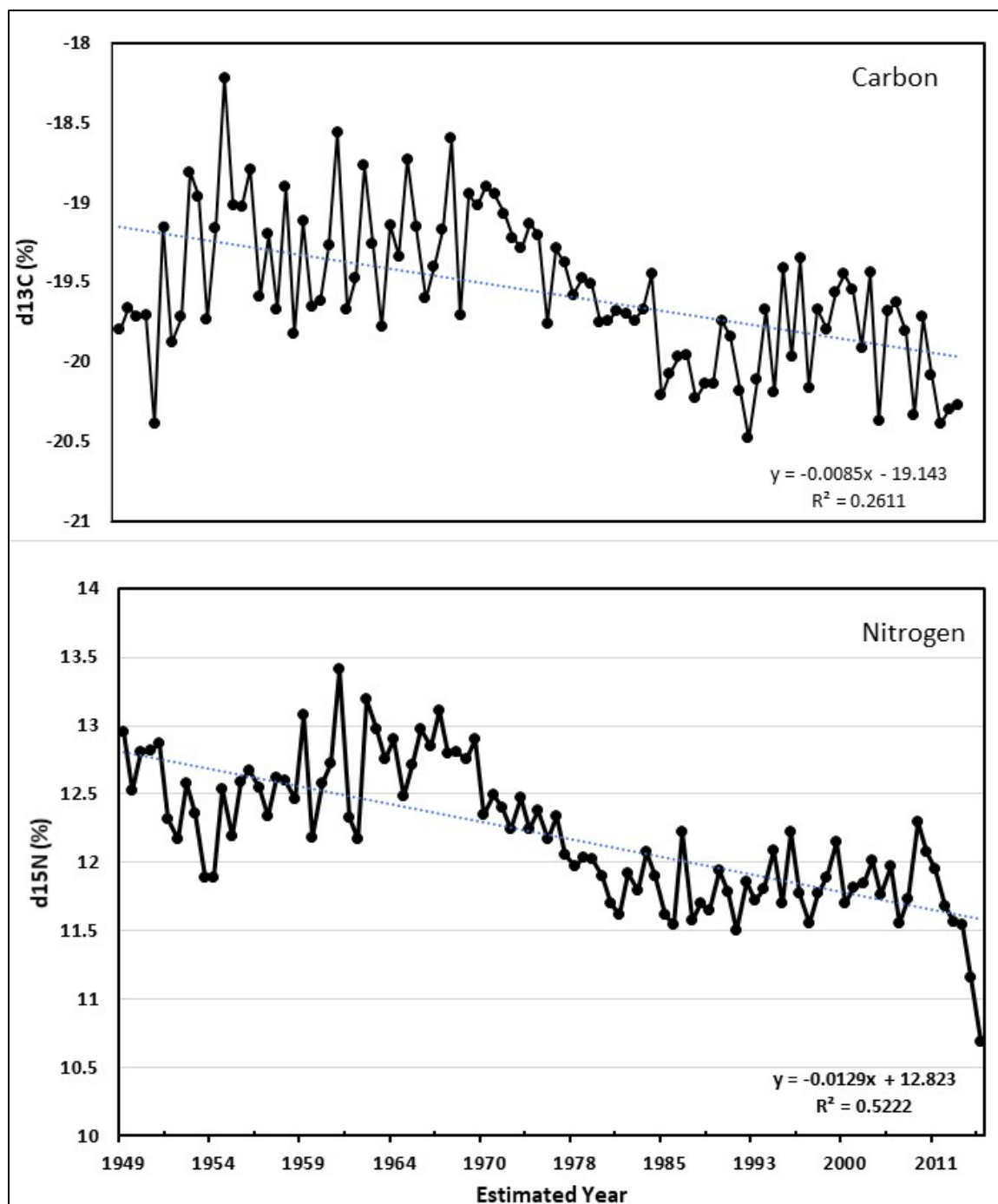


Figure 7. Multi-year stable isotope data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) taken from growth layers in the ear plug from birth to death. On the X-axis, the final growth layer (at far right) corresponds with the whale's death in summer 2016, while the distal end (at far left) corresponds with circa 1948.

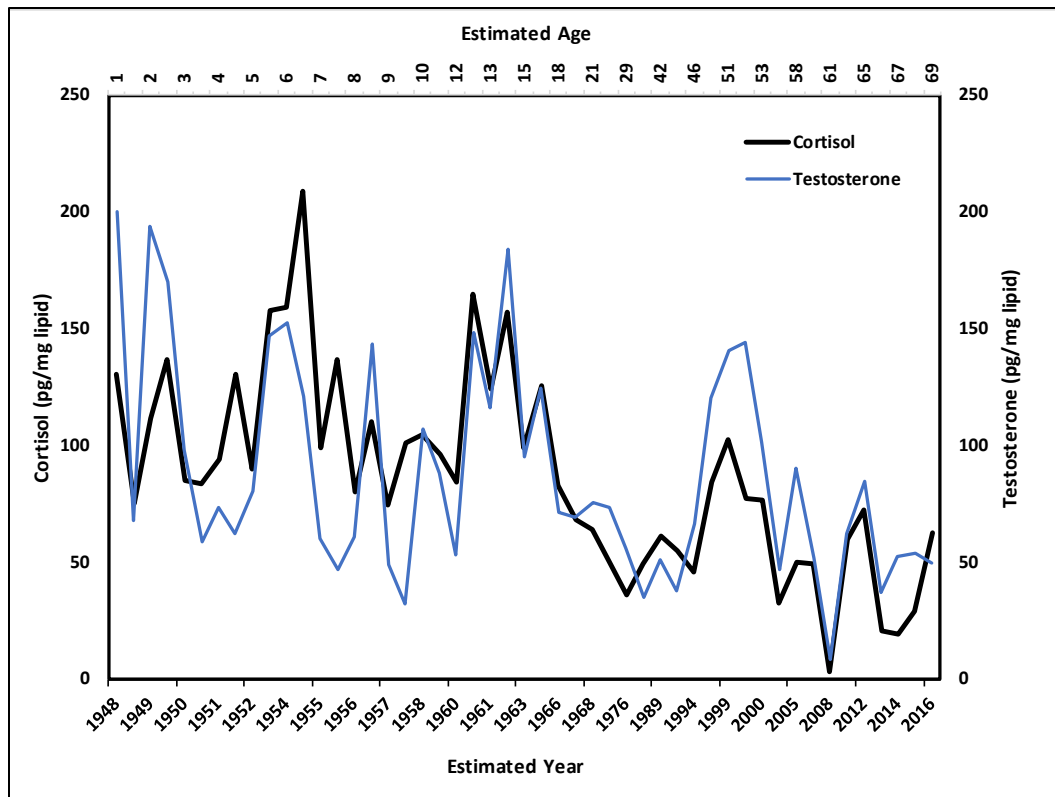


Figure 8. Cortisol and testosterone hormone profiles for Whale #441 along ear plug growth layer groups. Each point represents the hormone concentration in a single ear plug growth layer, reported in picograms of immunoreactive hormone per mg of lipid. Date was extrapolated using stable isotope analysis of ^{15}N (Fig. 7b).

Skeletal Abnormalities

Bone Inventory and Condition Assessment

Aside from a cursory examination of the carcass for evidence of trauma associated with human interaction or ship-strike, the skeleton of Whale #441 was not assessed during the necropsy due to time and logistical constraints. However, the carcass remained accessible during the following months and we returned on a few occasions to determine whether there was evidence of ship-strike, satellite tag remnants or scavenger activity. Over the summer and fall of 2016, we made visits to the carcass to make additional observations and collect samples. Patterns of scavenger activity are documented separately (Young 2018). Fractured bones that would have indicated trauma, potentially associated with ship-strike, were not detected. No satellite tag remnants were detected. However, on August 22, 2016, a portion of blubber that appeared to trigger a metal detector was collected and transported to the laboratory for further analysis. It was later concluded that naturally occurring magnetic rock in the gravel and sand at the stranding site were likely what triggered the metal detector.

As early as August 22, 2016 (58 days after the carcass was first located), examination of the carcass by NPS biologist Chris Gabriele and graduate student Michelle Fournet revealed abnormal bone formation on some of the vertebrae even as they were still covered with decaying connective tissue. Over the following year, we made several additional visits to the carcass to further document the bone lesions and look for contributing factors. Samples of rotting tissue attached to caudal vertebrae with obvious abnormalities were collected and sent to AVPS for viral and bacterial analysis. Over time, with weathering and putrefaction it became apparent that the skeleton of Whale #441 had multiple significant irregularities.

In the spring of 2017, with assistance from Amanda Murphy, a forensic anthropologist working temporarily at Glacier Bay National Park, we conducted a full examination of the remaining bones to describe the character and extent of the skeletal abnormalities. This included locating remaining bones, photographing them, taking measurements, and describing any abnormalities observed (Fig. 9). At that time, one year and one month after the necropsy of Whale #441, we located 41 bones including 22 vertebrae presumed scattered around the beach by wave action and largely free of soft tissue, bleached white by the sun (Fig. 10).

To understand how the inventoried bones fit together in the skeleton, hundreds of photographs taken of the decomposing carcass between 2016 and 2017 by several photographers were compiled, studied and annotated. Individual bones were assessed to determine the presence, nature, extent, and severity of lesions. In some cases, these photographs also provided information on the bones that were not collected for examination allowing us to understand whether they were normal or irregular. These observations were combined with the information gathered in the bone inventory and recorded in a spreadsheet (Table 3).

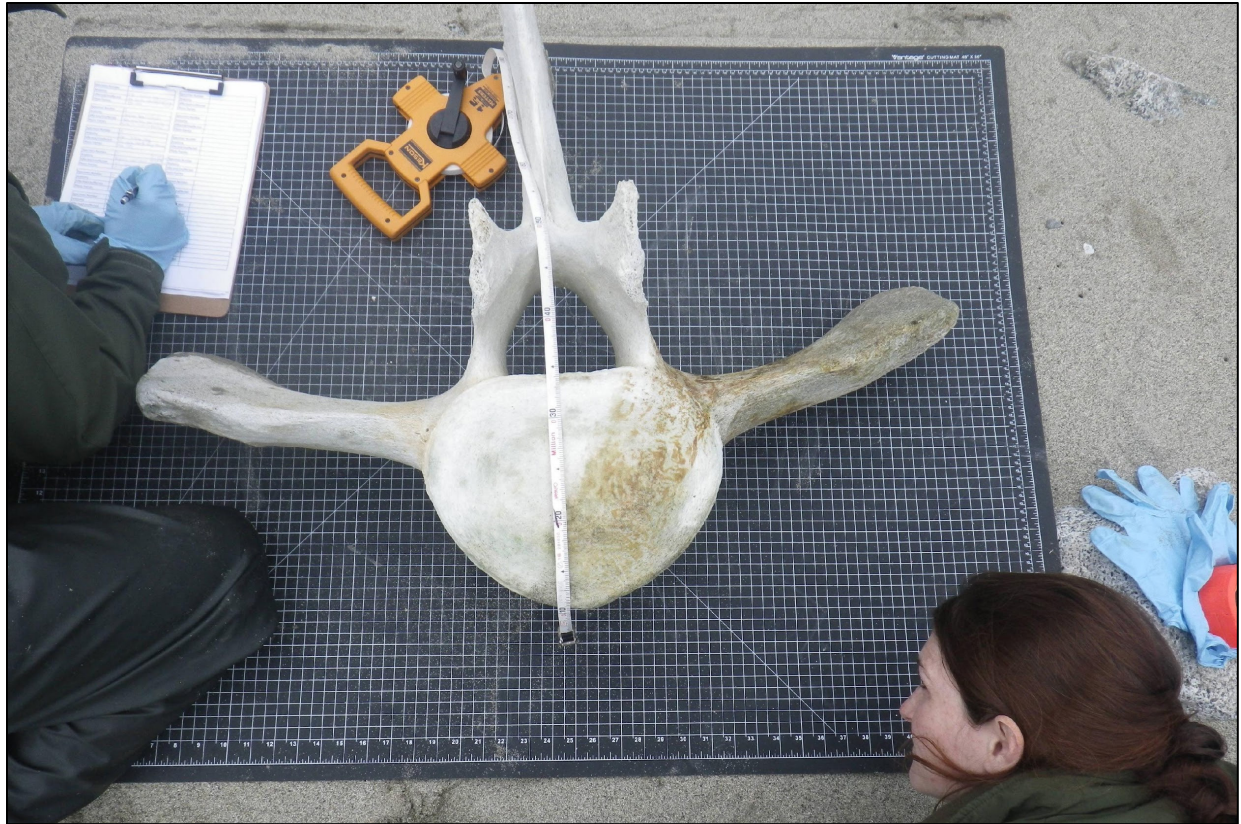


Figure 9. On June 29, 2017, we inventoried the bones remaining on the necropsy beach, measuring, and photographing each bone and taking note of any pathological changes.



Figure 10. Representative photos depicting the decomposition of the carcass. Top: On August 5, 2016, the carcass was found a little further down on the same beach. Soft tissue has decayed away from some of the thoracic cavity and the spine is severed at the atlanto-occipital joint. Bottom: By September 19, 2016, much of the soft tissue has decayed away revealing the bones of Whale #441.

Table 3. Summary of pathology found in bones of the spine.

Spinal section	Bone	Pathology	Source of Information	Description
Cervical vertebrae	C1	Age-related degeneration	Photographs	Rugosity on ventral surface, patches of plateauing/pitting especially on facets
	C2	Unknown	Photographs	One photo shows C2 and C3 apparently fused but this cannot be confirmed
	C3	Unknown	Photographs	One photo shows C2 and C3 apparently fused but this cannot be confirmed
	C4	–	–	–
	C5	–	–	–
	C6	Age-related degeneration	Photographs, inventory	Ossification on left lateral vertebral body
	C7	–	–	Not found, presumed absent
Thoracic vertebrae	T1	Age-related degeneration	Photographs, inventory	Degenerative changes to transverse processes, lipping or compression of facets
	T2	–	–	–
	T3	–	–	–
	T4	–	–	–
	T5	–	–	–
	T6	–	–	–
	T7	–	–	–
	T8	Ankylosing spondylitis	–	Extensive irregular bony proliferations on the ventral vertebral body, likely in response to the overlapping of T9
	T9	Ankylosing spondylitis	Photographs, inventory	Dramatic lipping overlapping on both T8 and T10 on the left side ventral, also overlapping T8 on the right side ventral
Lumbar vertebrae	T10	Ankylosing spondylitis	Photographs, inventory	Bony proliferation of ventral vertebral body with lipping cranially from ventral right
	T11	–	–	–
	T12	–	–	–
	T13	–	–	–
	T14	–	–	–
	L1	–	–	–
	L2	–	–	–
	L3	–	–	–
	L4	–	–	–

Table 3 (continued). Summary of pathology found in bones of the spine.

