NOAA Technical Memorandum NMFS-NWFSC-133

Wild Animal Mortality Investigation:
Southern Resident Killer Whale L-112
Final Report

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October 2016

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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Reference this document as follows:

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Executive Summary

On 11 February 2012, a 3.75-m juvenile female Southern Resident killer whale (SRKW, *Orcinus orca*), identified as L-112, stranded just north of the town of Long Beach, Washington. The following day the carcass was taken to a secure location at Cape Disappointment State Park for a full necropsy by biologists and volunteers from the Northwest Marine Mammal Stranding Network (Network). This report reviews the data from the necropsy, histopathology, and computed tomography (CT) examinations, as well as results from ancillary diagnostic tests. Available information on environmental data and human activities was compiled and evaluated to assess possible contributions to the loss of the animal.

Gross examination revealed that the whale was moderately decomposed but in good nutritional condition. Extensive subcutaneous bruising was present on the back of the head and neck, which extended deep into the adjoining musculature and tracked along the hypodermis to the throat region. On the right side, bruising extended to the anterior insertion of the pectoral fin. Initial estimates of time of death were from 4–10 days prior to the stranding.

A detailed dissection and CT scan of the head confirmed the gross observations of hemorrhage and revealed extensive gas and fluid accumulation. No skull fractures were noted. No fractures or dislocations of the bones of the middle and inner ear were seen. A CT scan of the cervical vertebrae was also performed. There was incomplete fusion of the dorsal aspect of the C7 vertebral body that was attributed to a congenital anomaly and was not considered clinically significant. No broken bones were found on preparation of the skeleton. There was, however, a long linear crack on upper right jaw tooth #13, but based on its gross appearance and orientation, it was not considered a consequence of terminal trauma.

Microscopic review of the sampled tissues was hindered by the extent of postmortem decomposition. Based on the degree of autolysis and mass spectrophotometry results of sampled tissue gases (Woods Hole Oceanographic Institution, Massachusetts), the gas accumulation (emphysema) throughout the carcass was attributed to putrefaction rather than gas bubble-type disease as previously reported in stranded beaked whales (family *Ziphiidae*) exposed to naval sonar or common dolphins (*Delphinus delphis*) with submerged blast injury. On dissection of the inner ear from the skull, a parasite (nematode) infection was observed around the right bulla, with associated inflammation detected microscopically. There was mild, chronic inflammation of the gastrointestinal tract and low- to intermediate-grade accumulation of scar tissue (fibrosis) in the heart, liver, and kidney. None of these were sufficiently severe to be assigned as a specific cause of death. Extensive molecular (polymerase chain reaction, or PCR), bacteriological, virological, and toxicological tests were undertaken, but no significant disease agents or pathologic entities were identified. The light bacterial growth recovered from harvested tissues was attributed to postmortem tissue invasion and overgrowth. Results from heavy metal and trace mineral analyses of the liver and kidney were largely within normal reference ranges, and persistent organic pollutant levels in blubber were within anticipated levels for SRKWs.

The nematodes found in the right bulla were identified as *Crassicauda* sp. Although ear infections with *Crassicauda* are more commonly recognized in smaller stranded cetaceans, detection in this animal is not unusual and would likely not have interfered with normal inner ear function (i.e., would not have caused deafness or vestibular dysfunction).

Stomach contents analysis revealed six fish eye lenses and nematode fragments identified as *Anisakis* sp. cf. *A. simplex*. PCR analysis of feces from L-112 detected Chinook salmon (*Oncorhynchus tshawytscha*) and halibut *(Hippoglossus stenolepis*), consistent with the dietary preferences of SRKWs.

We reviewed data collected by autonomous passive acoustic recorders deployed off the coasts of Washington, Oregon, and California by the NOAA Northwest Fisheries Science Center. In addition, we also reviewed an acoustic monitoring report on data from High-frequency Acoustic Recording Packages (HARPs) deployed by the U.S. Navy in the Navy's Northwest Training Range Complex, as well as reports from researchers of detections by acoustic recorders deployed in Haro Strait, Washington. These devices monitor underwater sounds, including ambient noise, anthropogenic sounds, and marine mammal calls. Killer whales were detected along the coast around the time of the stranding. Acoustic recordings of L Pod calls indicated the presence of a large group of L Pod whales near Point Reyes, California, on 30 January 2012, and near Fort Bragg, California, on 31 January 2012. On 5 February, acoustic recorders deployed by local researchers in Haro Strait, Washington, detected calls from K or L Pod whales on the west side of San Juan Island. Also on 5 February 2012, K or L Pod calls were detected off Westport, Washington, indicating multiple groups of K and/or L Pod whales in Washington waters. On 7 February 2012, a group of K and L Pod whales were photographed in Discovery Bay, Washington, but members of L-112's immediate family were not seen there. On 20 and 21 February, calls similar to those detected off Westport on 5 February were detected off Newport, Oregon.

Weather and sea-surface data for coastal Oregon and Washington waters (1–11 February 2012, coincident with the anticipated time of death of L-112) and drift patterns established for the Columbia River plume suggest that L-112's body had either been carried for some days in the Columbia River eddies or drifted north from farther south on the Oregon coast on the prevailing south winds and currents, before being cast on the Long Beach Peninsula. These data lead to a hypothesis that L-112 was somewhere between central and northern Oregon when she died. No other killer whales were reported stranded in association with this case, and there was no evidence of other dead mammals, birds, or fish in the area at the time of L-112's discovery.

Based on findings from the gross examination of the carcass and the absence of conclusive histopathology or ancillary test results, the investigative team identified blunt force trauma as the primary consideration for the acute death of the animal. However, the nature of the blunt trauma could not be determined. L-112 was hit, struck, or rammed in the head or neck, but the animate or inanimate source of the blow could not be determined based on postmortem examination.

NOAA received reports of sonar activities from hydrophone operators in Puget Sound preceding the stranding. Reporting parties expressed concerns that military activities may have been involved in the stranding. NOAA requested information on sonar, underwater explosive activities, and boat strikes in the Northwest from the Royal Canadian Navy, the United States Navy, the United States Army, and the United States Coast Guard. NOAA also requested information on human activities from additional organizations that operate or authorize activities off the coast. The Network investigators examined the circumstances of the stranding (single individual), environmental evidence, and information about human activities, and ruled out several possible sources of the traumatic injury, including the following:

1. Sonar and small underwater explosive activity was confirmed by the Royal Canadian Navy on 4, 5, and 6 February 2012 in Canadian waters off Vancouver Island and in the Strait of Juan de Fuca, but no marine mammals were observed during the training activities. The naval activities occurred approximately 340 kilometers to the north (downwind) of the stranding location, making blast injury as a result of explosive detonations from the exercise an unlikely contributor to the stranding.

2. The U.S. Navy reported that no domestic naval training activities involving sonar or explosives were conducted between 1 and 11 February 2012 in the Northwest Training Range Complex (which includes Washington, Oregon, and northern California).

3. The U.S. Army reported that no army-related training or military activities were carried out on the coast in early February.

4. Northwest Fisheries Science Center (NWFSC) hydrophone recorders off Newport, Oregon, and Westport and Cape Flattery, Washington, did not detect sounds from mid-frequency sonar or explosions in early February.

5. The U.S. Coast Guard reported that no vessel-related whale strikes were reported in early February from Oregon through Grays Harbor, Washington, and they were not aware of any explosives being used in the area. No ship strikes of any whales in Oregon or Washington waters were reported to NOAA.

6. The Fishing Vessels Operators Association reported that their member fishing vessels typically do not begin fishing off the lower coast (Washington, Oregon, and California) until March or April and were unlikely to be present during January to February 2012. No reports involving killer whales were submitted to NOAA's Marine Mammal Authorization Program during this time period.

7. The Army Corps of Engineers reported that there were no in-water construction projects involving pile driving or explosive activities, nor were there any scientific buoy installations or dredging projects being conducted along the Oregon and Washington coasts in this time period.

In conclusion, blunt trauma to the head and neck is the prime consideration for the cause of mortality. Despite extensive diagnostic evaluation, the cause of the head and neck injuries could not be determined.

Acknowledgments

This work was funded in part by the John H. Prescott Marine Mammal Stranding and Rescue Assistance Grant, as well as through additional NOAA funding for killer whale stranding response. We thank the numerous biologists, veterinarians, veterinary technicians, and Northwest Marine Mammal Stranding Network volunteers who assisted in this examination. We extend special thanks to Dr. McKlveen's staff (Claire Oliphant, Karli Anisoglu, and Noel Goldman) for their help with the CT scans. Special thanks also to the Portland State University and Seaside Aquarium teams that responded so quickly to L-112, arranged for her transport by Hill's Towing to a secure location, and helped with photography during the necropsy (Keith Chandler and Tiffany Boothe), and our sincere thanks to the Rangers at Cape Disappointment State Park for providing a necropsy site and help with logistics. Dalin D'Alessandro of the Northern Oregon/ Southern Washington Marine Mammal Stranding Network was a major player in the collection and distribution of tissues, initial stomach content analysis, and organizing and coordinating the necropsy. Thanks to William Walker of NOAA Northwest Fisheries Science Center for help with parasite identification. SRKW are listed as endangered under the Endangered Species Act, so response and investigation activities were accomplished under authorization granted in MMPA/ ESA Permit Number 932-1905, issued to the NOAA Fisheries Marine Mammal Health and Stranding Response Program.

Introduction

The Northern Oregon/Southern Washington Marine Mammal Stranding Network received a report of a stranded female juvenile killer whale (*Orcinus orca*) at 07:00 on 11 February 2012, 0.9 miles (1.4 km) north of Cranberry Road, Long Beach, Washington (lat 46.41°N, long 124.06°W). Keith Chandler of Seaside Aquarium, Seaside, Oregon, responded and collected Level A data, including photographs (Figures 1 and 2). He also had the animal moved (Figure 3) to a secure area in Cape Disappointment State Park, both to protect the carcass from vandalism and to provide a suitable site for the necropsy.

The whale was subsequently identified as Southern Resident killer whale L-112, based on photographs of the dorsal fin and saddle patch that biologists from the National Marine Fisheries Service (Seattle, Washington) and the Center for Whale Research (Friday Harbor, Washington) matched to catalogs of known killer whales.

To fully investigate the stranding of L-112, a member of the endangered Southern Resident killer whale population, a multidisciplinary team conducted a gross examination and full necropsy, including a suite of diagnostic tests. In addition, the team evaluated information on the sighting history of L-112 and environmental factors prior to the time of the stranding, to identify the geographic area and timing of mortality. This report compiles all available information on L-112, the gross and histopathologic findings, and additional test results, to inform our assessment of pathologic factors contributing to the whale's death. We also examine the context of the stranding, including both environmental factors and human activities, to assess the potential cause of death.

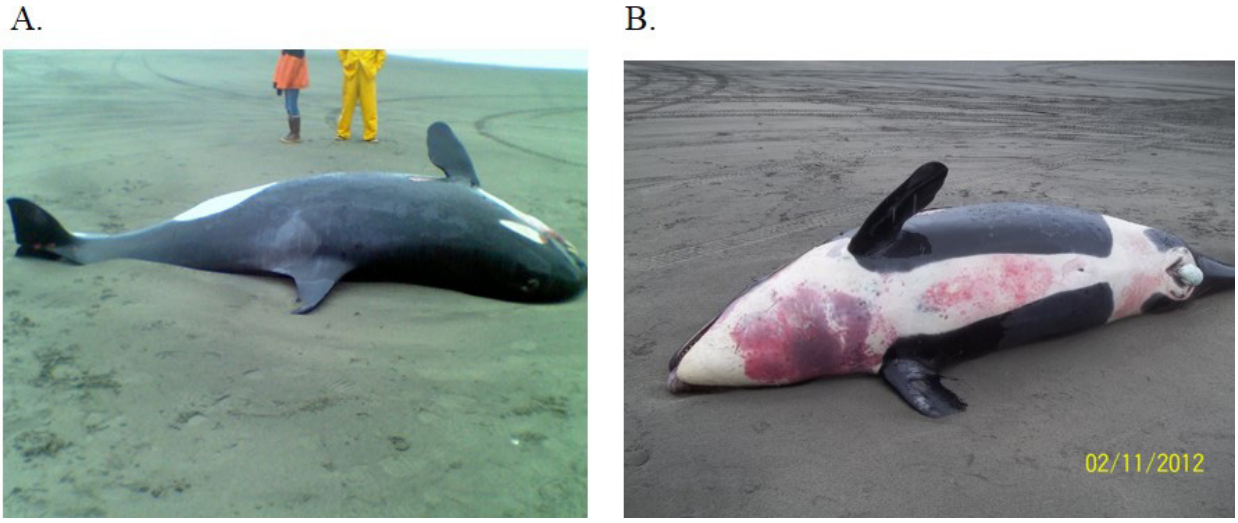

Figure 1. Female killer whale L-112 stranded on Long Beach, Washington, 11 February 2012. A) L-112 lying on right side. Note hemorrhage on left side of head near gape and "rub" posterior to flipper. B) Note hemorrhage on ventral surface of head, neck, and anterior chest.

Figure 2. Detailed photograph of extensive hemorrhage on left side of head.

Figure 3. The whale was moved to Cape Disappointment State Park for necropsy.

Background

The majority of Southern Resident killer whale (SRKW) sightings are documented during the spring and summer months (May through October) in the Salish Sea (the marine waters of Washington and southern British Columbia east of the entrance to the Strait of Juan de Fuca). SRKWs increase their use of the outer coastal waters in the fall through early spring as evidenced by their lack of occurrence in the Salish Sea. SRKWs are observed only intermittently from October to early January in Puget Sound. It should be noted that L Pod, the largest pod, is known to split up and travel for extended periods in sub-groups.

Killer whale sighting information is gathered from a number of sources, including the Center for Whale Research, whale watchers, researchers, and citizens who report through sighting networks such as Orca Network. The data is compiled into an "Orca Master" data set by the Whale Museum at Friday Harbor. The Center for Whale Research analyzes demographic and sighting data to compile an annual census of the SRKW population. Individuals in the population are identified using an alphanumeric code indicating kinship and birth order. Individuals may also be named.

Vocal communication is advanced in killer whales and is an essential element of the species' social structure. Killer whales produce numerous types of vocalizations for navigation, communication, and foraging (Dahlheim and Awbrey 1982, Ford 1989, Ford et al. 2000). Killer whale vocalizations comprise numbers and types of repetitive discrete calls which together are recognized as dialects (Ford 1991). Dialects are stable over time and unique to single pods. Call patterns are also distinctive within matrilines (Miller and Bain 2000). Similarity between dialects likely reflects the degree of relatedness between pods, with variation building over time as matrilines and pods grow and split (Ford 1989, Bigg et al. 1990, Ford 1991).

L-112 (Sooke) was first photographed with her mother, L-86, on 6 February 2009 off Victoria, British Columbia, by K. Balcomb III. He estimated at that time that she was 6 to 8 weeks old and born in December 2008. She was possibly sighted earlier—off Depoe Bay, Oregon, on 29 January 2009—but there are no photographic records to confirm this sighting. At the time of the stranding, L-112's immediate family consisted of L-86 (mother, age 21) and a 7-year-old male sibling, L-106. The extended family, which includes L-27, L-55, L-82, L-103, L-109, and L-116, forms a sub-group called the L4s, one of several different sub-groups of the larger L Pod.

The 2011 census of the Southern Resident killer whale population indicated that L Pod numbered 42 individuals on 31 December 2011, prior to the stranding. Most of the time, members of L Pod do not travel together as a single group. During her lifetime, L-112 and her immediate family traveled with several family groups that represented roughly half of the population of L Pod. Consequently, the L-112 sighting information below includes her immediate family, but not necessarily all of L Pod.

After the initial sightings in January and February 2009, L-112 was not seen again until 21 June 2009. Sightings continued throughout the summer of 2009, and the last sighting occurred on 9 October 2009 off the west side of San Juan Island. There were no documented sightings of L Pod during the winter of 2009–2010. On 25 May 2010, L-112 was seen in Haro Strait during L Pod's first summer appearance in the Salish Sea. The last sighting of L-112 in 2010 was 6 December off the south end of San Juan Island as the whales were heading west into the Strait of Juan de

Fuca. During the winter, all members of L Pod were sighted several times between 10 and 13 February 2011 in the San Francisco and Monterey Bay areas. L-112 was next documented on 29 May 2011 in the Canadian Gulf Islands, and photographed numerous times in Washington inland waters during the summer of 2011. The last images of L-112 prior to her stranding were taken on 21 October 2011 south of Discovery Island, British Columbia, heading toward San Juan Island. Subsequent movements of L Pod whales in late 2011 to early 2012 were detected by passive acoustic recorders, as shown in the Relevant Historical and Environmental Factors subsection.

Methods and Investigators

A multidisciplinary team responded to the stranding, conducted a gross examination, and reported basic information on a Level A data sheet (Appendix A-1). Investigators conducted a necropsy following a specific protocol developed by Raverty and Gaydos (2004), and collected a suite of samples for a variety of analyses to be performed at different reference laboratories. In addition, the investigators conducted computed tomography (CT) scans of the head, the bones of the middle and inner ears, and the cervical spine (post-skeletal preparation), with a subsequent detailed head examination. Following the necropsy, the skeleton was cleaned and prepared for display. To put the examination and sample analysis results in an ecological context, the investigators collected and requested information on environmental factors and human activities. Below is a list of methods used to investigate the stranding of L-112 and information on the individuals and organizations that participated in different aspects of the investigation.

Gross Exam and Necropsy

- Dr. Debbie Duffield, Portland State University, Oregon (Principal Investigator)
- Cascadia Research Collective, Washington
- Washington Department of Fish and Wildlife, Marine Mammal Investigations
- Seaside Aquarium, Oregon
- Seattle Seal Sitters, Washington
- Makah Fisheries Department, Washington
- NOAA Fisheries, Washington

Histopathology

- Oregon State University, Veterinary Diagnostic Laboratory
- Animal Health Center, Abbotsford, British Columbia, Canada

Bacteriology

- University of California, Davis
- Animal Health Center, Abbotsford, British Columbia, Canada

Virology

- University of California, Davis

Toxicology

- Animal Health Center, Abbotsford, British Columbia, Canada

Contaminants

- NOAA Northwest Fisheries Science Center, Washington
- Columbia Analytical Services (through Cascadia Research), Washington

Detailed Head Examination

- Dr. Joe Gaydos, University of California, Davis (Lead)
- Washington Department of Fish and Wildlife, Marine Mammal Investigations
- The Whale Museum, Washington

Computed Tomography

- VCA Veterinary Specialty Center of Seattle, Washington
- Animal Internal Medicine and Specialty Services, California

Stomach Content Analysis

- Biology Department, Portland State University, Oregon
- NOAA Northwest Fisheries Science Center, Washington

Parasite Identification

- University of Florida
- NOAA Northwest Fisheries Science Center, Washington

Gas Analysis for Effects of Blast Trauma

- Woods Hole Oceanographic Institution, Massachusetts

Skeletal Preparation

- The Whale Museum, Washington
- University of Washington, Burke Museum

Relevant Environmental Factors and Human Activities

- NOAA Office of Response and Restoration, Emergency Response Division, Washington
- NOAA Marine Mammal Authorization Program, Washington
- NMFS West Coast Region, Protected Resources Division, Washington
- NMFS Northwest Fisheries Science Center, Conservation Biology Division, Washington
- U.S. Air Force
- U.S. Army
- U.S. Army Corps of Engineers
- U.S. Coast Guard
- U.S. Navy
- Royal Canadian Navy
- Center for Whale Research, Washington
- Fishing Vessel Owners Association, Washington
- Orca Network, Washington
- The Whale Museum, Washington