Spinal section	Bone	Pathology	Source of Information	Description
Lumbar vertebrae (continued)	L5	–	Photographs	–
	L6	Lamellar ossification	Photographs	Small area of lamellar ossification on left side base spinous process, ring in caudal facet
	L7	Lamellar ossification	Photographs, inventory	Small area of lamellar ossification on left side base spinous process, spinous process is bifid
	L8	Lamellar ossification	Photographs	Small area of lamellar ossification on left side base spinous process
	L9	–	Photographs, inventory	Apparently normal
	L10	–	Photographs	Apparently normal
Caudal vertebrae	Ca1	–	Photographs	Apparently normal
	Ca2	–	Photographs	Apparently normal
	Ca3	Infectious spondylitis	Photographs, inventory	Moderate ossification of spinous process, prezygapophyses
	Ca4	Infectious spondylitis	Photographs, inventory, samples	Severe bony proliferations on left and right sides of vertebral body, spinous process leans right, caudal facet ruptured, bone collected for further sampling and study.
	Ca5	Infectious spondylitis	Photographs, inventory, samples	Severe proliferative ossification extending laterally from vertebral body including lateral processes with a large osteophyte on right dorsal aspect, cranial facet ruptured, bone collected for further sampling and study.
	Ca6	Infectious spondylitis	Photographs, inventory	Proliferative growth on the dorso-lateral left vertebral body
	Ca7	Infectious spondylitis	Photographs, inventory	Proliferative growth on the vertebral body especially left lateral, caudal facet ruptured asymmetrically
	Ca8	Infectious spondylitis	Photographs, inventory	Proliferative growth on the vertebral body especially severe on left lateral, large osteophyte extending caudally from right lateral, cranial facet ruptured asymmetrically.
	Ca9	Infectious spondylitis	Photographs	Severe proliferative growth on lateral vertebral body, large osteophyte extending from right side with possible overlap of Ca10.

Table 3 (continued). Summary of pathology found in bones of the spine.

Spinal section	Bone	Pathology	Source of Information	Description
Caudal vertebrae (continued)	Ca10	Infectious spondylitis	Photographs	Proliferative growth on the vertebral body.
	Ca11	Unknown	Limited photographs	This bone was still partially covered in tissue but showed no obvious signs of pathology
	Ca12	–	Photographs	Apparently normal
	Ca13	–	Photographs	Apparently normal
	Ca14	–	Photographs	Apparently normal
	Ca15	–	–	–
	Ca16	–	–	–
	Ca17	–	–	–
	Ca18	–	–	–
	Ca19	–	–	–
	Ca20	–	–	–
	Ca21	–	–	–
	Ca22	–	–	–

Three different pathological conditions were observed in the bones, primarily affecting the vertebrae; bridging between adjacent vertebrae, periosteal proliferative ossification, and ruptured vertebral facets (Table 3). Nine of the caudal vertebrae (Ca3 – Ca10) exhibited dramatic ossification involving the vertebral bodies and their lateral processes. Proliferative ossification was characterized by coral-like bony proliferations (Fig. 11) including some lipping or bridging between adjacent vertebral discs. Lesions including sinuses, cloacae, and those characterizing ruptured vertebral facets were observed in conjunction with proliferative growths (Fig. 12). The caudal facet of Ca4, cranial facet of Ca5, caudal facet of Ca7, and the cranial facet of Ca8 were all severely ruptured, characterized by extensive erosion with cavities. The condition in the caudal vertebrae is suggestive of infectious spondylitis and does not resemble normal age-related degeneration. In humans, cadmium exposure can produce bone resorption and malformations (Alfvén et al. 2000) but we were unable to collect kidney and liver samples to assess cadmium levels in this whale. Cadmium is a toxic heavy metal widely used in industry, which enters the marine environment through air pollution and river discharge and has been observed to bioaccumulate in mollusks and crustaceans (Engel and Fowler 1979).

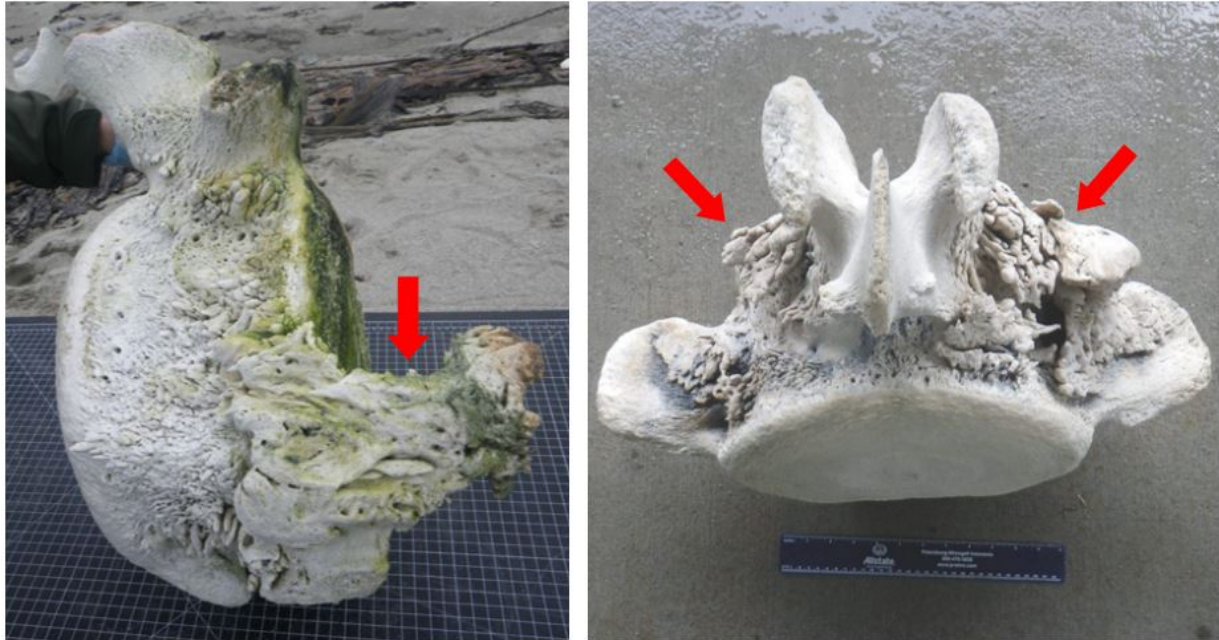


Figure 11. Proliferative ossification on the right lateral aspect of vertebra Ca8 (left) and the dorsal aspect of vertebra Ca5 (right). Note proliferative growth of Ca8 extending across the intervertebral gap where it would have interacted with Ca7.

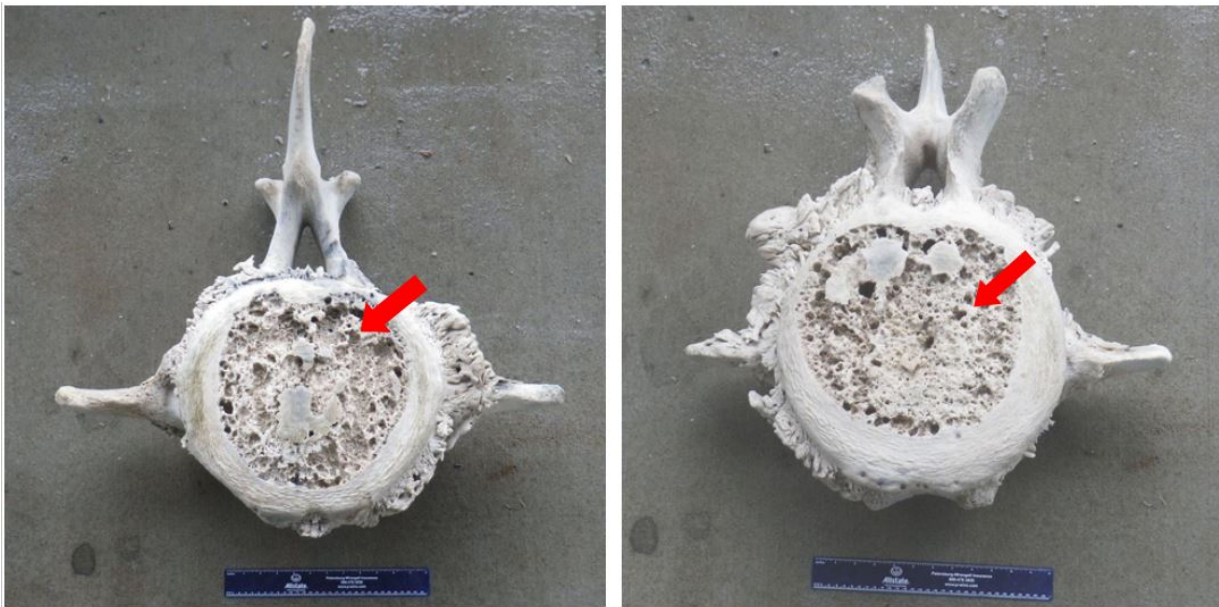


Figure 12. The caudal facet of vertebra Ca4 (left) and the cranial facet of vertebra Ca5 (right) show evidence of severe osteolytic lesions that may be indicative of disc herniation.

In thoracic vertebrae T8, T9, and T10 (Fig. 13), a distinct pathologic lesion was observed. In these bones, large, smooth bony proliferations from the ventral vertebral bodies bridged intervertebral discs. This condition resembles ankylosing spondylitis although none of the examined vertebrae were actually fused, or diffuse idiopathic skeletal hyperostosis (DISH), which affects the thoracic spine in

humans (Kompanje 1999). In humans, the vertebral and lateral processes are not typically involved and the vertebral end plates (facets) of the affected bones are intact. In T9, the most dramatically affected bone, the proliferations (syndesmophytes) extend both cranially and caudally from the ventral vertebral body, extending between the intervertebral space and overlap the adjacent vertebrae (Fig. 11). T8 shows extensive irregular bony proliferations on the ventral vertebral body, likely in response to the overlapping syndesmophyte of T9. T10 shows a similar syndesmophyte extending cranially from the right ventral vertebral body with a curved shape suggesting a bulged disc between T9 and T10. Although the syndesmophytes are quite large, none appear to have fully fused adjacent vertebrae. The facets of these thoracic vertebrae were intact but exhibited lipping and facial rings that may indicate slight degeneration of the intervertebral disks. Furthermore, the ventral protrusions in T9 and T10 are smooth in contrast to the jagged, coral-like protrusions marking the lesions in the affected caudal vertebrae. This difference may indicate that the thoracic bone growths developed more slowly or were the result of a distinct pathologic entity or a combination of both processes.

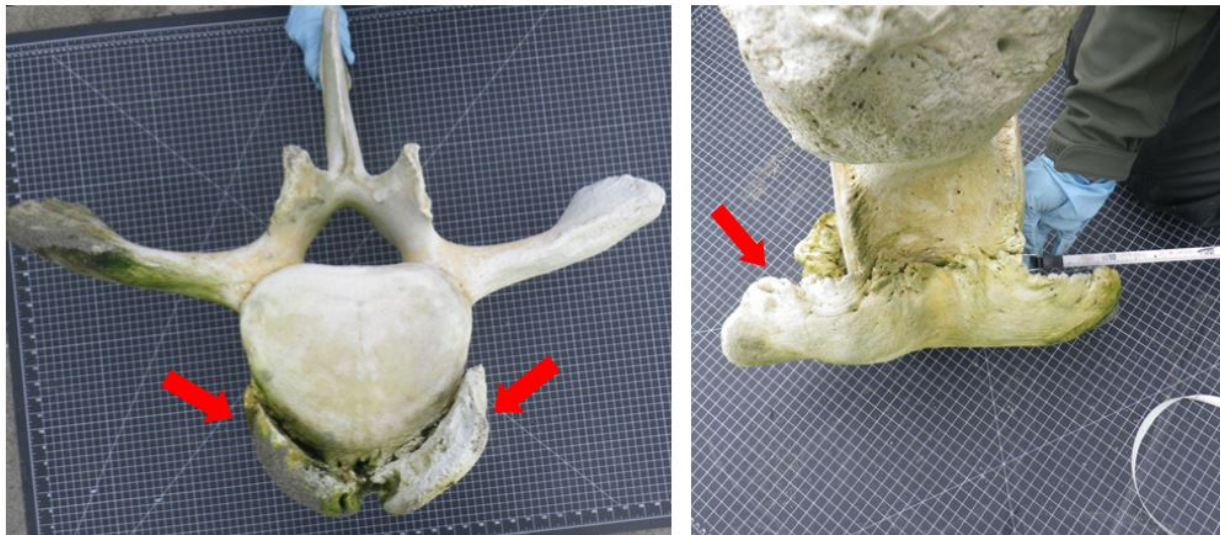


Figure 13. Thoracic vertebra 9 shown in cranial aspect (left) and detail of ventral syndesmophyte (right). Note the relatively smooth surface of the syndesmophyte in comparison to the proliferative growth on the caudal vertebrae in Fig. 11.

Thoracic vertebrae 11 through 14 and all the lumbar vertebrae appeared normal grossly. Lumbar vertebrae 6, 7, and 8 had lamellar bone growth on the left dorsolateral aspect of the vertebral body (Fig. 14). This button-like lamellar bone growth may be the result of normal age-related degeneration or ossification of ligaments. Lumbar vertebra 8 also has a bifid spinous process (bifid means it develops from two separate centers of ossification), which is normally only true of cervical vertebrae. This could be related to occult spina bifida, some irregular spinal curvature, geriatric change or may represent a normal anatomic variation in the species.