Law Enforcement Investigation

- NOAA Office for Law Enforcement, Washington

Requests for information on relevant factors included:

- L-112's life history and movements
- L Pod movements in 2011 and 2012
- L Pod demographics and population census
- Ocean currents and temperature, weather, and wind patterns
- Columbia River eddies and drift patterns
- Chinook salmon (*Oncorhynchus tshawytscha*) run timing
- Human activities, military exercises, marine mammal incidental take during fishing, vessel collisions, and in-water construction

Results

A postmortem examination of L-112 was performed on 12 February 2012, led by Northern Oregon/Southern Washington Marine Mammal Stranding Network primary responder D. Duffield (Portland State University). A complete photographic series of the necropsy has been archived by Portland State University, the Washington Department of Fish and Wildlife, and the Cascadia Research Collective. An initial brief necropsy report was prepared by Portland State University to accompany submission of histopathology samples. A comprehensive necropsy report with morphometrics and complete sample allocation was prepared for the group by the Cascadia Research Collective and the Washington Department of Fish and Wildlife, and a Marine Mammal Mortality Investigation Gross Report dated 19 March 2012 was prepared by D. Lambourn (Washington Department of Fish and Wildlife), J. K. Gaydos (SeaDoc Society, University of California, Davis Wildlife Health Center, and San Juan County Marine Mammal Stranding Network), D. Duffield (Portland State University), J. Huggins (Cascadia Research Collective), and T. McKlveen (VCA Veterinary Specialty Center of Seattle). These reports are included in Appendices A-2, A-3, and A-4). The Southern Resident Killer Whale L-112 Stranding Progress Reports issued by NOAA on 2 April 2012 and 12 May 2012 are included in Appendices B-1 and B-2.

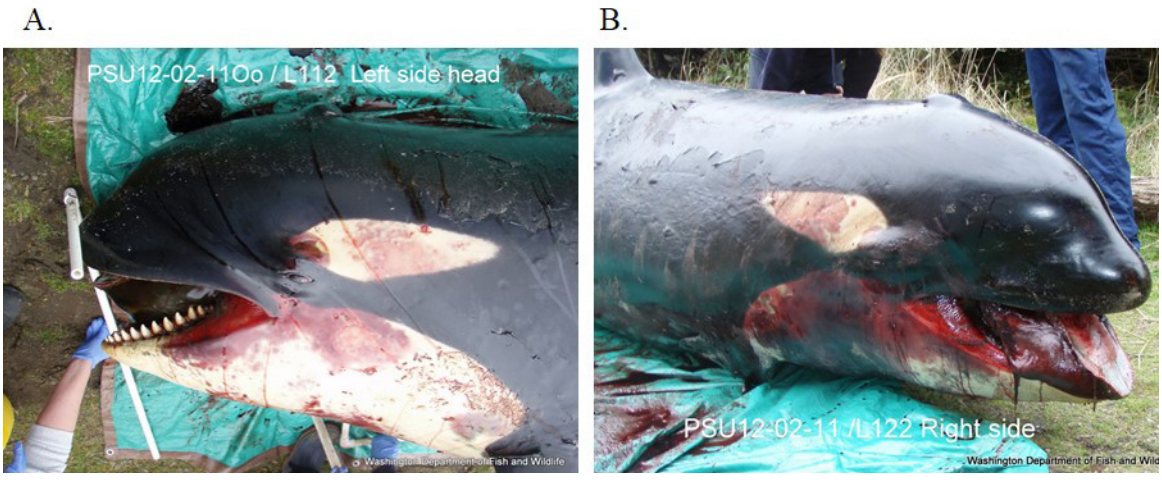

Figure 4. Bruising on L-112's head. A) View of bruising on left side of head. B) Extensive congestion, possibly hypostasis, is apparent along right side of head and thoracic wall.

Gross Examination and Findings

History

External examination of L-112 revealed that the carcass was moderately distended with gas, and that there was minimal scavenging (superficial and mostly from birds) on the left, exposed side of the body. The carcass was in moderate to late-moderate postmortem condition (code 2.5 to 3.0). There are varying opinions on the exact state of decomposition because the abdominal cavity appeared more decomposed than the thoracic cavity, and the skin was just starting to dry, peel, and slough. Based on the external examination, the initial estimated time of death was from two to seven days prior to discovery. The estimated window for time of death was later expanded to as long as 10 days based on the degree of postmortem autolysis noted on histopathology.

External Examination

The whale was in good body condition, with a 4.5-cm dorsal blubber thickness and fat noted around the heart. Throughout the ventrolateral aspect of the head, neck, and chest, there was variably extensive red mottling of the skin. Along the left dorsolateral aspect of the head, subcutaneous bruising extended from approximately 5 cm above the eye, rostrally to the level of tooth #6 on the lower jaw, and caudally to the midlevel of the neck (Figure 4). Other, smaller areas of hemorrhage were observed in the middle of the left eye patch and immediately anterior to the insertion of the left pectoral fin. Extensive bruising and swelling were also observed on the right lateral side of the head and neck (Figure 5) approximately 5 cm above the eye to the level of tooth #4 on the right lower jaw, and extended down the right side of the body, past the insertion of the right pectoral fin to the midlevel of the thoracic wall. Severe bruising spanned the ventral lower jaw, almost to the inside of the left lower mandible (Figure 5). The eyes were intact and slightly protuberant. Throughout the head, chest, and down the right lateral side of the body there was extensive hemorrhage and edema in the skin, blubber, subcutaneous tissues, and muscles (Figure 6), as well as in the lung and heart. Hemorrhage was also noted in the thyroid gland; subscapular, suprascapular, mediastinal, and reproductive lymph nodes; tongue, esophagus oropharynx, and pharyngeal musculature; liver; and around the spinal cord, at the foramen magnum and lumbosacral junction.

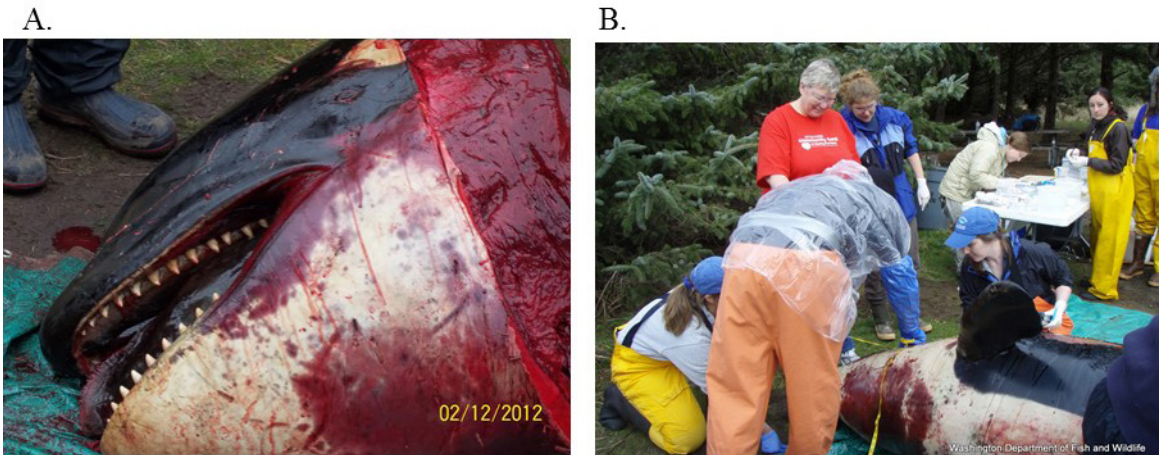

Figure 5. Bruising on ventral side of L-112's head. A) Close-up of ventral bruising on lower jaw. See also Figure 2. B) Extensive bruising on ventral aspect of lower jaw and chest.

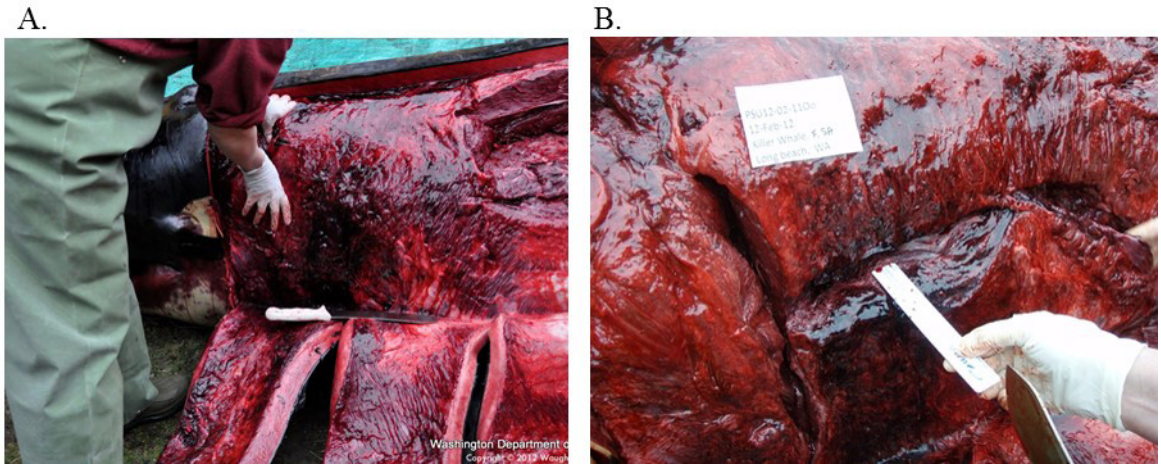

Figure 6. Hemorrhage in L-112's tissues. A) Hemorrhage in soft tissues extending under the scapula on the left side. B) Hemorrhage extending deeper in the chest wall musculature.

Two small, healed superficial longitudinal linear scars were present in the skin along the right torso, one immediately caudal to the trailing edge of the dorsal fin and the second dorsolateral to the anus. Subcutaneous swelling was present around the genital slit (Figure 7), and the anterior margin of the blowhole was raised and swollen. The tongue was markedly swollen, congested, and edematous (Figure 4B), and a portion of the right side appeared collapsed. Twelve teeth were erupted from the right and left mandibles as well as from the right and left maxillae. On the left mandible, tooth #10 was deviated more medially than the other teeth, but it appeared to have erupted in this direction because it was firmly held by the periosteal ligament and there was no associated bruising, inflammation, or signs of trauma. The upper right tooth #13 had a vertical linear fracture that extended to the level of the gingival, and had minimal separation of the opposed tooth margins.

Figure 7. Subcutaneous swelling around the genital slit with partial evagination of vagina.

Musculoskeletal System

No broken bones were observed on gross examination or upon flensing and cleaning the skeleton; however, anomalies were observed in the cervical vertebrae by CT imaging and bone preparations. Small linear fissures or splits were noted on some of the ribs after the skeleton was rearticulated. The splits run parallel to the length of the ribs from the vertebrae to the sternum, with no evidence of callous formation or bone response. At the time of necropsy, no hemorrhage or bruising was evident in the intercostal muscles or associated with the costal periosteum. Antemortem trauma of ribs would more likely produce transverse fractures (i.e., perpendicular), or at an oblique angle to the long axis of the bone. The observed splits were likely an artifact of skeletal preparation.

Digestive System

In the base of the mouth, medial to the right mandible and ventral to the tongue, there was a large, triangular 7 cm in diameter dark green-brown emphysematous area that protruded up to 16 cm above the surrounding tissues. A similar appearing and smaller area (~3 cm long) medial to the left mandible was also noted. Brown-green linear tracks extended from the mandibular symphysis caudally toward the mandibular ramus and pharyngeal area. The mandibular or acoustic fat of the left mandible was dark red, and the right mandible fat appeared more autolyzed and darker, suggesting passive congestion. Removal of the mandibles and the hyoid apparatus revealed air-filled, sponge-like brown material immediately rostral to the tympanic bulla on the right, and to a much lesser extent on the left, side of the head. Serosanguinous fluid (presumptive blood) was evident in the lumen of the oropharynx, as well as at the junction of the nasopharynx. The left pharyngeal muscles were hemorrhagic and edematous. The forestomach lining was diffusely detached, sloughed in one piece, and was compressed into a ball inside the lumen of the main compartment. In the glandular compartment, there was focally extensive erosion of the mucosa with a few 8 × 6 cm ulcers. Approximately 30 nematodes were interspersed within the detached forestomach lining and there was a moderate amount of ingesta within the small and large intestines. Feces were present in the colon.

Respiratory, Cardiovascular, Hemolymphatic, and Urogenital Systems

Approximately 3 L of dark red serous fluid were in the right, and to a much lesser extent in the left, thoracic cavities. Both lungs were congested, and with the right craniobronchial lobe there was moderate, focally extensive subpleural edema and hemorrhage. There were approximately 50 mL of dark red-black serous fluid within the pericardium, and the epicardium was edematous (Figure 8). On the cut surface, the myocardium was green (autolysis) and there was no blood within the lumen of the heart or peripheral large caliber blood vessels (Figure 8), suggestive of hypovolemia secondary to hemorrhage in the head and neck regions. Although tissue gas accumulation and compression of the heart and great vessels may have displaced the blood cranially into the neck and tongue (bloat), it is also possible that hypostasis, because of position in the water column prior to stranding, may also account for the lack of blood. The kidney and spleen were friable and tan brown (autolysis). The urinary bladder was empty.

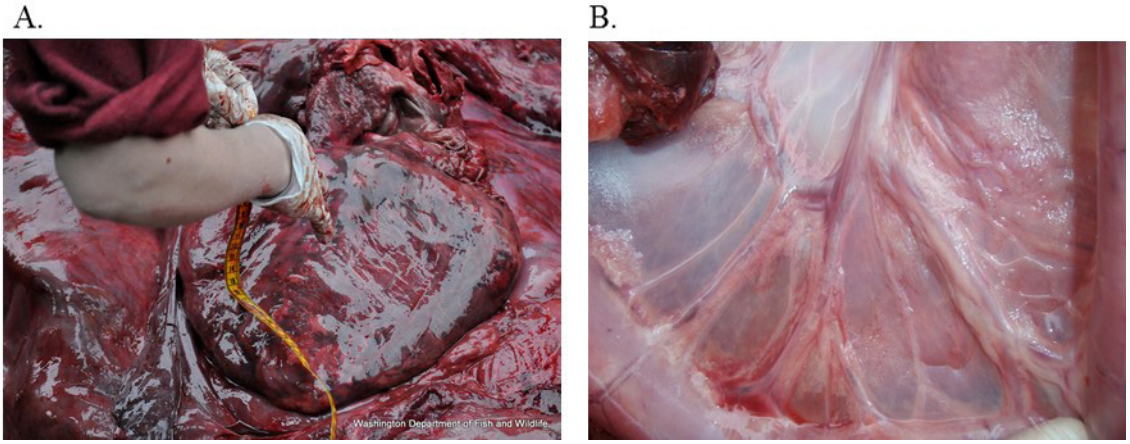

Figure 8. Heart, arteries, and veins of L-112. A) Red serous edema in heart lining. B) Blood was absent from arteries and veins as seen here in the mesenteries, although there were clear, well delineated spaces (presumptive gas emboli). Gases were consistent with decomposition.

Nervous System

During the necropsy, the atlanto-occipital junction was transected, and the head was frozen intact. Imaging studies were conducted 23 February 2012, and the head was dissected 6–7 March. On initial incision of the neck, large amounts of dark red serous fluid (~2 L) and variably sized fragments of brain poured from the foramen magnum (Figure 9). The head dissection (Appendix A-3) revealed extensive subcutaneous bruising around the left, and to a lesser extent the right, eye. The fascia and blubber surrounding the melon (dorsally and laterally) were diffusely pink to red. There was more intense red discoloration of tissues immediately rostral to the blowhole and lateral diverticulae (multiple sacs associated with the blowhole), which extended up to 27 cm toward the snout. The pink to red color was darker on the right side than it was on the left. The right craniofacial muscles adjacent to the melon, immediately dorsal to the maxilla, were dark red. Focally extensive hemorrhage was evident in the connective tissue on the right side of the head at the junction of the blowhole's rostral vestibular sac and the melon (Figures 2 and 5). Approximately 5–10 cc of serosanguinous fluid were present frozen in the left nares within the blowhole, likely an artifact of the head's position when frozen, and the right nares was clear.

While the bullae are not fused to the skull, they are suspended by ligaments in the peribullar cavity. Dissection of the tympanic bullae revealed that the right bulla was less firmly attached to the skull and significantly looser than the left bulla. After removal of the tympanic bullae, one small (1–2 cm) nematode and approximately 12 slightly longer (2–4 cm) worms were found in the area of the skull adjacent to the tympanic bulla, including the peribullar sinus, fibro-venous plexus, and surrounding peribullar soft tissue of both bullae. Red serous fluid was present in both peribullar sinuses. Dorsal to the right and left bullae were two small mineralized fragments. The left fragments were approximately 2.5 × 2 cm and 1.5 × 1.5 cm, and the right fragments measured 4 × 2 cm and 1.5 × 1.5 cm and were displaced into the calvaria. The edges of all four pieces were irregular but well-rounded and did not appear to be fractured bone fragments or remnants.

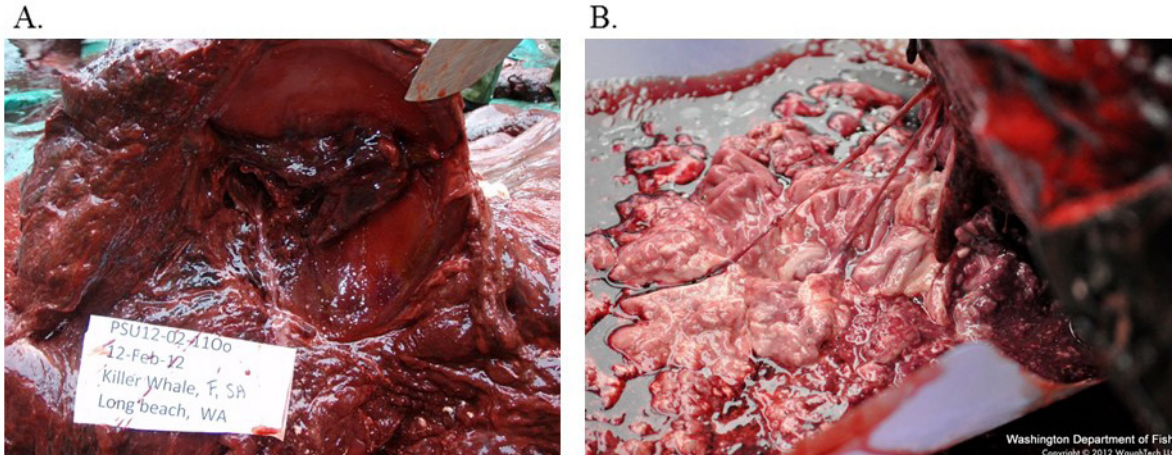

Figure 9. Skull and brain of L-112. A) Tissues of the calvaria were dark red and filled with red serous fluid. B) Copious dark red serous fluid and fragments of brain flowed from the foramen magnum.