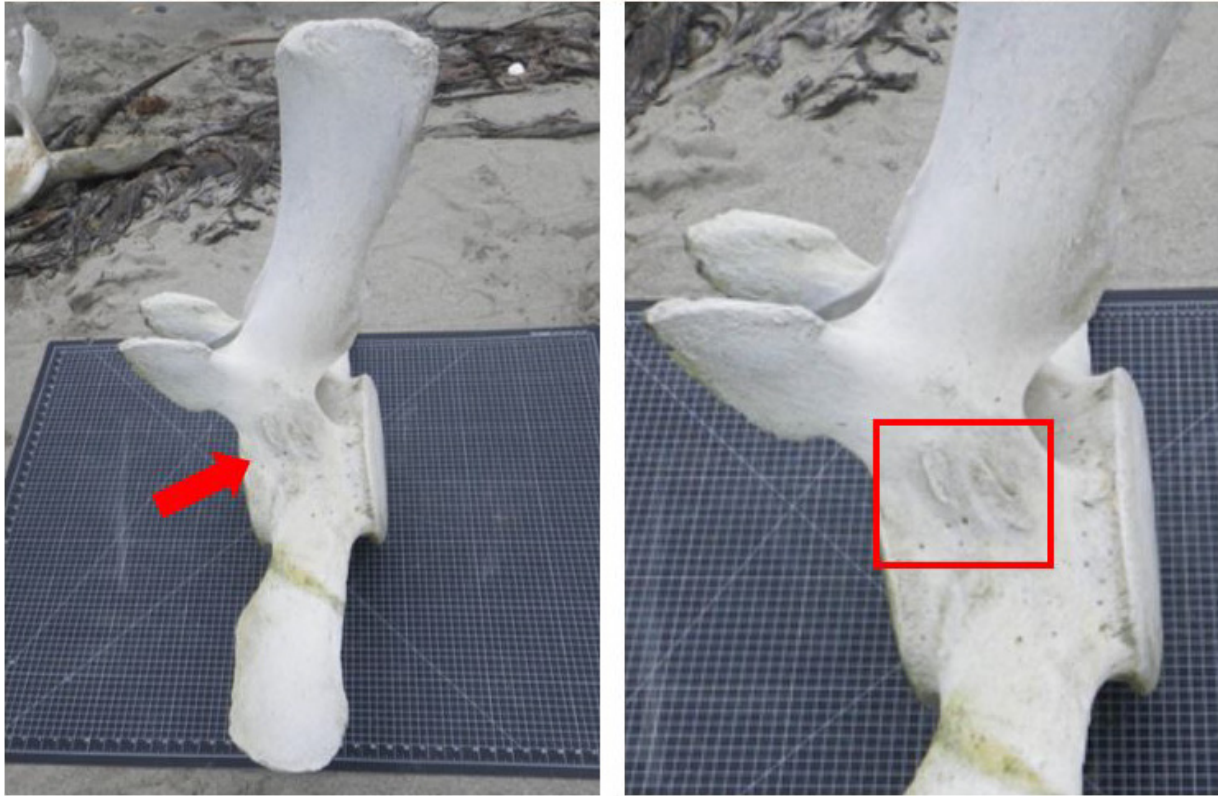


Figure 14. Lumbar vertebra 6 presents lamellar bone growth on the left dorsolateral vertebral body.

To summarize, the ankylosing spondylitis in the thoracic vertebrae is smooth, limited to the ventral aspect of the vertebral bodies, does not involve the vertical or lateral processes, and the vertebral facets are all intact. In contrast, the severe infective spondylo-osteomyelitis in the caudal vertebrae is marked by ruptured facets, and dramatic porous ossification of the vertebral bodies and lateral processes. In conclusion, the fact that the vertebrae in the central region of the spinal column appear grossly normal and the different characteristics of the thoracic and caudal spine suggests that there may be two separate processes underlying these lesions. Figure 15 highlights the affected bones in a diagram of a typical humpback whale skeleton. A dotted red circle shows the approximate location of the satellite tag and subsequent subcutaneous swelling.

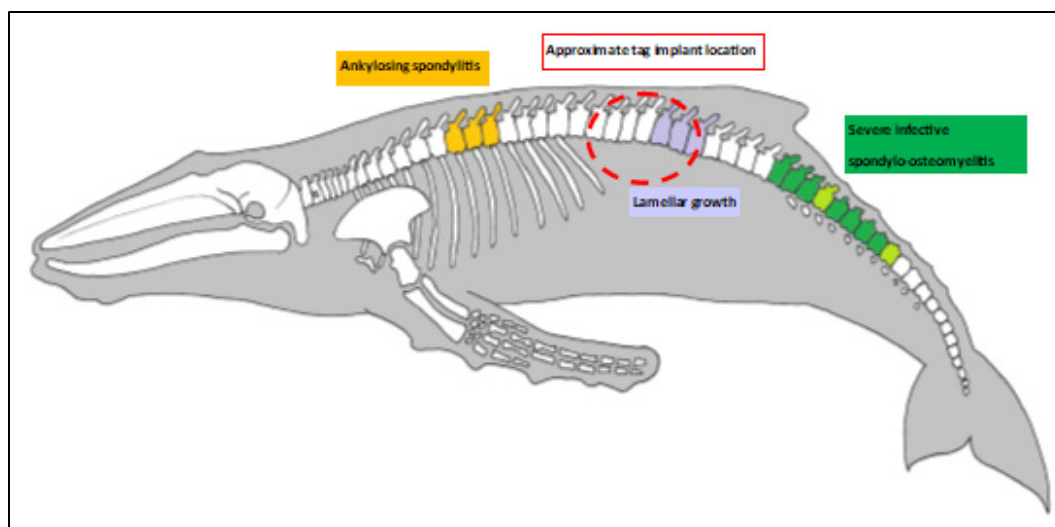


Figure 15. Graphic representation of skeleton indicating affected bones. Thoracic vertebrae 8, 9, and 10 are shown in yellow (ankylosing spondylitis), lumbar vertebrae 6, 7, and 8 are shown in purple (minor lamellar growth on left dorso-lateral), and caudal vertebrae 3 through 10 are shown in green (severe infective spondylo-osteomyelitis). The red circle shows the approximate location of the satellite tag implanted in 1999.

Differentiating between normal age-related degeneration and what may be pathologic is challenging in the bones recovered from stranded whales. Glacier Bay National Park has a valuable resource to help approach this challenge: an articulated skeleton exhibit of a female humpback whale (Whale #68, nicknamed Snow) that died when ship-struck in 2001 at the age of 45. Comparing the bones of Whale #441 to Whale #68's clean and articulated skeleton, we can begin to assess the range of variation of bone conditions that occur in an adult humpback. The lesions described above in T8-T10 and Ca3-Ca10 in Whale #441 (Figs. 11, 12) are not found in any Whale #68's bones. Many of Whale #68's vertebrae show a slight ring in the facet; however, none are ruptured and there is no evidence of lipping at the margins. In contrast, the condition of Whale #441's caudal vertebrae shown in Fig. 13 probably indicates disc herniation.

Another notable distinction between the bones of Whale #441 and Snow was their apparent oil content. We suspect that the bones of Whale #441 had less oil content; they presented a white, oil free appearance 15 months after death without any cleaning efforts aside from exposure to the sun. By contrast, some bones in the Whale #68 skeleton exhibit continue to exude oil after resting on the beach for 18 months and being cleaned with a variety of methods over the course of 13 years. Blubber thickness and other necropsy findings suggest that Snow was in excellent body condition, while Whale #441 was in poor condition. The apparently lower oil content in Whale #441's bones corroborates other findings indicating poor nutritional condition. Whale #441's poor nutritional condition is not surprising given that 13% of observed whales were emaciated during the 2016 annual population monitoring (Neilson et al. 2017) and he died during the Northeast Pacific marine heatwave that was associated with widespread ecological disruption (Piatt et al. 2020).

Comparative Cases Found in the Literature

Opportunities to examine fully flensed whale skeletons are uncommon. As a result, there is little information on how prevalent such lesions are among large whales. Spondylo-arthritis has been documented in other baleen whales. Many of these have come from museum specimens, although a few are from stranded whales still on the beach. Stranded humpback whales surveyed in Brazil over a 10-year period revealed pathological changes in 24% of skeletons (Groch et al. 2012). Kompanje (1999) described an adult female fin whale (*Balaenoptera physalus*) with spondylo-arthritis in which the cervical and first thoracic vertebrae were irregularly fused by ventral syndesmophytes. These syndesmophytes did not share the smooth appearance of the thoracic lesions found in Whale #441.

Groch et al. (2012) describes a 9.18 m juvenile humpback whale stranded in Brazil with similar “exuberant irregular bony proliferations on the entire circumferences” of two caudal vertebrae. Additionally, the vertebral endplates exhibit severe erosion similar to that found in the caudal vertebrae of Whale #441. In this case, the animal was a juvenile that had been necropsied with no identified cause of death; however, they did note “an abnormally high incidence of whale lice”. Groch classified this lesion as diskospondylitis after Kompanje (1999).

Bone Pathology Lab Results

Samples of bone lesions were sent to AVPS for histological analysis and further testing. Histopathology of the bones recovered after the postmortem examination revealed a massive fungal infection within areas of abnormal bone, and that this fungus was morphologically distinct to the fungus identified in the lymph node (Appendix B). Detection of two morphologically distinct fungi may represent a dual infection or possible postmortem colonization and proliferation of the bone.

Implications on Health and Death

Bone abnormalities similar to those documented in this report have been documented in terrestrial and marine mammals and have been attributed to geriatric change, infectious agents, trauma, inflammation, auto-immune disorders, and other entities. At present, little is known about the progression of this condition in whales so we cannot estimate whether this infection developed over the course of several months or several years. To address the possibility that entanglement in fishing gear could be related to Whale #441's vertebral injuries, we looked for evidence of entanglement as determined in a systematic study of entanglement scarring in Southeast Alaska (Neilson et al. 2009). In that study, based on 2003–2004 photos, Whale #441 was coded as "E3" indicating a high probability of prior entanglement with minor tissue damage. Whale #441's caudal peduncle scars were typical of most whales in Southeast Alaska and did not suggest a severe entanglement that would have led to spinal deformities (J. Neilson, pers. comm. September 2020). No subsequent field observations of Whale #441 subsequent to this report suggested additional entanglement events. The observed changes in the bone are consistent with an infectious or inflammatory process, and were likely to have occurred as a consequence of infection of the tag site, although this is not conclusive. The extent and severity of the bone lesions suggest they may have influenced the mobility and health of Whale #441 and contributed to his decline in condition and ultimate death. It is thought that the ankylosing of the thoracic vertebrae, which would have resulted in reduced mobility and pain, may be indicative of long past and ongoing vertebral instability. We speculate that the spinal lesions

Whale #441 experienced may have limited his ability to bend like a typical humpback whale at the start of a dive, perhaps contributing to the tendency noted in his sightings record to not lift his flukes out of the water when diving.

The proliferative growth and ruptured disks in the caudal vertebrae also likely resulted in pain and reduced mobility. Impaired mobility may have impeded the ability of this whale to travel, maintain social contact with conspecifics, breed, or effectively pursue and capture prey, leading to reduced body condition over a long period of time. Additionally, whales typically shed external parasites by performing surface active behaviors (such as breaching or peduncle slapping), thus Whale #441 may have been subject to carrying a high parasite load as a result of his reduced mobility. It is possible that these osteologic abnormalities may be a primary cause of (or a contributing factor in) the extended period of chronic stress suggested by his endocrine data.

Conclusions

Evidence of nutritional stress, including emaciation and elevated ^{15}N in the most recently grown baleen and ear plug tissue suggests that poor nutritional condition was the proximate cause of the death of Whale #441. The ultimate cause of this poor condition is much more difficult to pinpoint. Repeated visits to the carcass revealed bone abnormalities that likely limited Whale #441's mobility and thus contributed to his poor condition. While we cannot conclusively state that the 1999 tag injury caused the bone anomalies, the proximity of the tag site to the infected bones suggests that during or after tag deployment, a microbial invasion may have occurred with secondary infection and extension to the underlying bone and draining lymph nodes (prescapular lymph node). However, it is not possible to confirm this route of infection in absence of apparent gross pathology to demonstrate a dissecting or tracking infection from the tag wound to the vertebrae.

The large scale ecological disruption and elevated levels of harmful algal toxins (HABs) resulting from the Northeast Pacific marine heatwave in 2013–2016 likely played a role in Whale #441's poor condition and ultimate death. The whale's feces had relatively high levels of domoic acid and saxitoxin compared to those found in other Alaskan humpback whales, but without known baseline values for large cetaceans and how these levels impact normal behavior and function in whales, it is impossible to say how great a factor HAB toxicity was in the whale's death. The whale's heavy external parasite load, dermatitis, toxoplasma, bacteria, yeast and diatom infections in the lymph nodes, and two different bone infections, along with an extended period of elevated adrenal stress hormones and reduced testosterone, are evidence of long term health issues. We recommend baleen stable isotope and endocrine profiles as standard analyses in future whale strandings, given that baleen plates are relatively easy to collect, store, and ship, and the valuable physiological context that the baleen profiles provided. The lifetime ear plug endocrine and stable isotope profiles further illuminated the timeline and additional factors contributing to this individual's health and eventual death. This collaborative work focused on an individual whale demonstrates the power of combining physiological, pathological, and life history data to advance understanding of baleen whales.