Removal of a large triangular section of the occipital bone for access to the calvaria revealed cerebrum encapsulated by meninges in the left side and a large portion of dura that was adherent to the calvaria in three to four places. The right cerebellum and cerebrum were mostly lost, with portions of brain tissue draining into the left bulla. Roughly 20 cc of dark red to brown serosanguinous fluid were between the dura and the calvaria (epidural). This fluid was consistent with the fluid that was noted during the initial necropsy. The sutures on the right side of the calvaria appeared to be looser than on the left side, and red serous fluid was leaking through the suture areas.

Histopathology

Histopathology was performed on tissues harvested at the time of necropsy and subsequent to the head dissection. Severe autolysis hampered microscopic assessment of virtually all examined samples. In the multiple lymph nodes and throughout the small intestine and mediastinum, there were numerous microcavitations (emphysema) with no associated bleeding (hemorrhage) or inflammatory infiltrate. As fat emboli were also a consideration for the clear areas, cryostat sections were prepared of affected tissues and stained with Oil Red O. There was no indication of fat embolization in any of the examined tissues. Varying amounts of bacterial overgrowth were observed throughout the tissues. Within the heart muscle, kidney, and liver, there was a small amount of scar tissue, and in a few kidney tubules there were scattered protein casts (tubuloproteinosis). Histologic sections of the tissues associated with the right bulla disclosed moderate inflammatory infiltrate. Sections of the nonglandular stomach compartment revealed pronounced thickening of the inner lining (stratified squamous epithelia), and in a few areas there were intranuclear inclusions suggestive of an underlying viral infection. Throughout the liver, there was moderate biliary ductular hyperplasia with occasional bridging, periductular fibrosis, and cholestasis. A small number of skeletal muscle fibers featured intracellular protozoa morphologically consistent with *Sarcocystis* spp., and in a few sections of skeletal muscle there was variably extensive interstitial accumulation of acellular to hypocellular proteinaceous material. No other significant findings were identified within the examined tissues. Histopathology reports are in Appendices C-1 and C-2.

Microbiology, Molecular Studies (Polymerase Chain Reaction), Trace Minerals, Contaminants, and Biotoxin Analyses

Bacterial overgrowth secondary to postmortem decomposition likely hindered recovery or detection of any significant pathogens. Samples of lung, spleen, lymph node, brain, cerebrospinal fluid, meninges, liver, colon, heart, and kidney, together with blowhole and mammary gland swabs, were submitted to reference laboratories for routine and special microbiology (Appendices D-1, D-2, D-3, and D-4). Small to moderate growth of *Edwardsiella tarda* were recovered from select tissues, a few *Micrococcus* sp. were recovered from the spleen, and moderate growth of alpha *Streptococcus* sp. were cultured from the colon. No *Salmonella* sp., *Campylobacter* sp., or *Yersinia* sp. were recovered in selective media. Anaerobic culture recovered large numbers of *Clostridium perfringens*, *C. sordelli*, and *C. difficile* from the colon, and heavy growth of *C. septicum* was isolated from the discolored skin. Because of the grossly noted hemorrhage and emphysema within the neck and head skeletal musculature, immunofluorescence for clostridial toxins to rule out clostridial myositis was pursued, and proved negative for *C. chauvoei, C. noyvi,* and *C. sordelli*. Polymerase chain reaction (PCR) of pooled tissues proved negative for herpesvirus, *Brucella* spp., canine distemper virus, West Nile virus, and influenza virus, and results of trace mineral and vitamin A analyses of the liver were within acceptable reference limits.

Liver and kidney tissues were analyzed by Columbia Analytical Services (CAS) in Kelso, Washington (contracted by Cascadia Research Collective), and Prairie Diagnostic Services (PDS), Saskatoon, Saskatchewan, for the following elements: aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), methyl mercury (MeHg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), and zinc (Zn). Results are presented in Table 1. Tests on the ingesta for biotoxins, domoic acid, and saxitoxin were negative, and a test stripe for *Cryptococcus* spp. did not detect antibodies in postmortem heart blood.

PCR of the heart proved positive for Apicomplexa (M. Grigg, National Institutes of Health); however, close evaluation of the myocardium did not reveal any discernible protozoa.

The NWFSC completed an analysis of persistent organic pollutants (POPs, e.g., polychlorinated biphenyls [PCBs] and their congeners, polybrominated diphenyl ethers [PBDEs], DDTs, and chlordanes; Appendix D-1). The relative percentages of five lipid classes in the blubber and POP concentrations are shown in Tables 2 and 3. The sample had no free fatty acids present, which indicates that the blubber sample was not subject to decomposition significant enough to alter toxicological analysis (Appendix D-1). The blubber/skin decomposition does not always correlate with the decomposition noted for internal organs. The internal organs of the animal are more likely to decompose because of increased temperature in the carcass compared to the outermost tissues (e.g., the blubber).

Table 1. Trace metal concentrations in liver and kidney tissues of L-112 compared to reference values for odontocetes and mysticetes from published literature. Unless otherwise noted, results are in parts per million (ppm), where *ww* = wet weight, *dw* = dry weight, and *nd* = not detected.

		L-112			Odontocetes		Mysticetes	
		CAS[a,b]		PDS[b]	Range[c]		Range[c]	
Element	**Unit**	**Liver**	**Kidney**	**Liver**	**Liver**	**Kidney**	**Liver**	**Kidney**
Aluminum	dw	3.1	10.2					
	ww	0.93	3.06				6.4–150	1.6–9.3
Arsenic	dw	0.56	0.83					
	ww	0.168	0.249				0.47–0.7	0.01–2.7
Cadmium	dw	0.812	7.98		<0.05–175	<0.05–426	0.56–18.61	1.93–208.9
	ww	0.244	2.39		nd–11.5	2.19–18	0.06–6.2	0.14–6.1
Calcium	ww			2				
Cobalt	ww			0.009				
Copper	dw	28.2	7.33		5.3–260	5.7–73	3.37–228	7.68–65
	ww	8.46	2.2	9	6.02–16.3	1.68–4.53	0.63–25	0.45–4.9
Iron	ww			175				
Lead	dw	0.0755	0.0387		<1–3.2	<1–3.6	0.78–3.62	0.34–6.12
	ww	0.0227	0.0116				0.02–0.27	nd–0.1
Magnesium	ww			213				
Manganese	ww			2				
Mercury	dw	33.8	7.94		0.6–344	0.9–105		
	ww	10.1	2.38	12.4	0.31–97.8	0.21–10.4	0.009–0.12	nd–0.06
Methyl mercury	dw	1.83	1.98		0.8–3	0.2–2		
	ww	0.549	0.594					
Molybdenum	ww			0.28				
Nickel	dw	0.38	0.38		0.2–1.5	<0.2–1.2	1.53–2.13	1.07–3.29
	ww	0.114	0.114				nd–0.35	nd–0.21
Selenium	dw	21.4	8		0.6–99	0.1–57		
	ww	6.42	2.4	5.18			0.35–3.4	0.34–2.1
Silver	dw	2.73	0.061					
	ww	0.819	0.0183				0.01–0.02	0.01–0.03
Zinc	dw	306	121		40–684	44–201	59.07–209.09	66.21–212.15
	ww	91.8	36.3	98	59.8–93.5	15.3–30.9	1.6–160	32–110
Vitamin A	µg/g			1844.4				
Vitamin E	µg/dL			829.2				

[a] Results from CAS were dry weight, and were converted to wet weight for comparison to literature using average moisture content of 70%.

[b] CAS = Columbia Analytical Services. PDS = Prairie Diagnostic Services.

[c] Values from literature were not converted; wet and dry weight range values for a single element are from different sources.

Table 2. Animal information, total lipid, and lipid classes in blubber (0–2 cm depth) of L-112 and some other juvenile Southern Resident killer whales. *SALE* = stearic acid laurel esters; *TG* = triglycerides; *FFA* = free fatty acids; *CHOL* = cholesterol; *PL* = phospholipids.

Pod ID number	Age at sampling	Age class	Sex	Collection date	% lipid TLC-FID[a]	% of total lipid				
						SALE	TG	FFA	CHOL	PL
L-112[b,c]	3 years	Juvenile	F	02/12/12	51.73	19.8	80.2	0	0	0
L-98[b]	4 years	Juvenile	M	03/13/06	56.39	13.1	83.2	2.7	0	0
J-39[d]	3 years	Juvenile	M	05/23/06	40.85	13.8	84.4	1.8	0	0
J-38[e]	4 years	Juvenile	M	06/08/07	20.9	16.1	81.1	2.7	0	0
K-36[e]	4 years	Juvenile	F	12/14/07	18.3	14.1	81.5	4.4	0	0
K-42[c]	4 years	Juvenile	M	11/27/12	27.6	16.9	83.1	0	0	0

[a] Percent lipid and lipid classes determined using thin-layer chromatography/flame ionization detection method (Ylitalo et al. 2005).
[b] Necropsy blubber sample; other samples biopsy blubber samples.
[c] NWFSC, unpubl. data.
[d] Data from Krahn et al. (2007).
[e] Data from Krahn et al. (2009).

Table 3. Concentrations of persistent organic pollutants in blubber (0–2 cm depth) of L-112 and some other juvenile Southern Resident killer whales.

Pod ID number	ng/g, wet weight						ng/g, lipid weight					
	HCB	ΣCHLDs	ΣDDTs	ΣHCHs	ΣPCBs	ΣPBDEs	HCB	ΣCHLDs	ΣDDTs	ΣHCHs	ΣPCBs	ΣPBDEs
L-112[a,b]	630	4,100	31,000	390	20,000	2,400	1,200	7,900	60,000	750	39,000	4,600
L-98[a]	250	4,600	44,000	300	24,000	1,900	440	8,200	78,000	530	43,000	3,400
J-39[c]	650	2,100	9,800	530	14,000	6,000	1,600	5,100	24,000	1,300	34,000	15,000
J-38[d]	250	1,100	5,000	210	8,600	3,000	1,200	5,300	24,000	1,000	41,000	14,000
K-36[d]	360	2,200	17,000	320	11,000	2,800	2,000	12,000	93,000	1,700	60,000	15,000
K-42[b]	180	770	5,400	100	4,700	970	650	2,800	20,000	360	17,000	3,500

[a] Necropsy blubber sample; other samples biopsy blubber samples.
[b] NWFSC, unpubl. data.
[c] Data from Krahn et al. (2007).
[d] Data from Krahn et al. (2009).

Computed Tomography of the Head and Bullae

On 23 February 2012, CT scans were performed on the intact head and subsequently on the separated bullae and cervical vertebra (Appendices E-1, E-2, and E-3). Findings from the scans of the entire head indicated extensive gas accumulation in the soft tissues and fat. The absence of the right cerebral hemisphere and right cerebellum of the brain was secondary to the loss of tissue during disarticulation of the head. Significance is uncertain based on imaging alone, but unilateral loss of brain tissue is unusual. Bilaterally, small mineralized deposits were detected dorsal to the bulla and suggestive of otoliths, dystrophic mineralization, or parasitic granulomas with right-sided displacement through the foramina into the calvaria, possibly because of loss of supporting tissues. In the left, and to a much lesser extent the right, osseous bulla (middle ear), there was fluid or soft tissue accumulations, which may be attributed to blood, infectious pathogens, nematodes, inflammatory debris, polyp-like material, postmortem accumulation of fluid, or engorged mucous membranes. Sinusitis was noted and likely related to parasite migration.

Gas Examination

Because of concerns of possible blast injury and seismic or sonar exposure, coupled with the grossly noted gas accumulation in multiple tissues on necropsy, four samples of air bubbles from the heart and two aliquots from the mesenteric vein were collected (per Bernaldo de Quiros et al. 2011) in Vacutainers (Becton, Dickinson and Company, Franklin Lakes, New Jersey) and a Monoject tube (Medtronic, Minneapolis, Minnesota) and shipped in a sealed, pressurized container with a barometer and altimeter to Y. Bernaldo de Quirós, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, for mass spectrometry analysis. Gas bubble analysis yielded consistently higher carbon dioxide to hydrogen and nitrogen levels in all samples, compatible with putrefactive gases. However, the animal was considered too decomposed to detect gas embolism chemically (Pierucci and Gherson 1968, Pierucci and Gherson 1969, Bajanowski et al. 1998, Bernaldo de Quirós et al. 2011; Appendix D-4).

Parasitology

The nematodes found in the right bulla were identified as *Crassicauda* sp. by H. Stockdale Walden of the University of Florida College of Veterinary Medicine (Appendix D-3), and the nematodes recovered from the forestomach were *Anisakis* sp. cf. *A. simplex*.

Food Habit Information

Ingesta from the stomach and intestines and feces from the colon of L-112 were collected at necropsy and examined for prey remains. The forestomach contained 17 cc of brown mucoid fluid. The main and pyloric chambers contained approximately 35 cc of pink salmon-colored pasty material with six small fish eye lenses. A fecal sample obtained from the colon was screened genetically for all prey species known to be in the diet of resident-type killer whales (see Hanson et al. 2010 for methods). Only Chinook salmon and halibut (*Hippoglossus stenolepis*) DNA were detected. Stock-specific information for the Chinook salmon could not be determined, as the DNA was too degraded.

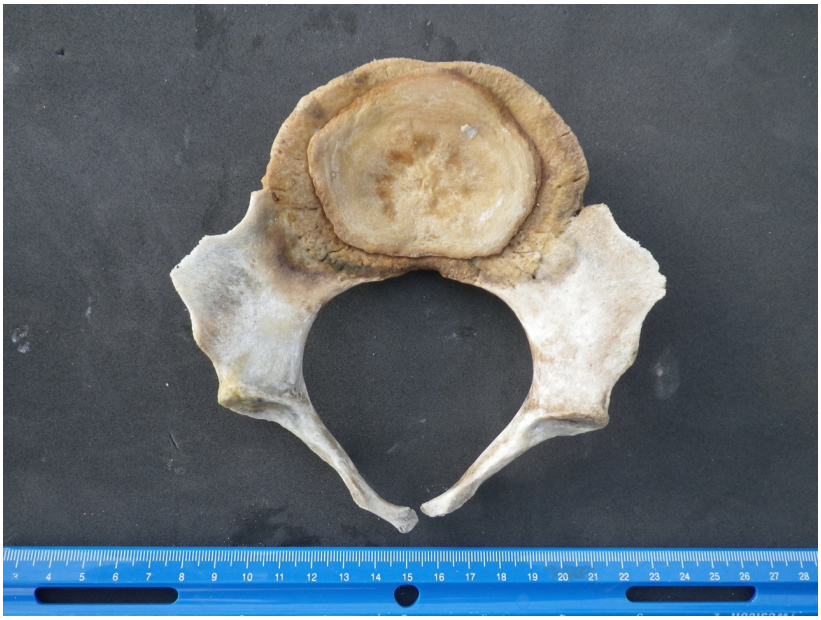

Figure 10. The dorsal aspect of cervical vertebra C7 showing incomplete fusion of the lamina and incomplete formation of the spinous process.

Skeletal Examination

Skeletal flensing, cleaning, and disarticulation revealed no bone fractures. However, CT scan of the cervical vertebrae detected a defect in the dorsal lamina, and no dorsal spinous process, of C7. Based on follow-up gross examination of the vertebra, it was concluded that this malformation was likely congenital and preexisting to the stranding and likely not due to physical trauma (Figure 10). In the right maxilla, there was a vertical crack of tooth #13 which extended from the apical tip to below the dental ligaments (Figure 11). There was no indication of odontodystrophies, maxillary osteomyelitis, developmental anomalies, periodontal disease, caries, or host response to the defect. The cause of the crack is undetermined. Shearing chips, consistent with biting, were also observed on some teeth. Tooth #6 on the right mandible showed the most dramatic chip, but the cause was not determined.

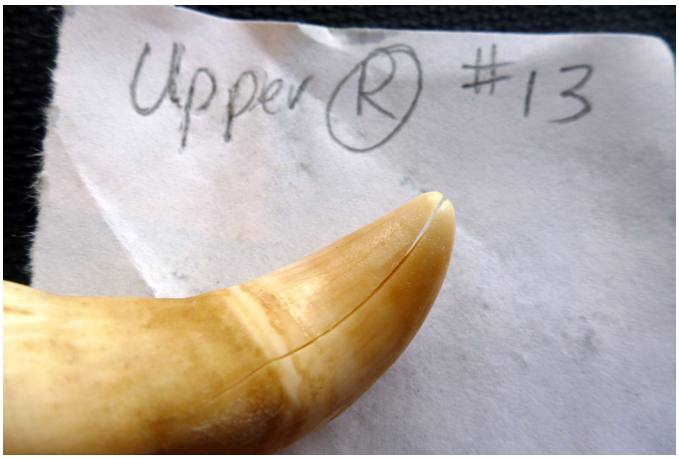

Figure 11. Linear vertical fracture in the upper right jaw tooth #13; the defect extended to the pulp cavity.

Relevant Historical and Environmental Factors

L Pod movements in 2011–2012

In fall 2011, the NWFSC deployed seven autonomous passive acoustic recorder moorings off the coasts of Washington, Oregon, and California (Figure 12), and these arrays were recovered in the summer and early fall of 2012. The recorders were set to record for 30 seconds every 10 minutes during their deployment. The recorder moored off of the Columbia River failed in mid-November 2011. The mooring for the Cape Flattery inshore recorder failed in early February, and the recorder was recovered near Tofino, British Columbia, in April 2012.

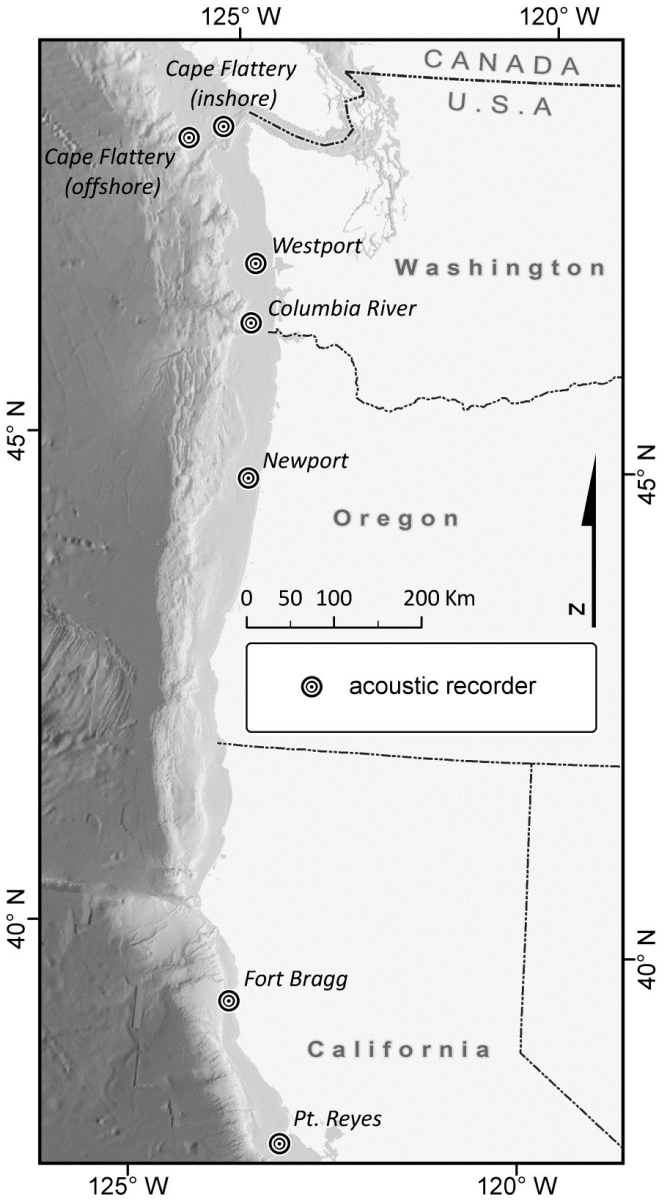

Figure 12. Location of acoustic recorders deployed by NWFSC in 2011 and 2012.