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Appendix A. Necropsy

CASE NUMBER: 2016094

OWNER: Glacier Bay National Park DFO

VETERINARIAN: Dr Stephen Raverty

DATE: June 28, 2016

GROSS FINDINGS

An adult male humpback whale with minimal reproductive activity is presented dead June 28, 2016 in poor post mortem (code 3.5) and fair to moderate body condition. There are reduced subcutaneous and visceral adipose stores and the animal is fairly muscled. There is generalized subcutaneous, thoracic and abdominal bloat and the penis is protuberant. On cut surface, the blubber stroma is prominent, dull yellow white and little oil oozes. The mandible is displaced to the right and the right body of the mandible is partially buried in the sand; there is massive distention of the tongue and oral mucosa by dark red fluid which readily drains on incision. Approximately 1 m caudal to the mandibular symphysis, there is a prominent, transverse skin fold which spans the mandibles and caudally is bound by massive fluid accumulation. Within the midventral region of the buccal pleats, there is a 15x10 cm multinodular sessile botryoid mass. There are large numbers of ectoparasitic crustaceans (whale lice) throughout the carcass with scattered superficial erosions, ulcerations and occasional small subcutaneous nodules. There are scattered cookie cutter shark scars. In the left dorsolateral aspect of the thoracic cavity involving ribs 4–10 and corresponding to the past tag implant site, there is massive focally extensive acute subcutaneous and blubber hemorrhage which peripherally merges to more normal blubber. The subjacent skeletal musculature is distended and on incision of the fascia, there is a moderate amount of frank hemorrhage and edema which oozes from the cut surface. Extending from the superficial aspect deep into the affected muscle, the fibers are individualized by abundant dark red slightly opaque fluid. The left retropharyngeal lymph nodes are moderately enlarged, multinodular and abscessing. The stomach is displaced cranially to the level of ribs 6–8 and the exposed portion is empty, the intestines are distended and taunt with gas and have a small amount of mucoid chyme multifocally adheres to the mucosa. The colon contains a moderate amount of feces and there is approximately 30 ml of turbid yellow brown urine in the bladder. There were no other apparent gross internal or external lesions.

Biological Data:

- Total length: 13.9 m
- Blubber thickness, anterior insertion of dorsal fin:
 - Dorsal: 8.5 cm
 - Lateral: 6 cm
 - Midventral: 11 cm

COMMENTS

Post mortem change and dorsal recumbency hampered gross examination of this animal. Due to the position of the animal it was difficult to assess the nutritional status. On incision of the blubber there was loss of oil and prominent fibrous connective, however, post mortem change and bloat may have contributed to seepage of oils through the epidermis. The epaxial musculature was also difficult to assess and further evaluation may await histopathology. Corresponding to the past tag implant site wound within the left dorsolateral region of the thorax, there is focally extensive subcutaneous and muscular hemorrhage and edema. These changes are more consistent with an acute agonal or terminal event, rather than chronic resolving tag wound. The hemorrhage may be attributed to blunt force trauma, possibly related to ship strike, attempted predation or some other process. Although close dissection of the affected area did not reveal any chronic inflammation, scar tissue or retained tag pedicles or barbs, it is possible that the position of the animal or hemorrhage and edema may have obscured a more chronic process related to long past tagging. The number of whale lice was significantly greater than other stranded humpbacks (particularly healthy ship strike) and may suggest generalized debilitation or immunosuppression; throughout the skin, there were areas of erosion and ulceration suggestive of compromised healing response. Involving the left retropharyngeal lymph nodes, there was generalized lymphadenomegaly with abscessation consistent with a localized bacterial infection. Bacterial colonization and invasion may have occurred within the naso- or oropharynx, from the skin (whale lice, cookie cutter shark bites, or tag wound), via the lymphatics or some other source and microbial identification may provide valuable insights into virulence and pathogenesis of infection. Skin lesions have previously been recognized in humpback whales and typically comprise vesiculobulbous to ulcerative entities; in this animal, within the mid ventral region of the pleats, there was a large bossylated mass, which may represent an infectious or benign neoplastic entity. Further evaluation is pending histopathology and ancillary studies.

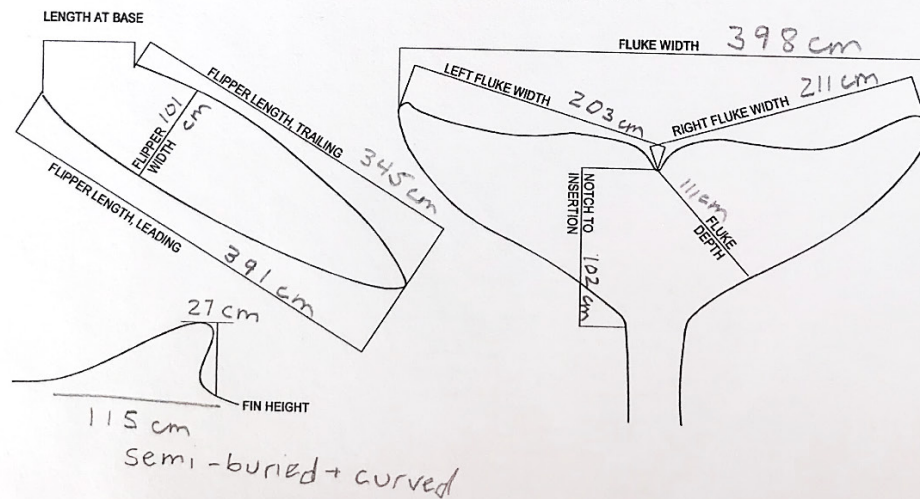
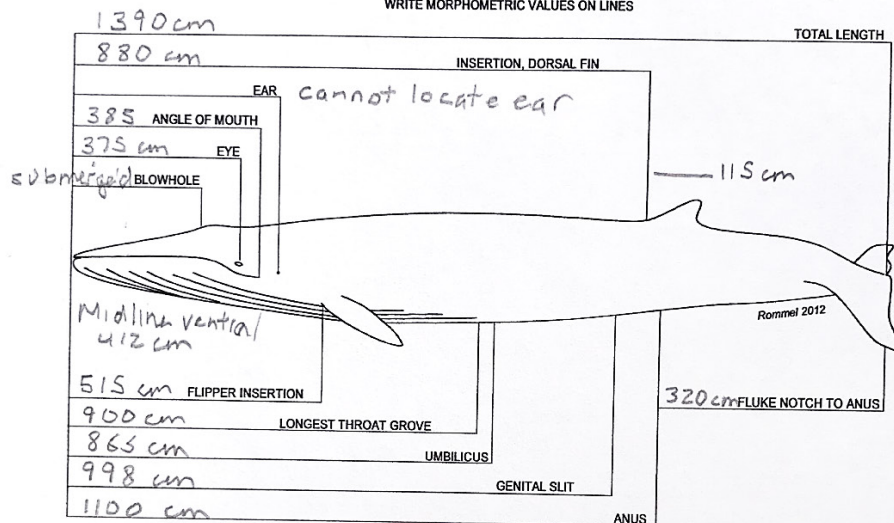
GROSS DIAGNOSES:

- 1) Carcass: Presumptive emaciation, generalized, moderate, with prominent stroma and little oil in the blubber
- 2) Thorax, left dorsolateral region: Hemorrhage, subcutaneous and blubber, marked, focally extensive with edema involvement of the subjacent skeletal musculature
- 3) Lymph node, left retropharyngeal: Lymphadenopathy, marked, diffuse with multifocal abscessation
- 4) Skin: Dermatitis, moderate, multifocal to coalescing, erosive and ulcerative with subcutaneous nodules and florid proliferation of ectoparasitic copepods, *Chalimus* spp

Balaenopterid External Morphometrics

Field ID# 2016094 Date 6/28/2016 Observer S. Raverty

WRITE MORPHOMETRIC VALUES ON LINES



CETACEAN GROSS NECROPSY FORM

(transcribed from handwritten forms)

Case: 2016094 **Species:** *Megaptera novaenangliae*

Date of Necropsy: 6/28/2016 **Sex:** Male

Location of Necropsy: North Passage, Dundas Bay area, Alaska

Date of Stranding: 6/26/2016

Stranding Location: Point Carolus, Icy Strait, Alaska

Pathologist: Stephen Raverty

Assistants: Janet Neilson, Christine Gabriele, Louise Taylor-Thomas, Jennifer Helm, Tania Lewis, Luke Williams, Michelle Fournet. Additional helpful bystanders: Anya Maier, Christina Senac (photos), Linnea Lentfer, Hank Lentfer. Additional photography by Sean Neilson, Andrew Peacock.

Data Recorder: Louise Taylor-Thomas

Case History: Floating dead whale reported by Paul Johnson (M/V Yakobi) on 6/26/2016, carcass towed to shore and secured by NPS later that day.

Necropsy arranged by NMFS with Dr. Stephen Raverty, DVM. Necropsy began 11 AM, crew departed from beach 16:35. Great weather conditions; clear skies, light breeze.

External Examination

Body condition: Fair body condition.

Postmortem code 3 to 3.5. Moderate decomposition: carcass is slightly bloated, tongue and/or penis protruding, skin may be starting to peel or slough, eyes are sunken, tissues feel less firm.

External structures and observations: Penis distended, significant bloating, internal organs partially liquefied. Very few barnacles.

Searched for cause of in vivo swelling / lump but it was buried in the sand. Dorso-lateral left side. Abnormal pink stuff underneath the blubber. #484 skeletal muscle sample is the possible impact site. Observations inconsistent with chronic inflammation.

Internal Examination

1) Body Cavities (chest and Abdomen)

2) Cardiovascular system

Heart muscle liquefied (not 100% certain because accessed through ribs, elbow deep).
Probably heart, palpated chest cavity

3) Respiratory System

Samples collected from lung tissue

4) Reproductive system

5) Urinary System

Urine samples collected in syringes. 20 L of urine, turbid yellow-brown

6) Immune System

lymph nodes retropharyngeal (5 samples collected). Cascelating, Brucella potential, lymphadenopathy with abcessation. Pus-filled indicative of bacterial content.. Close to skin, significant.

7) Endocrine System

8) Central Nervous System

9) Sensory Organs

Left eye collected on 6/27/2016

Ear plug collected by C. Gabriele and A. Maier and stored in formalin

10) Digestive System

Intestines are gas inflated diffusely. Stomach protruded into midlevel of thoracic cavity but the diaphragm appeared intact “bloat”. Colon contained a moderate amount of copper-brown fecal material

11) Musculoskeletal System

Right mandible displaced (two personnel worked 1+ hour to locate)

12) Additional Comments or Observations

Animal was in dorsal recumbancy, slightly tilted to the right, head partially buried in sand. Satellite tag site was inaccessible but we attempted to sample the swelled area visible *in vivo*.

Left side observations:

Fair body condition. massive abdominal and thoracic bloat. Great deal of gas released. Intermandibular bruising. Retropharyngeal lymph node casealating. Left mandible intact

Right side observations:

Bruising on right ventral snout. Right mandible displaced: wondered in notes whether palatine bone was attached to nasal bones.

Dorsal and ventral observations:

Transverse inversion, lower jaw invaginated 85 cm caudla to tip of rostrum. Subcutaneous bruising on peduncle. Barnacles in ventral pleats. Sampled mandible, intermandibular muscle

Blubber Thickness: at anterior insertion of dorsal fin

- D 2: 8.5 cm (low confidence, difficult)
- D 3: 6 cm
- D 6: 11 cm

Head Observations:

Left side of head: Search for earplug taking longer than expected (?). Muscle appears to separate from skull easily. L hemorrhaging at rear of skull, occipital vertebra C1 and skull muscle.

Postmortem decomposition. Left mandible periosteum hemorrhage

Right side of head: Foreshortening of right mandible. Haemorrhage of masseter muscle. Right mandible displaced to the right, oriented to the sand and buried.

LIST OF SAMPLES:Large Pail of formalin

Mandible

Mammary tissue, unlabeled

3 pc stomach, unlabelled

good blubber

bruised blubber

mid tag site (blubber?)

skeletal muscle from tag site

fascia from tag site

cranial skeletal muscle

skeletal muscle at cranial margin

Jar of formalin

1 barnacle

1 skin tag

1 lateral blubber

Deep skeletal muscle mid

Cranial deep skeletal muscle

Skeletal muscle from cranial margin

Fascia at tag site

Bronchii

Jar of formalin

Left side ear plug

Whirl-paks – formalin

3 bags of ear lymph labelled as earwax or earplugs

1 scar

possible bone or ear plug fragments

blood vessel

On Ice

2 skin tags

2 scars

3 @ 5 ml cryovials of urine

2 nalgenes of blood

2 vials feces

1 nalgene of urine

1 barnacle
2 lateral blubber
5 retropharyngeal lymph nodes
1 bone shards (ear plug)
2 skeletal muscle
2 urinary bladder
2 heart muscle
2 lung

Appendix B. Histopathology Report

Alaska Veterinary Pathology Services (AVPS)

Kathy Burek, D.V.M, M.S.

Diplomate, A.C.V.P.