In 2011 and 2012, the U.S. Navy funded a contract to conduct acoustic monitoring off the northern Washington coast within Quinault Canyon (site QC: lat 47.50°N, long 125.35°W) and off Cape Elizabeth (site CE: lat 47.35°N, long 124.72°W). The goal of the monitoring effort was to characterize vocalizations of marine mammal species present in the area, determine their seasonal presence, and evaluate the potential for impact from naval operations. Two HARPs were deployed in December 2011 and were recovered in July 2012. Recordings were obtained from December 2011 through January 2012 at site CE and through July 2012 at site QC. Killer whale whistles, pulsed calls, and echolocation clicks were detected intermittently between 7 December 2011 and 4 January 2012 at both HARP locations, but the calls were not attributed to a specific killer whale ecotype. Additional killer whale whistles and echolocation clicks, not identified to ecotype, were detected at the Quinault Canyon site on 24 January and 8 February.

On 2 November 2011, L Pod was last sighted in inland waters with J and K Pods off Race Rocks, Canada. On 15 December 2011, S19 calls (used by all members of L Pod) were detected on the recorder moored near the head of the Juan de Fuca Canyon about 10 miles (16 km) west of Cape Flattery, Washington. The next detection of L Pod was on 26 January 2012 on the recorder moored a few miles southwest of Fort Bragg, California. On 27 January, L Pod-specific calls were detected on another NWFSC recorder located a few miles southwest of Point Reyes, California. Additional L Pod calls were again detected on the Point Reyes recorder on 30 January and then on the Fort Bragg recorder on 31 January. This series of detections (which, unlike in 2011, were short in duration) indicates relatively quick southbound movement of the whales on 26 and 27 January and subsequent northbound movement on 30 to 31 January. Call types heard indicate the main group of L Pod animals was present, which could have included L-112. Between 08:00 and 09:00 on 5 February 2012, S16 calls (used by both K and L Pods) were heard on the recorder located about 5 miles (8 km) west of Westport, a distance of 531 miles (854.6 km) north of the Fort Bragg recorder, indicating an average speed of approximately 4.5 miles (7.2 km) per hour if this was the same group of whales detected off California in late January. After the 15 December 2011 detection, SRKW calls were not detected by the Cape Flattery offshore hydrophone again until 4 March 2012.

Also on 5 February, hydrophones on the west side of San Juan Island detected K and L Pod calls near Limekiln Point at 10:55, and Andrews Bay later that evening (22:45). The traveling distance between Westport and Limekiln Point (200+ miles, or 322+ km) is too far for the group heard at Westport to travel and be detected at Limekiln that same morning, indicating that at least two groups of K and/or L Pod whales were transiting in Washington waters on 5 February.

Part of K Pod (K16s and K18s) and part of L Pod (L2s, L9s, L54s) were photographed during an unprecedented appearance in Discovery Bay, Washington, on 7 February 2012 at 15:45. The L4s (the sub-group including L-112's matriline) were not seen with this group. It is not known whether the group observed in Discovery Bay included individuals from the group recorded off Westport on 5 February or the group detected at San Juan Island that same day. If the animals seen in Discovery Bay came from the outer coast group, the travel speed to cover the 233 mile (375 km) distance between Westport and Discovery Bay during the intervening time would have averaged 4.3 miles (6.9 km) per hour. This is within the range of the average traveling speed for SRKWs and similar to the 4.5 mph (7.2 km/h) rate of travel calculated for the trip between Fort Bragg (30–31 January) and Westport (5 February), a speed typical of the summer/fall range.

On 20 to 21 February 2012, SRKWs (K and/or L Pods) were detected on the Newport, Oregon, recorder. The next detection of L Pod was on 27 February on the Westport, Washington, recorder, followed by a 2-month period in which there were 16 SRKW detections and one visual sighting off Westport and one additional acoustic detection off Newport in mid-March. The acoustic recordings support the hypothesis that a group of whales, possibly including the L4 sub-group and L-112, were present and could have been transiting in the area of the Columbia River plume during the time frame of the mortality and subsequent stranding.

Between the stranding date (11 February) and 1 July 2012, all of the remaining members of the L4 sub-group (L-27, L-55, L-82, L-86, L-103, L-106, L-109, and L-116) except L-112 were observed alive and were included in the 2012 census.

Wind and Currents

Using data from the National Data Buoy Center,[1] we reviewed weather and sea surface data collected by buoys near the mouth of the Columbia River (Station 46029) and further north near Neah Bay, Washington (Station 46087), for the coastal waters of Washington and Oregon from 1–11 February 2012 (the period from the estimated time of death until the stranding date), and found that prevailing winds and currents near the mouth of the Columbia River were southerly with some east–west variability in the days prior to the stranding. For the same period, wind directions near the entrance to the Strait of Juan de Fuca were generally more easterly. Current models (Center for Coastal Margin Observation and Prediction, CMOP) and data from drift card studies provided some insights into local influences on drift patterns near the Columbia River plume. NOAA Office of Response and Restoration, Emergency Response Division (ERD), advised that current conditions off the Long Beach Peninsula, Washington, are largely influenced by eddies created by flows from the mouth of the Columbia River. In the period prior to the stranding, eddies would have flowed northward under the influence of the prevailing winds, allowing floating debris trapped in them to be deposited on Long Beach.

ERD further advised that floating debris arriving from the open sea to the west or north of Long Beach would have been carried northward by the current to be deposited elsewhere on the Washington or British Columbia coasts, not on Long Beach near the mouth of the Columbia River. Figure 13 depicts patterns of surface drifters deployed by the University of Washington off the mouth of the Columbia River in 2005. These patterns illustrate the eddy circulation in the region. The cyan surface drifter tracks (from 17 August) represent conditions that are most similar to the winds and currents off the Washington and Oregon coasts in February 2012. The tracks further substantiate the potential for objects floating in the plume to be deposited on Long Beach. Moreover, drift patterns from the prevailing winds and currents for this period indicated a northward flow along the Washington and Oregon coasts, so that a floating object from far off of the Washington coast or farther to the north would be unlikely to have been deposited on the southern end of the Long Beach Peninsula.

[1] http://www.ndbc.noaa.gov/

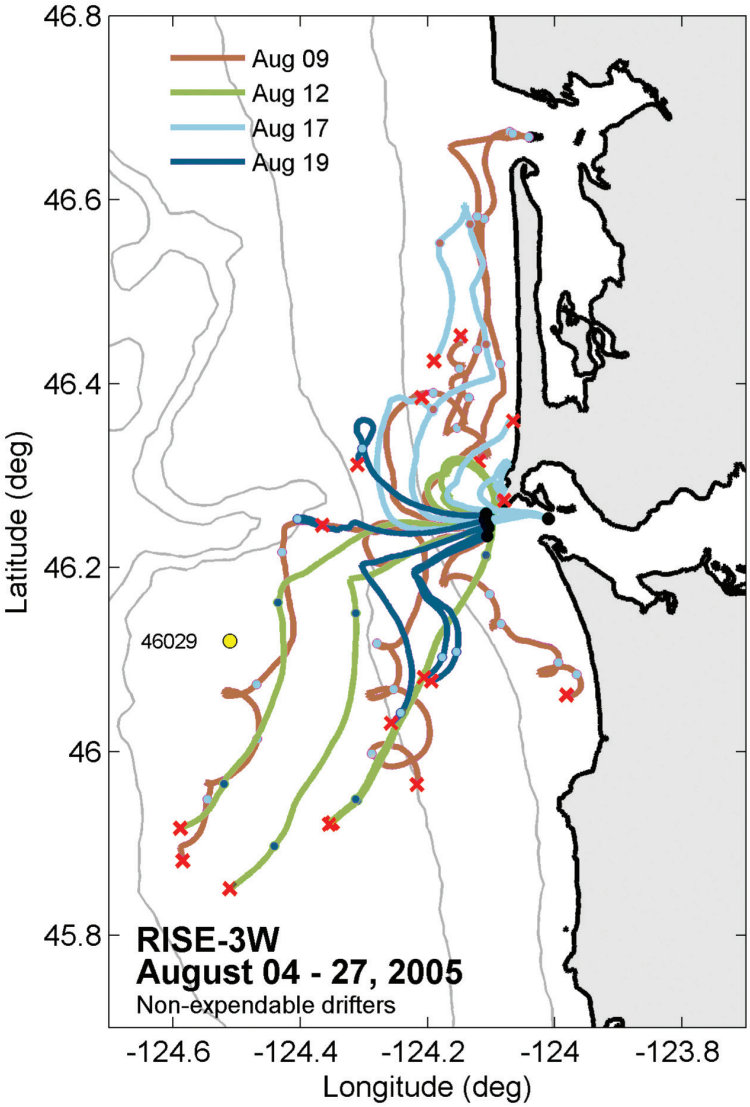

Figure 13. Patterns of drift for surface drifters deployed by the University of Washington off the mouth of the Columbia River, 9–19 August 2005.