23834 The Clearing Dr. / Eagle River, AK 99577

Work/Cell: (907) 242-2566/ Fax: (907) 696-3565 / Email: AVPS.KBH@gmail.com

FINAL HISTOPATHOLOGY

SPECIES: Humpback whale (*Megaptera novaeangliae*)

SEX: Male **AGE:** Adult (66 years by ear plug)

ANIMAL ID/NAME: 2016094

CONTACT: Christine Gabriele

VETERINARIAN: Stephen Raverty

CLINIC: NMFS / MMHRSP / NOAA – National

ADDRESS: 1315 East-West Hwy. F/PR

Silver Spring, MD 20910

Ph: 3017132322

Fax: 3017130376

Email: Teri.Rowles@noaa.gov

CASE NUMBER: V16-074

SAMPLE DATE: 06/28/2016

TRIM DATE: 09/18/2016

DATE FINAL: FINAL 6/21/18.5/20/20

PATHOLOGIST: Kathy Burek

NUMBER OF BLOCKS: 44

PENDING: nothing

PERMIT INFO. 18786 PERMIT HOLDER: MMHSRP

SPECIMEN: multiple tissues PRESERVATION: 10% NBF

GROSS DIAGNOSES

1. Presumptive emaciation / poor body condition, generalized, moderate, with prominent stroma and little oil in the blubber
2. Thorax, left dorsolateral region: Hemorrhage, subcutaneous and blubber, marked, focally extensive with edema involvement of the subjacent skeletal musculature (Consistent with ship strike)
3. Lymph node, left retropharyngeal: Lymphadenopathy, marked, diffuse with multifocal abscessation
4. **Musculoskeletal system (found long after initial necropsy)**
 - a) **Multifocal vertebral body bony proliferation consistent with severe, chronic neoplasia or osteomyelitis**

5. Integument:

- a) Dermatitis, moderate, multifocal to coalescing, erosive and ulcerative with subcutaneous nodules and florid proliferation of ectoparasitic cyamids
- b) Heavy Cyamid parasitism
- c) 15x10 cm multinodular sessile botryoid mass – ventral midline at pleats

HISTOPATHOLOGIC DIAGNOSES

1) SYSTEMIC:

- a) Atrophy of fat, generalized, marked

2) MUSCULOSKELETAL:

- a) Multifocal, severe, chronic osteomyelitis with intralesional fungal hyphae
- b) acute degenerative myopathy with contraction band necrosis, mandible

3) INTEGUMENT:

- a) Dermatitis, chronic active, eosinophilic, severe, skin tag
- b) Focal dermatitis with infolding and pseudo-epitheliomatous hyperplasia, chronic, lymphoplasmacytic with intra epithelial intracytoplasmic inclusion bodies (poxvirus?)
- c) Ulcerative dermatitis, chronic active focally severe
- d) Cellulitis, focal, pyogranulomatous, severe with intralesional yeast – area of the ear plug

4) LYMPHOID:

- a) Chronic, severe, lymphadenitis with intralesional bacteria, and yeasts (bacterial – *Edwardsiella tarda*, *Granulicatella balaenopterae*; Fungal – *Malassezia restricta*)

SUMMARY DIAGNOSIS

This animal was in poor body condition and had a very chronic, severe osteomyelitis involving several of the Thoracic and Caudal vertebrae likely due to a fungal infection. There was also a yeast and bacterial lymphadenitis in the prescapular LN. There were also significant levels of domoic acid and saxitoxin in both urine and feces.

RECOMMENDATIONS

1) Special stains

- a) Slide #26 – PAS and Giemsa DONE
- b) Slide 24 – PAS DONE

2) EM on block # 27 CANCELLED

3) Histopathology, Brucella and fungal PCR on samples collected later from the bones DONE

4) Specials: PAS and GRAM in Slide #45, 47, 51 DONE

5) Recut and coverslip #50 – Not possible due to bone / rocks and 47

6) Viral culture at DFO – CANCELLED

7) Fungal PCR on Bone at ILLINOIS – DONE

BRIEF HISTORY

This Male, Adult Humpback whale from Icy Strait, Gustavus, AK (58.301378 N ; -136.239008 W) SEA was necropsied on 06/28/2016. This animal was a known animal whale identified by fluke as Festus or #441. Post mortem state was Fresh autolysis.

GROSS FINDINGS

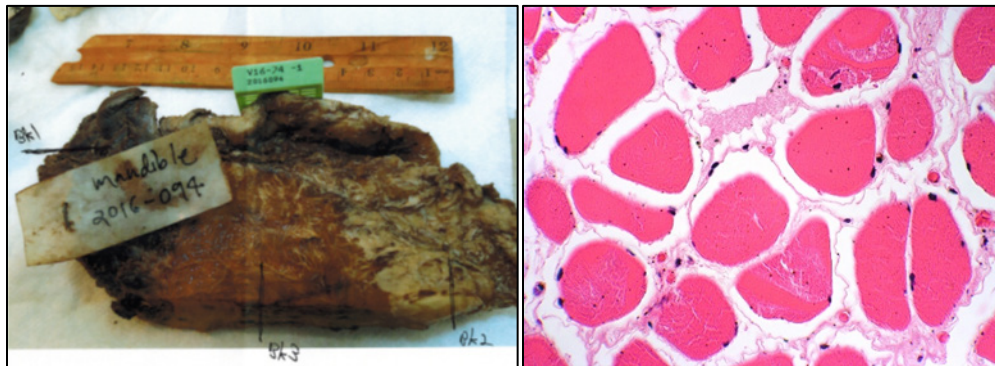
See Necropsy report

HISTOPATHOLOGIC FINDINGS

60 tissues were trimmed into 44 blocks on at HCS. The following tissues were examined

Slide 1 – Mandible Lesion

There is heavy postmortem overgrowth. Myofibers are widely separated by what appears to be edema. Myofibers are highly variable in size and eosinophilia. Many demonstrate fragmentation. There is also frequent contraction band necrosis acute degenerative myopathy with contraction band necrosis.



Tissue sample (left) and 40X (right)

Slide 2 – Mandible Lesion

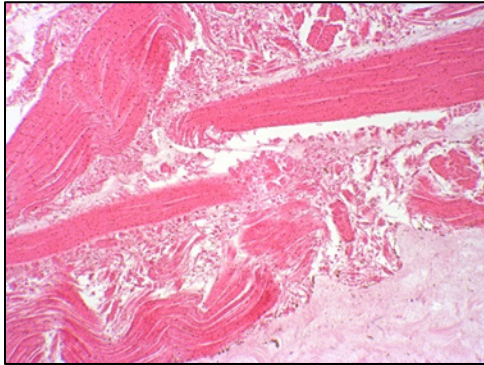
This tissue is composed of many collagenous bands separating lobules of adipose tissue. The adipocytes appear shrunken with some possible serous change, though this could be postmortem artifact.

Slide 3 – Mandible Lesion

This tissue is composed of intersecting bands of dense collagen (fascial tissue). Some of the tissue along the edges appears shredded with irregular debris on the surface (postmortem artifact?). There is no associated hemorrhage or inflammation.

Slide 4 – Left Mandible Lesion

This tissue is composed of bands of degenerated skeletal muscles separated by amorphous lightly eosinophilic material. Intact red blood cells are not appreciated. There are large numbers of postmortem bacteria within the blood vessels. Many of the muscle fibers are disintegrating and contraction bands are not evident (postmortem change).



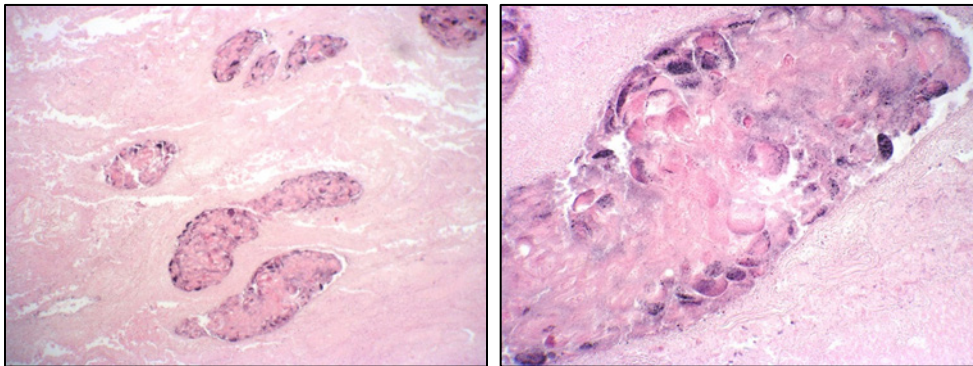
40x

Slide 5 – Tag site – Muscle

In this tissue, there are bands of skeletal muscle separated by amorphous eosinophilic material. Neither free intact red blood cells nor contraction bands are appreciated.

Slide 6 – Lung

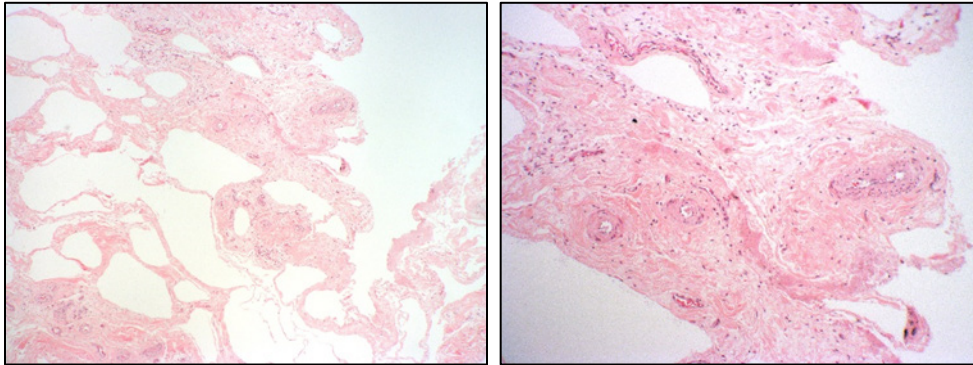
There is marked autolysis. There are pockets of large round oval-shaped structures which are partially mineralized. These appear to be within blood vessels or bronchi. They are not in the pattern to be cartilaginous rings



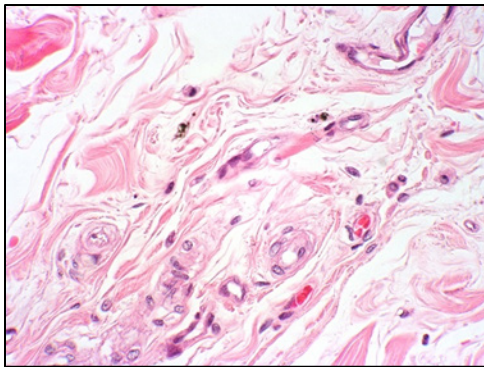
10x (left), 40x (right)

Slide 7 – Fascia – tag site

This tissue is in surprisingly good postmortem condition. It is composed of clear areas separated by collagenous stroma. Many of these bands of stroma are highly vascularized occasionally in the pattern almost reminiscent of a rete system with all different vessel types ranging from arteries through veins with many small delicate capillaries. There are rare foamy macrophages but no other inflammation.



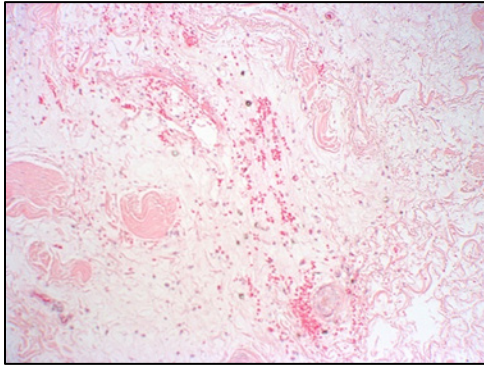
4x (left), 10x (right)



40x

Slide 8 – Fascia

This is similar to slide 7. There is one region in which there is very small numbers of free red blood cells (acute hemorrhage).



10x

Slide 9 – Bronchus

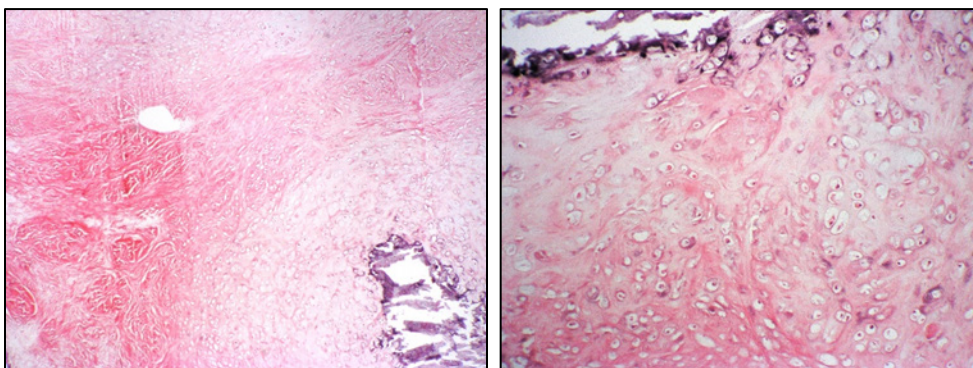
There are more of the round balls of the mineralized structures as indicated in slide 6. These appear to be within blood vessels. They are similar to the mineralized cells within the bronchial cartilage. Although structures are markedly disrupted by autolysis.

Slide 10 – Oropharynx

The epithelium has sloughed. There are no obvious free intact red blood cells within the tissue.

Slide 11 – Oropharynx

In this section, there is a large nodule within the deeper tissue composed of irregular nodules of partially mineralized cartilage. (Could this be part of the larynx?).

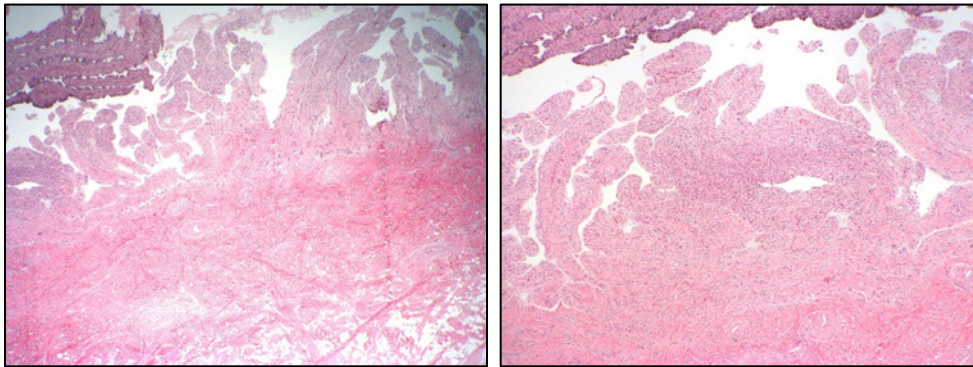


4x (left), 10x (right)

Slide 12 – Lesions – Possible tag site

This tissue is composed of epithelium and superficial subcutaneous tissue. The subcutaneous tissues are composed of many irregularly shaped and sized collagen bundles separating small amounts of adipose tissue. There are focal areas of acute hemorrhage within this area. In the epithelium, there are moderate to focally severe amounts of inflammation within the dermal papillae. Most of these cells are eosinophils. In the central core of this section, this inflammatory reaction extends throughout the entire dermal papilla and into the superficial dermis.

Dermatitis, chronic, focally severe, eosinophilic.



2x (left), 4x (right)

Slide 13 – Lesions – Possible tag site

This is the deeper part of this area. It is composed primarily of subcutaneous tissue down to the deep fascial layers. Again, there are bands of very highly vascularized tissue running through areas of adipose. There is also amorphous eosinophilic material separating tissues with no obvious intact red blood cells. No foreign body was appreciated.

Slide 14 – Muscle – gingival margin?

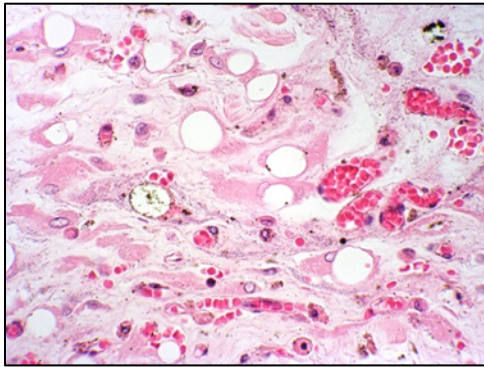
This tissue is composed of skeletal muscle bands separated by amorphous to granular eosinophilic debris. There are no obvious intact RBCs. The muscle fibers are fragmented and there are rare suggestions of contraction band necrosis.

Slide 15 – Muscle WNL

This tissue is composed of muscle bundles separated by light fibrovascular stroma and very little fluid. There is no fragmentation or evidence of contraction band necrosis. There is heavy postmortem overgrowth.

Slide 16 – Skin – Jaw

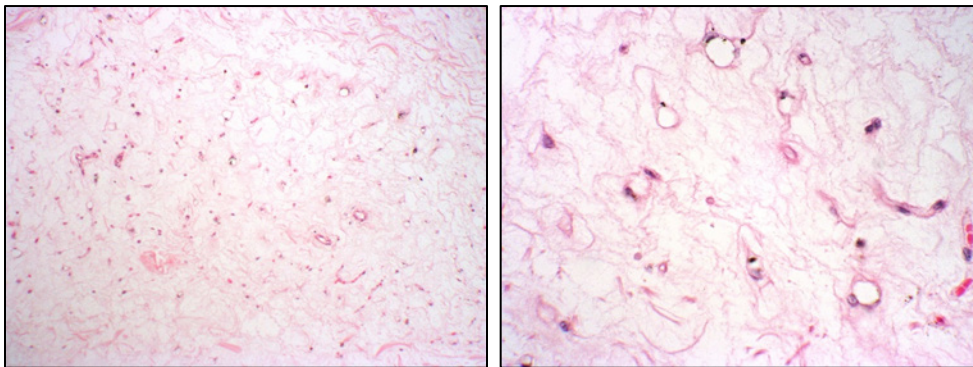
Is a section of epithelium which has sloughed from the underlying dermis. the tissues are markedly congested, and fibers are separated by fluid. There are very rare free red blood cells within the tissue. Some of the adipocytes have abundant eosinophilic cytoplasm.



40x

Slide 17– Blubber – Mid tag

Mostly adipocytes do not contain significant lipid droplets. The ones that do contain lipid droplets have a distinct rim of eosinophilic cytoplasm. **Atrophy of fat, generalized, marked.**



10x (left), 40x (right)

Slide 18 – Blubber – WNL

Marked atrophy of fat

Slide 19 – Muscle – Cranial

There is mild fragmentation but no contraction band necrosis.

Slide 20 – Lesions – Possible tag site margin

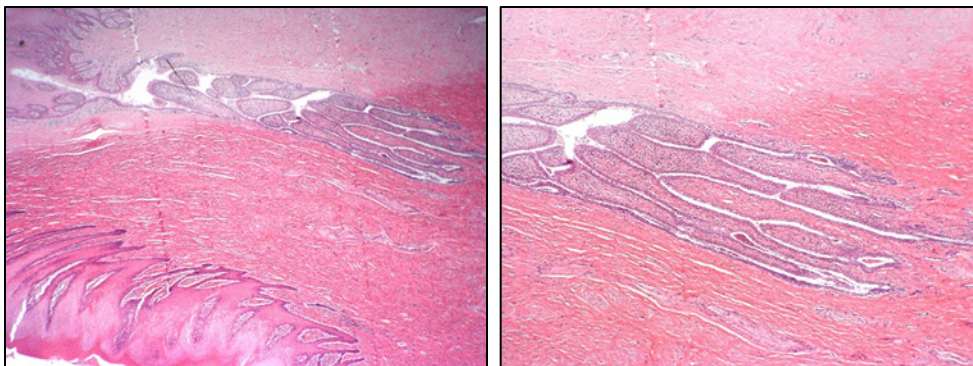
There is minor acute hemorrhage. There is one very large blood vessel in the section. Otherwise it is composed of atrophied adipose tissue in collagen.

Slide 21 – Lesions

This tissue is composed of dense dermis with a cleft fully lined by epithelium. On one edge, there is pseudoepitheliomatous hyperplasia extending the dermis and chronic inflammation composed of lymphocytes and plasma cells subjacent to the epithelium. there are no inclusions to suggest papilloma virus or poxvirus. the main larger cleft extending into the epithelium is also mildly inflamed.



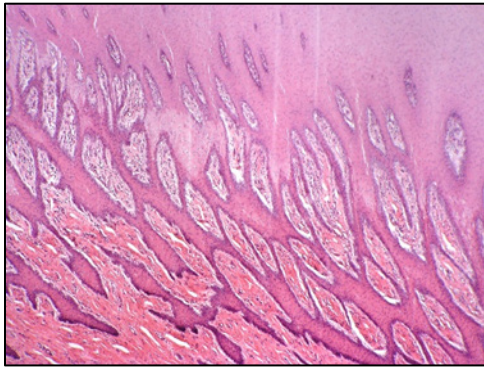
Lesion photo



2x (left), 4x (right)

Slide 22– Lesions

In this section. There were extensive areas of pseudoepitheliomatous hyperplasia with broad branching and anastomosing rete pegs extending into the underlying dermis. This is a mild lymphoplasmacytic inflammation.



4x

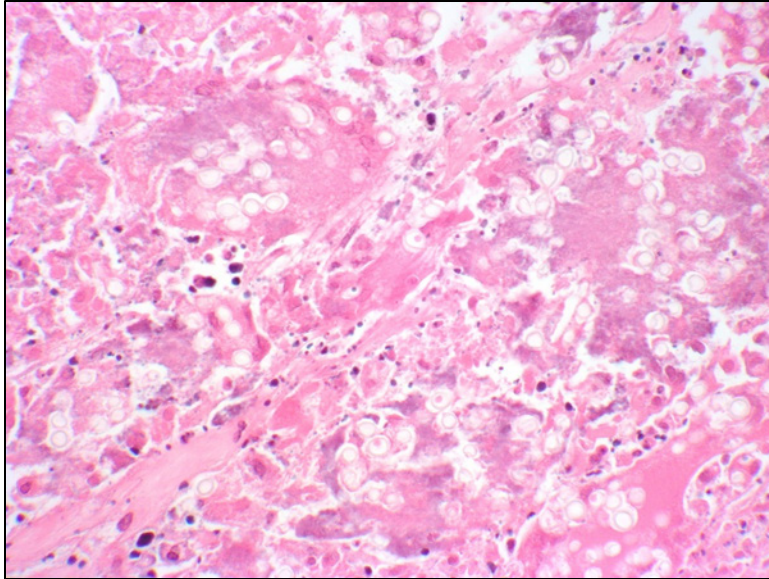
Slide 23– unidentified tissue

This tissue has lost most cellular detail due to autolysis. It does appear to be composed of bands of either muscle or collagen separated by looser tissue. It is most likely more fascia or subcutaneous tissue.

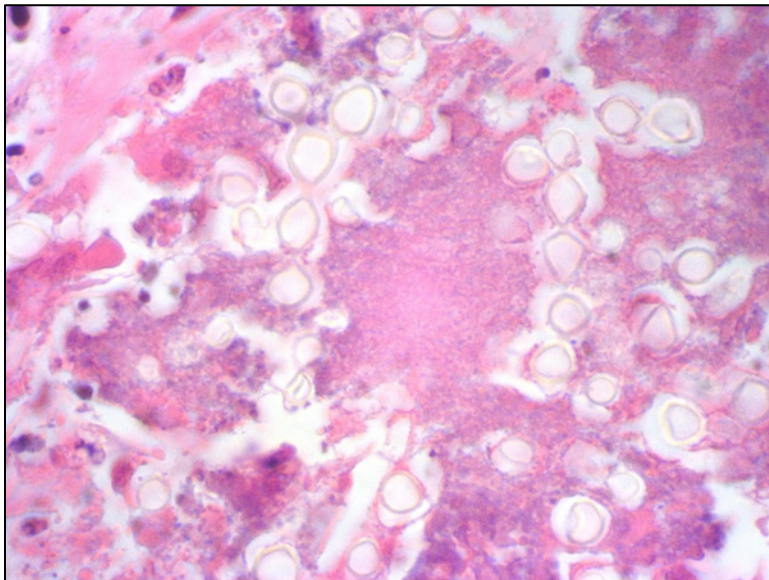
Slide 24– Untagged (probable lymph node)

This is a round, encapsulated structure composed of islands of tissue, debris and mineralized material. These islands are composed of multinucleated cells, some degenerated cells, fibrin, necrotic debris and yeast-like organisms

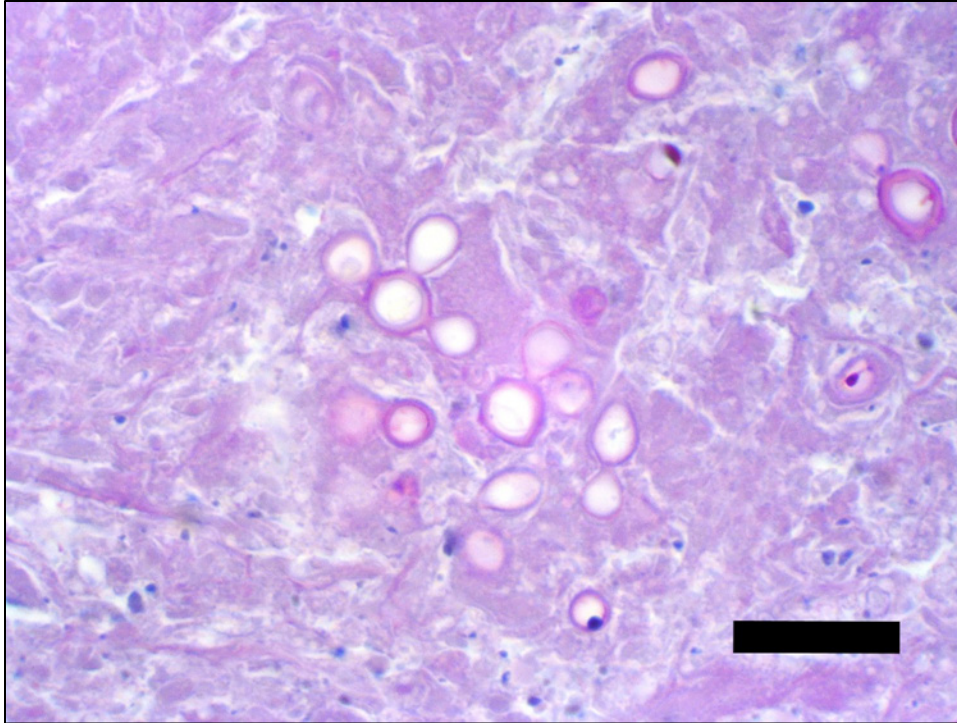
These organisms often form chains of round to oval to lemon shaped refractile bodies with narrow-based budding and occasionally a central small eosinophilic body. These bodies are approx. 7.8x 10.4µm.



40x



100x



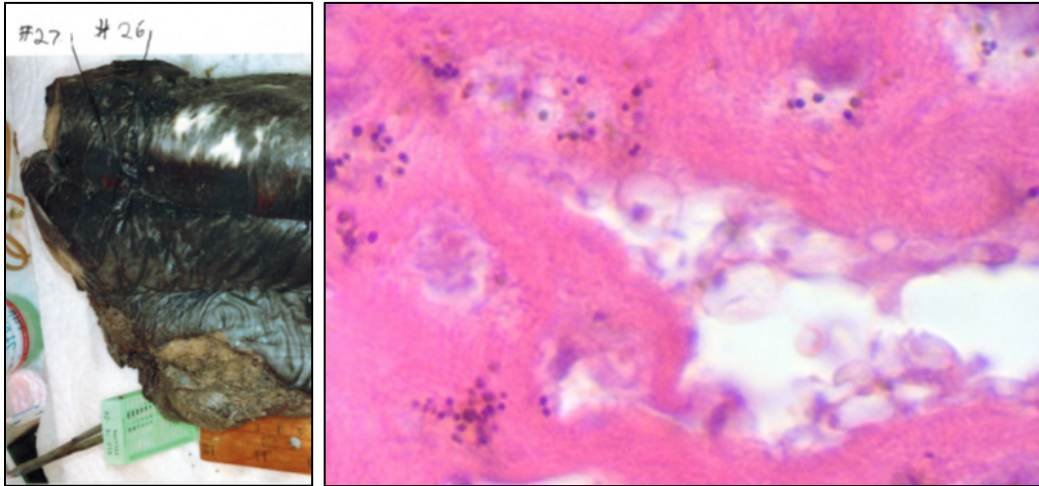
100X. Bar = 20 microns. Mycetoma (probable lymph node), composed of granulomatous inflammation and yeasts or fungi. PAS Stain – There are many narrow based budding yeasts which are roughly ovoid or "lemon" shaped. Occasionally they will form a rosette like pattern. And approximately 7.5 μ m long.

Slide 25– Unknown tissue

There is too much autolysis to identify this tissue.

Slide 26– Skin section

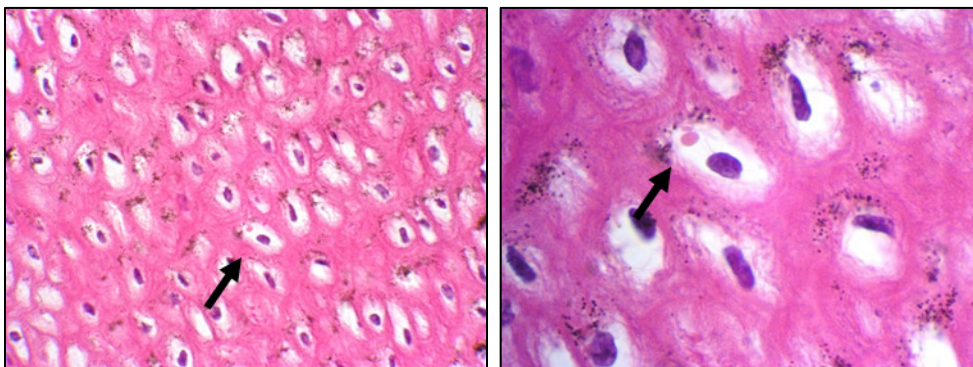
This is a section of skin in which there is hyperplasia of the epithelial rete pegs. This is characterized by irregular anastomosing fusion and thickening of the rete pegs. There is also very minor lymphocytic plasmacytic inflammation within the tips of the dermal papillae. Many the epithelial cells are vacuolated. In one section, there is marked thickening of the stratum corneum and in one area, this has become clefted, forming an intra-corneal vesicle. There are occasional aggregates of clear bodies with a single basophilic nucleus within the cornified cells adjacent to the cleft. (ciliates? Protozoa? Other?). These do not stain with PAS or GMS.



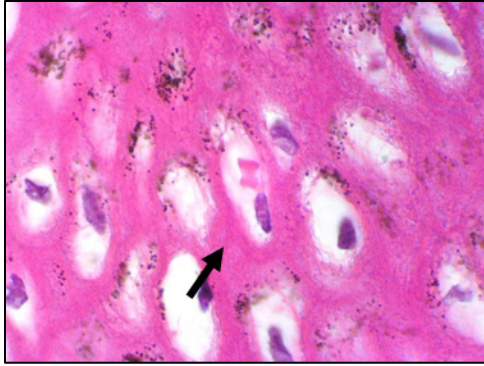
Skin photo (left), 100x (right)

Slide 27– Skin section

This section of epithelium is massively hyperplastic with wildly thickened and anastomosed rete pegs. The surface is much roughened and within the disturbed epithelium are more of the small clear bodies (protozoa? Ciliates?) as described in slide 26. There are also very rare eosinophilic intracytoplasmic inclusions in vacuolated epithelial cells (arrows). These were demonstrated by EM to be keratin inclusions with no virus present.



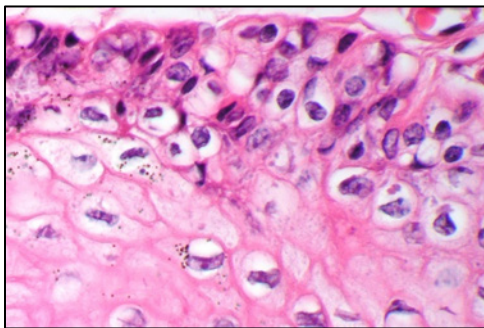
40x (left), 100x (right)



100x intracytoplasmic inclusion.

Slide 28– Skin section

This section is a piece of dermis with an in-folded, epithelium lined tract. There is minimal inflammation. The epithelium lining has an exuberant amount of deeply eosinophilic cornified epithelium under which is markedly vacuolated epithelium. Many of the vacuolated cells have an intracytoplasmic inclusion (viral inclusion or degenerative cytoskeletal materials).



40x

Slide 29– Skeletal muscle unknown site

In this section, the muscle fibers are widely separated by edema fluid and is otherwise unremarkable.

Slide 30– Encapsulated mass

See slide 24 there are massive numbers of fungal organisms

Slide 31– Unknown tissue

This is a cannot be identified due to postmortem change

Slide 32– Barnacles

This is a section of dermis and epithelium on which is an attached parasite. In the superficial dermis, there is very little inflammation. Inflammation present is composed of pigmented macrophages and rare eosinophils. This organism is heavily mineralized internally, has a thick cuticle, has a pseudocoelom lined by polymyarian and coelomyarian musculature lining the walls as well as internal striated skeletal muscle.

Slide 33– Possible Ear plug

This section is composed of collagen separated by wispy connective tissue. There is an encapsulated area within which are accumulations of necrotic debris, multinucleated giant cells with phagocytosed yeast-like organisms identical to those described in slide 24. **Cellulitis, focal, pyogranulomatous, severe with intralesional yeast**

Slide 34– Possible Ear plug

See above – pyogranuloma

Slide 35– Possible Ear plug

This tissue is composed of very dense collagenous fibrous tissue. There is also a central core of pyogranulomatous debris and yeast like organisms.

Slide 36 – Scar

This is a section of epithelium with a central ulcer and an underlying region of very well organized, inflamed granulation tissue. The ulcer is colonized by which appear to be mixed bacteria / primarily cocci. There are also pockets of necrotic debris and possible ciliates similar to slide #26 deep within the tissue. Request Giemsa and PAS stain.

Some of the superficial vessels have fibrin clots within them. The epithelium adjacent to the ulcer is highly vacuolated and hyperplastic with exocytosis of neutrophils. Ulcerative dermatitis, chronic active focally severe.

Slide 37 – Ear plug fragments

This tissue is composed of amorphous eosinophilic and slightly Brown tinged material (grossly and histologically consistent with ear plug).



Ear plug fragments

Slide 38 – Blood vessel

There is marked autolysis. There is a central large vessel surrounded by very dense collagenous material.

Slide 39 – Fascia – Tag site

See above

Slide 40 – Skin tag

Dermatitis, chronic active, eosinophilic, severe, skin tag.

Slide 41 – Muscle – Cranial deep

Marked autolysis. Muscle fibers are widely separated by amorphous presumably fluid material. Intact RBCs are not appreciated.

Slide 42 – Muscle-Cranial deep – Margin

This is muscle with muscle bundles separated by clear spaces, atrophied adipocytes and rare macrophages. The blood vessels are packed full of bacteria.

Slide 43 – Muscle-Cranial deep mid

Markedly autolyzed muscle.

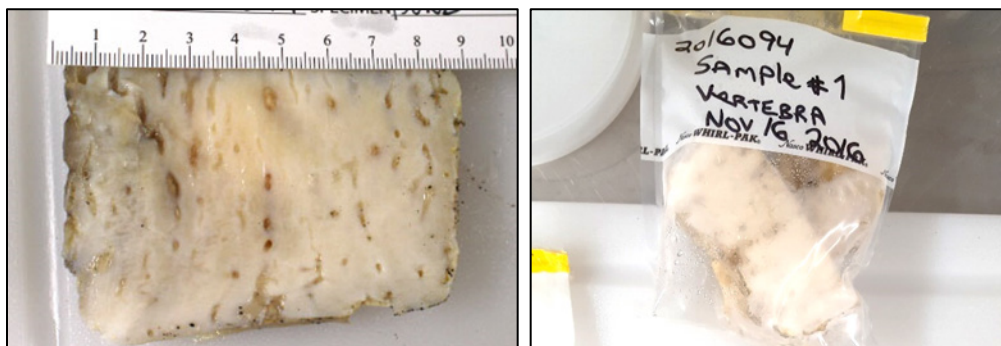
Slide 44 – Skin

This is a section of appears like a polyp with a central core of inflamed collagen covered by a layer of thick epithelium.

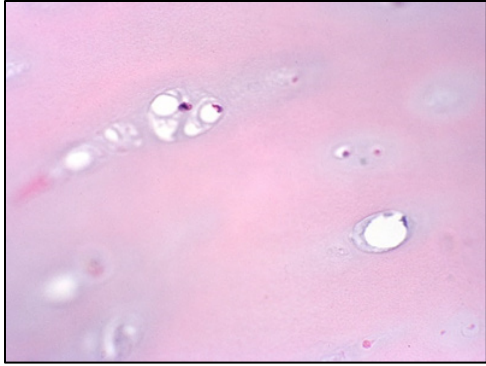
Slide 45 – Bone Sample #1 11/16/16

The tissue is consistent with cartilage. Characterized by abundant ground substance within which are embedded lacuna containing generally one sometimes multiple cells. There are scattered patches of what appear to be consistent bacterial colonies as well as mixed bacteria and what appear to be Protozoa. There is no cellular reaction (postmortem change). No fungal organisms consistent with that seen in the retropharyngeal lymph node are found.

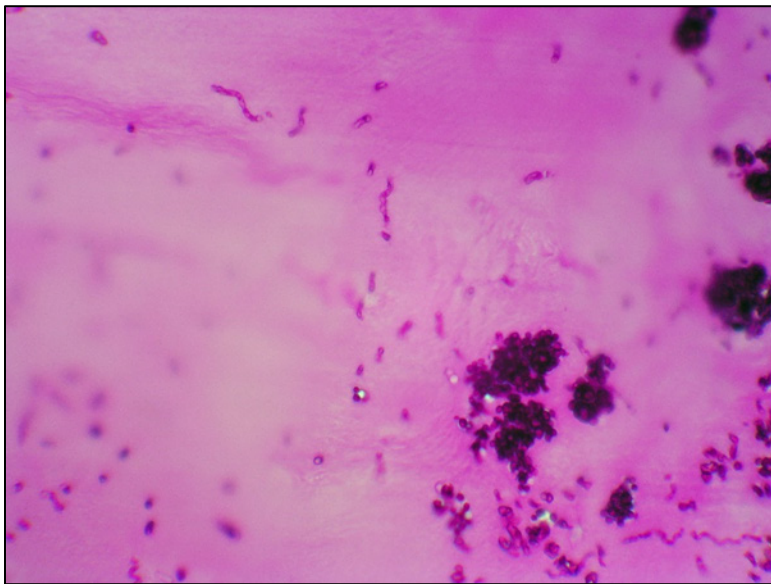
These Structures do not stain with gram stain; however, the PAS stain demonstrates fungal hyphae and yeast-like structures infiltrating throughout the tissue. The hyphae have irregular, non-parallel walls with rare septae and irregular angles.



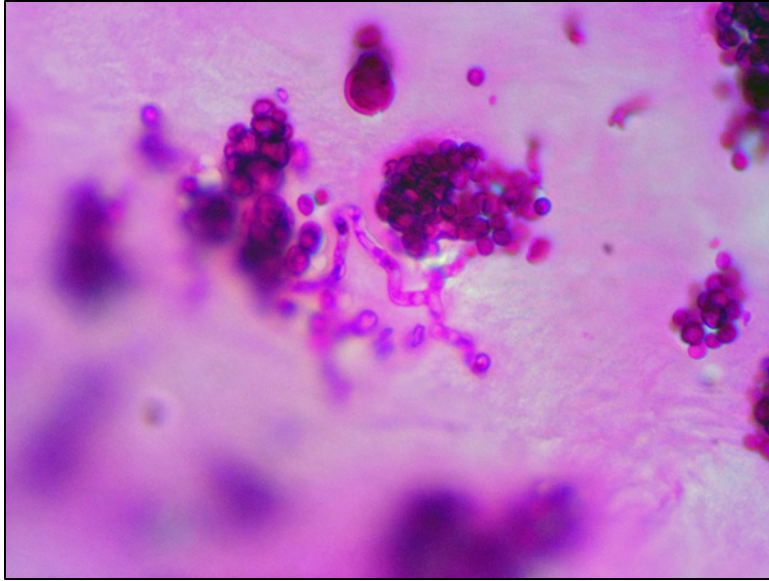
Bone sample fragments



40x



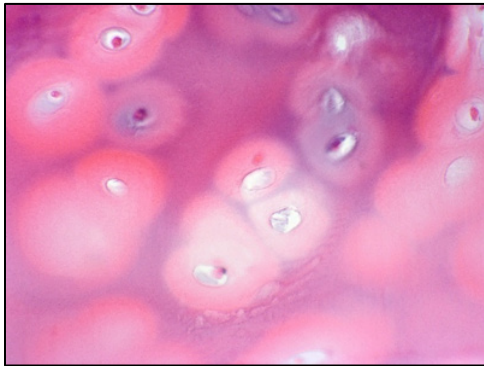
40x



100x

Slide 46 – Bone Sample #1 11/16/16

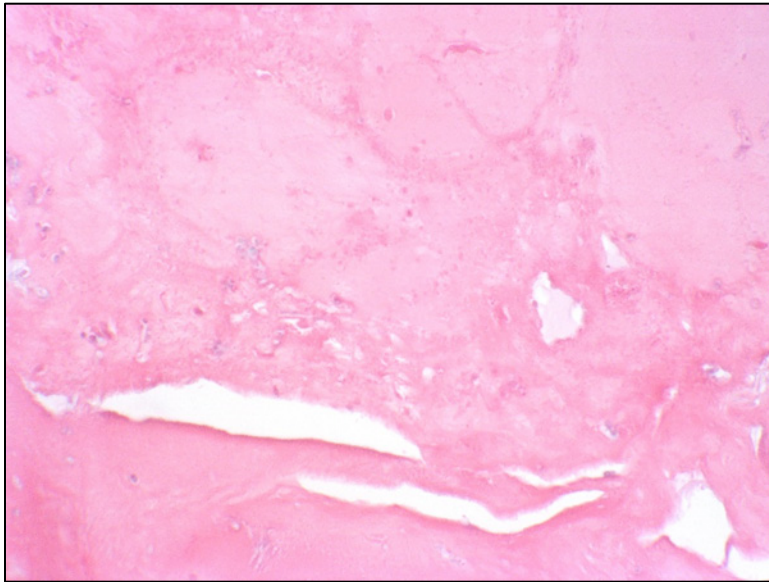
In some areas, the ground substance is discolored deeply basophilic more consistent with formation of bone and there is evidence of heavy striations in the cutting likely related to fragments of bone within the tissue.



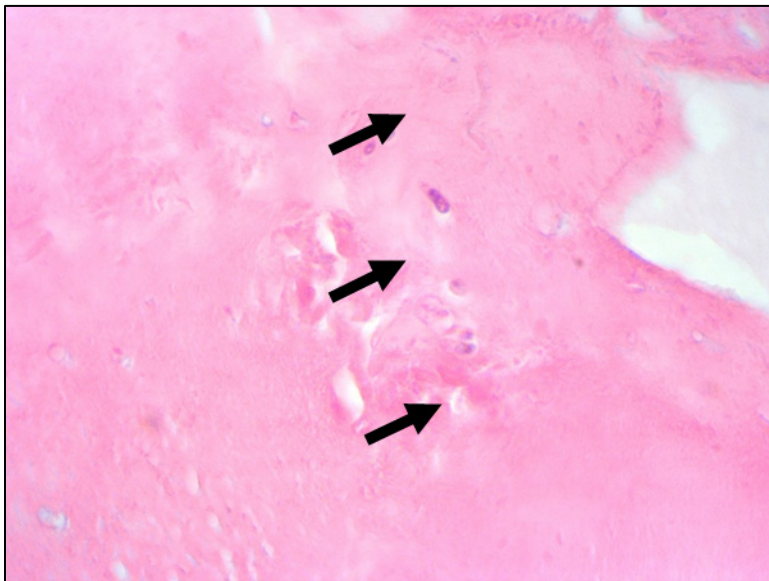
40x

Slide 47 – Bone Sample #1 11/16/16

In this section, there is a disrupted area of the cartilage within which are refractile round oval to possibly hyphae of fungi. With postmortem change is not possible to determine whether inflammatory cells are also present. PAS and Silver stain.



40x



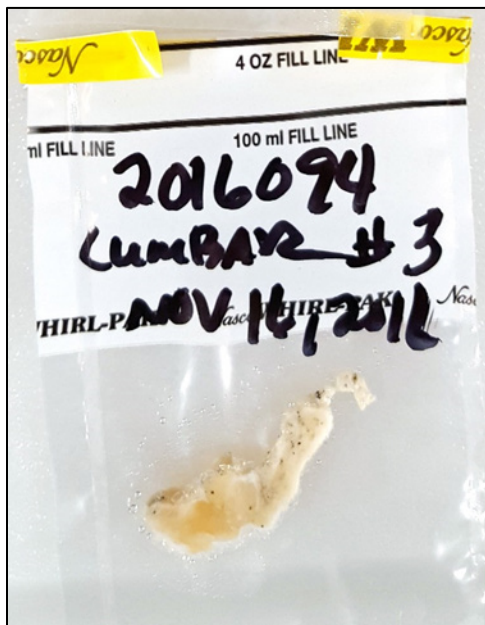
100x. There are many gram-positive rods consistent with Clostridia on gram stain. Many of these areas are riddled with fungal hyphae similar to that described above. (Arrows)



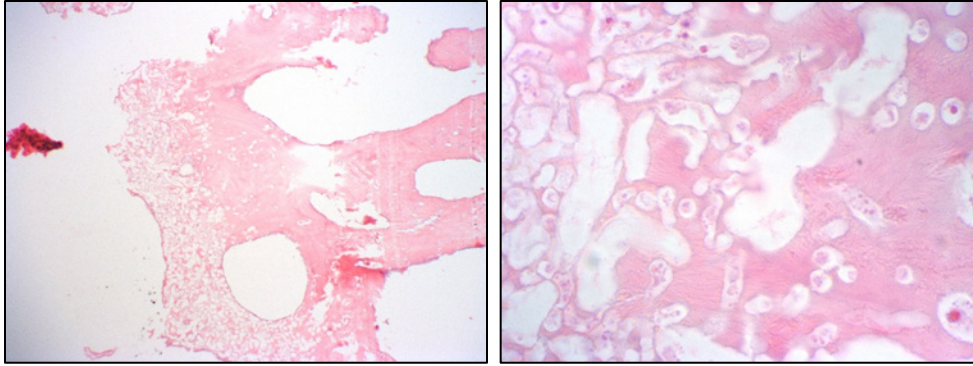
40x

Slide 48 – Bone – Lumbar #3 11-16-16

This is woven bone with loss of most of the cellular structure due to postmortem change. The edges are “moth-eaten” with some disruption that appears to be cellular (could be protozoa, or inflammatory cells...). Suspect osteomyelitis.



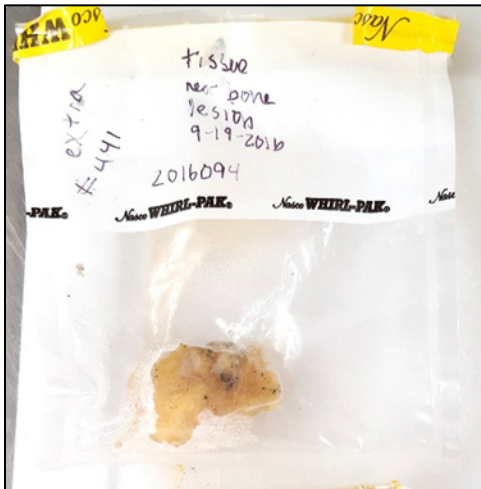
Bone fragment



10x left, 40x right

Slide 49 – Tissue near bone 9-19-16

This is composed of parallel layers of very dense collagen with no indication of inflammation (well healed granulation tissue).



Tissue sample

Slide 50 – Tissue near bone 9-19-16

These are very dense interlacing bundles of collagen (scar / Granulation tissue).

Slide 51 – Skin And blubber

Inadequate cover slipping. There are massive amounts of sand, etc. in the section.

Slide 52 – Bone From infected vert caudal 4

Woven bone; There are mixed bacteria on the surface (postmortem change) but no suggestion of inflammatory cells.

Slide 53 – Bone From infected vert caudal 4

Very dense interwoven bundles of collagen. Some areas form lacunae suggestive of fibrocartilage (proliferative reparative response).

Slide 54 – Bone From infected vertebra (U)

Woven bone

Slide 55 – Bone From infected vert caudal 4

The edges are again very mottles with s suggestion of cells within the peripheral tissue along with heavy mixed bacterial growth. Postmortem change? Or osteomyelitis.

Slide 56 – Bone From infected vert caudal 4

Too much sand – couldn't cut

ANCILLARY DATA**Table B-1.** Bacteriology.

Lab short name	Sub type of test	Sample tested	Lab_RPT_No	Agents	Pos/Neg	Measured result
Athens Vet Diagnostic Lab	PCR	Lesion Swab, RPLN, Tissue near bone, Osteomyelitis sample, and lesion 1 (cartilage / bone)	A17-13999 A17-38934	Brucella sp.	NEG	–
	Aerobic	RPLN	A17-13998	Edwardsiella tarda	POS	Light
	Aerobic	RPLN	A17-13998	Gram + bacilli Light Beaded, branching org; Granulicatella Balaenoptera by 16S rRNA gene 1406 bp sequence	POS	light
	Fungal PCR	RPLN Osteomyelitis bone	A17-13998 A17-38934	NEG	–	–
	Fungal PCR	RPLN	A17-13998 A17-38934	NEG	–	–
U of IL	FUNGAL PCR	RPLN	Navicula Vara (a diatom!) and Malassezia restricta	POS	–	–
	FUNGAL PCR	Multiple sections of bone	OPEN	NON-DIAGNOSTIC	Too much postmortem overgrowth to characterized	Too much postmortem overgrowth to characterized

Table B-1 (continued). Bacteriology.

Lab short name	Sub type of test	Sample tested	Lab_RPT_No	Agents	Pos/Neg	Measured result
UCDAVIS – MICRO	Aerobic	RPLN	16M09999	Edwardsiella tarda	POS	–
CANADA – S. RAVERTY	PCR	RPLN	16-5437	Brucella sp. and Morbillivirus	NEG	–
	Aerobic	RPLN	16-5437	Open	NEG	–

Table B-2. HABS.

Lab short name	Type of test	Sub type of test	Sample tested	Lab_RPT_No	Agents	Pos/Neg	Measured result
NWFSC – HABS	HABs	Abraxis (saxitoxin)	Feces	18227_17 DQ27	PSPT – STX	POS	100.18 ng/g
	HABs	Domoic Acid ELISA	Feces	18227_17 DQ27	Domoic acid	POS	356 ng/g
	HABs	Abraxis (saxitoxin)	Urine	16507_17 AW40	PSPT – STX	Neg	BDL
	HABs	Abraxis (saxitoxin)	Urine	16507_17 BG4	Domoic acid	POS	2.7 ng/g

Table B-3. NUTRITION: THIAMINE – Auk Bay Laboratory see comments.

Sample tested	Lab_RPT_No	Agents	Pos/Neg	measured result	Comment – Normal – chicken liver =
Muscle	373116	Thiamine	LOW?	0.04 nmol/g	14.06 nmol/g
Muscle	373116	Thiamine (Total)	LOW	1.11 nmol/g	16.3 nmol/g
Muscle	373116	Thiamine monophosphate	WNL?	0.22 nmol/g	0.00 nmol/g
Muscle	373116	Thiamine pyrophosphate	LOW?	0.85 nmol/g	2.23 nmol/g

Table B-4. PARASITOLOGY.

Lab short name	Type of test	Sub type of test	Sample tested	Agents	Pos/Neg
NIH – Mike Grigg	Parasitology	PCR	Heart	Sarcocystis monodontidis	POS
NIH – Mike Grigg	Parasitology	PCR	Heart	Toxoplasma gondii	POS

COMMENT: The most interesting finding in this animal as a severe lymphadenitis and cellulitis in which were very large numbers of an unknown yeast or fungus. The parameters and shape of this organism matched most closely with *Lacazia loboi*, which is very odd since that occurs only off the coast of Florida and south America in the Atlantic Ocean and causes primarily dermatitis and subcutaneous lesions. The next closest would be *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*. This can cause systemic fungal disease however occurs primarily in central and south America. This was a chronic illness, so it is possible this animal could have traveled to these areas to pick up this organism but still very surprising. This organism was identified as the yeast *Malassezia restricta* by PCR.

This animal was also very thin and had evidence of atrophy and degeneration of the fat stores. This could either be due to lack of prey, but in this case, could also be related to chronic disease.

The lesion shown in slides 26–28 was suggestive of a pox-like proliferative lesion with intralesional intracytoplasmic inclusion bodies. I'm not sure which lesion on the animal these slides are from (see photo). Since I'm not sure where it was I'm not sure whether I have frozen tissue for this lesion. Electron microscopy indicated though that the inclusions were composed of degenerated keratin vs viral.

I have also not cut in yet the various samples from the vertebra yet for testing and histopathology. It would be very interesting if this is also affected by this organism. Hopefully the post mortem change will not render those samples unreadable. They will be submitted for fungal and Brucella PCR.

ADDITION: 6/21/18

Of interest, there was also an unusual bacterium characterized from the lymph node, *Granulicatella Balaenoptera*, which was sequenced by 16S rRNA gene 1406 bp sequence. This organism and others in the *Granulicatella* have occurred in some human cases of vertebral osteomyelitis, valvular endocarditis, septicemia and other systemic infections and is a possible emerging pathogen (*Christensen and Facklam, 2001; Liao et al., 2004; Rosenthal et al., 2002; Senn et al., 2006; Woo et al., 2003*). It would be good to do the same work up if we see a beaded gram-positive bacterium in some of these later samples. Gram stains have been ordered.

Histopathology of the later samples did not demonstrate fungal / yeast-like organisms similar to those seen in the lymph node. There were aggregates of bacteria and either yeast, fungi or protozoa, which are most likely post mortem contamination, however the cellular detail was very poor. This makes it very difficult to be definitive about the significance of organisms in a very rotten sample.

ADDITION: 7/26/18 and 9/10/8/18

Special stains supported a massive fungal infection within the areas of abnormal bone from the lumbar area. This supports the idea that the vertebral lesions were due to a chronic fungal osteomyelitis. This fungus had hyphae or pseudohyphae and had a very different morphology from the lymph node fungus. This fungus was more suggestive of a *Candida* type of yeast with pseudohyphae and blastoconidia.

At AVDL, The osteomyelitis lesions and the retropharyngeal lymph node was negative by Fungal PCR, however at Univ of Ill, the lymph node was positive for *Navicula Vara* (a diatom!) and *Malassezia restricta*. The shape and size of the object in the lymph node fits both *Navicula* sp. and *Malassezia*, however *Malassezia* has been shown in other species to be a pathogen which usually cause skin disease, but can become systemic in rare cases in humans with immunosuppression and a variety of chronic conditions (Gaitanis et al., 2012). At University of Illinois the bone samples had too much postmortem overgrowth to identify a fungus.

It is interesting that this animal had two separate lesions with fungi or yeast. This suggests possible immunosuppression or overwhelming exposure to the pathogens. Cases of *Candida* osteomyelitis have been described, with vertebra being the most common site of (Arias et al., 2004; Gamaletsou et al., 2012). Unfortunately, the fungal PCR on bone indicated too much post mortem overgrowth to be diagnostic.

ADDITION: 5/20/20

Domoic acid (DA) and saxitoxin (SXT) levels were both positive in urine and feces in this animal with the fecal DA and SXT quite high compared to other animals. The significance of this finding in terms of the mortality are unknown, but it is reasonable to suggest they could have been a factor in the immediate death.

Table B-5. Lymph node organism.

Organisms	Size	Pathology	Geologic dist.
Our organism	Approx. 7.5 µm long	–	–
<i>Sporothrix schenckii</i>	2–6 µm	Lymphocutaneous form; dermal to SQ nodules and rarely spreads to LNs	Worldwide in temperate and tropical areas.
<i>Lacazia loboi</i>	5–12 µm	SQ and dermal masses	Florida, Brazil Mexico – Atlantic tropical
<i>Paracoccidioides brasiliensis</i> and <i>Paracoccidioides lutzii</i> .	5–15 µm	Lymphadenitis, pneumonia, other systemic granulomatous	central and South America (from Mexico to Argentina) showing high prevalence in Brazil, Colombia, Venezuela and Argentina
<i>Navicula Vara</i>	7–8.5 µm x 3.5–4.5	Diatom – no known pathology	Arctic benthic
<i>Malassezia restricta</i>	about 7 x 2.5 microns	Yeast – skin and systemic disease	–

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NPS 132/175241, April 2021

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