Earthquakes

NOAA Fisheries received comments requesting that they investigate the possibility that earthquake(s) may have contributed to the stranding. Minor earthquakes (magnitude 2.6 to 4.0) were detected off the Oregon coast near Coos Bay on 22 January and on 2, 3, 4, and 7 February 2012 (information on all of these events can be found by searching the USGS Earthquake Hazards Program's Earthquake Catalog[2]). A light magnitude 4.8-5.6 earthquake was recorded off Vancouver Island, Canada, on 4 February 2012. Minor earthquakes are considered common in the region, and some can be felt but seldom cause damage. There is little data currently available to assess the impact or contributions of small-magnitude earthquakes to killer whale strandings.

[2] http://earthquake.usgs.gov/earthquakes/search/

Table 4. Average sea surface temperatures (SST) offshore of southern, central, and northern Oregon, 1–11 February, 2011–2013.

Latitude, Longitude	Location	Average SST 2011	Average SST 2012	Average SST 2013
43.00°N, 125.20°W	southern Oregon	9.9°C 49.8°F	9.86°C 49.7°F	10.0°C 50.0°F
45.00°N, 124.80°W	central Oregon	9.76°C 49.5°F	9.06°C 48.3°F	9.73°C 49.5°F
46.00°N, 124.00°W	Columbia River	9.16°C 48.4°F	8.96°C 48.1°F	9.50°C 49.1°F

Sea Surface Temperature

Sea surface temperature data for 1–11 February 2012 were obtained using NOAA's Comprehensive Large Array-data Stewardship System (CLASS). Sea surface temperature is defined as the skin temperature of the ocean surface water and is generated every 48 hours for North America. Three locations were investigated: approximately 37 miles (59.6 km) off southern Oregon, approximately 38 miles (61.2 km) off shore of central Oregon, and at the mouth of the Columbia River. The average sea surface temperatures at each location for the period 1–11 February 2011, 2012, and 2013 are presented in Table 4. Sea surface temperature data from each 48 hour cycle are available on the CLASS website.[3]

Average sea surface temperatures varied little (<1° C) from 2011–2013, and no large-scale temperature anomalies were noted.

Acoustic Recordings

On 6 February 2012, researchers monitoring hydrophones deployed in the inland waters of Washington detected sounds identified as military mid-frequency sonar and possibly explosions. The researchers linked the sounds to a Canadian Navy exercise in the Strait of Juan de Fuca involving the HMCS *Ottawa*. The researchers accessed Automatic Identification System (AIS) data from Marine Traffic to retrace the movements of the HMCS *Ottawa* as it departed and returned from the North Pacific off Vancouver Island in the days prior to the exercise in the Straits. Reports of the sonar detections and accompanying impulsive sounds were published in the media and prompted considerable public interest and concern over potential sonar impacts to SRKWs. The concern intensified with the discovery of L-112 stranded on Long Beach five days later (on 11 February).

[3] http://www.class.ncdc.noaa.gov/saa/products/search?datatype_family=SST14NA

External Inquiries for Information

NOAA Fisheries solicited information from a variety of sources (for an example, see Appendix F-3) to identify whether human activities may have contributed to the injuries observed during the postmortem examination and diagnostic assessment of L-112. Activities of particular interest included military exercises, vessel traffic and collisions, incidental take in fisheries, in-water construction activities involving demolition or blasting, and earthquakes, particularly in the 10–30 days preceding the stranding (Appendices F-1, F-2, F-3, F-4, F-5, F-6, F-7, and F-8).

NOAA Fisheries requested information on naval activities from the Royal Canadian Navy (RCN). The RCN confirmed the use of sonar and small underwater charges in Canadian waters west of Vancouver Island and in the Strait of Juan de Fuca. On 4 February, Canadian naval exercises using a small (1.4-kg) explosive charge and sonar were conducted in Canadian waters approximately 85 miles (136.8 km) northwest of the Strait of Juan de Fuca. According to the Navy report, the "kill radius" for a human diver from the type of charge used is approximately 15 yards [13.7 m]. Sonar was operated for approximately eight hours at this general location. A similar exercise occurred approximately 90 miles (144.8 km) northwest on 5 February, when two 1.4-kg charges were deployed (one in the morning and one in the afternoon), and sonar was operated for approximately 11 hours in this general location. After the offshore exercises, the HMCS *Ottawa* returned to the Strait of Juan de Fuca using sonar while in transit to Constance Bank. On 6 February 2012, two 1.4-kg explosive charges were deployed in the morning as part of an anti-submarine warfare exercise near Constance Bank. In each case, the HMCS *Ottawa* adhered to their Marine Mammal Mitigation Policy prior to deploying the small charges and while using ship's sonar. Marine mammals were not detected in the area of the exercises by shipboard lookouts or passive sensors, according to the RCN's report (Appendix F-7).

NOAA Fisheries also contacted the U.S. Navy regarding naval exercises in the Northwest Training Range Complex. The U.S. Department of the Navy, Office of the Chief of Naval Operations, responded to our request and confirmed from their records that there were no naval exercises involving mid-frequency sonar use or explosives deployment in the Northwest Training Range Complex between the end of January and 11 February 2012 (Appendix F-6). The offshore area of the Northwest Training Range Complex includes surface and subsurface operating areas extending generally west from the coastline of northern California (approximately Cape Mendocino), Oregon, and Washington for a distance of approximately 250 miles (463 km). In addition, we received a copy of Marine Physical Laboratory Technical Memorandum MPL-TM 542, *Passive Acoustic Monitoring for Marine Mammals in the Northwest Training Range Complex 2011–2012* (Kerosky et al. 2013). The passive monitors (HARPs) detected sporadic explosions, attributed to seal bombs (underwater firecrackers) associated with fishing activity off Cape Elizabeth, in mid-to-late January 2012. We were unable to identify the source of these sounds.

NOAA Fisheries contacted the U.S. Army to determine whether any military assets (Army or Air Force) from Joint Base Lewis-McChord (JBLM) may have been involved in training or other military activities on the coast during the time prior to the stranding. The Chief of Staff at JBLM responded that no military training, shipping, in-water construction, explosive events, or other potential perturbations involving JBLM units took place in the area during that time frame (Appendix F-4).

NOAA Fisheries also contacted the United States Coast Guard. The Coast Guard conducts search and rescue activities, engages in homeland security and law enforcement tasks, maintains aids to navigation, and trains for these missions in the Lower Columbia River and marine waters off the coasts of southern Washington and northern Oregon. NOAA contacted them to see whether training exercises or civilian activities (e.g., shipping, resource exploration, fisheries, or in-water construction) along the Oregon coast to as far north as Ledbetter Point, Washington, may have been conducted by or reported to the Coast Guard from 1–11 February 2012. The Coast Guard reported that they did not learn of any whale strikes or other impacts to whales by Coast Guard assets or commercial vessels during this time frame (Appendix F-5). During that period, Coast Guard activities included search and rescue, law enforcement, aids to navigation work, and training exercises. Small boats and cutters made over 100 voyages in the area and on the Columbia River, Oregon. No major cutters transited the area and there were no reported whale strikes. There were 116 large (300+ gross tons) vessel movements that arrived or departed Sector Columbia River's area of responsibility, which encompasses the Oregon coast north to Grays Harbor, Washington. We did not attempt to reconstruct the courses and speeds of these vessels from historical AIS data. Additionally, the Coast Guard was not aware of any explosives being used in the area during that period.

Regarding fisheries activities that the members of L Pod may have encountered, NOAA Fisheries reviewed reports received by the Marine Mammal Authorization Program from commercial fishing vessels between January and February 2012 and found that no incidental mortality or injuries involving killer whale(s) were reported anywhere on the West Coast during this time frame. We also contacted the Fishing Vessel Owners Association to see if they had received any reports of encounters with whales. The Fishing Vessel Owners Association responded that vessels are not typically on the water and fishing in February, and reported no interactions between whales and fishing vessels. Vessels that are part of the Association are smaller, between 50 to 85 feet (15.2–25.9 m) in length, with crews of three to six persons, and travel an average speed of 7–12 knots (Appendix F-8).

The United States Army Corps of Engineers (USACE) is authorized by Congress to regulate activities that may impact wetlands and waters of the United States. In response to NOAA Fisheries' request, the U.S. Army Corps of Engineers' Regulatory Office, based in Portland, Oregon, provided information on permit actions along the coast of Oregon north to Leadbetter Point, Washington, for the 5-year period prior to February 2012. Permit activities in the area were primarily beach grass removal on dunes, installation and maintenance of scientific buoys, and dredging. Permits for dredging activities are typically issued with special conditions and require additional consultation if marine mammals frequent the area in which the work will be completed. On February 5, 2013 the ASACE confirmed by email that during the time frame of January 2012 through February 2012, they were not contacted by any permit holder notifying them that marine mammals were present in the permitted work area. There were no permitted in-water construction projects involving blasting or pile driving in the coastal waters of Oregon or near the Columbia River mouth that would have produced noise disturbance during the time frame that we investigated.

Law Enforcement Investigation

An initial investigation into cause of death was undertaken by the NOAA Office of Law Enforcement. No subjects or witnesses with knowledge of the circumstances associated with or leading to the death of L-112 were identified. The case was closed as a result of a lack of evidence to support that a crime had occurred.

Discussion and Conclusions

Killer whale strandings provide a rare opportunity to improve our understanding of whales' natural history, diet, reproduction, threats, disease, and mortality. With the listing of the SRKWs as endangered in 2005, interest and resources have increased the number of complete investigations of killer whale strandings, particularly along the U.S. West Coast (Barbieri et al. 2013). Stranding investigations were identified as an important action in the Southern Resident Killer Whale Recovery Plan (NMFS 2008). This report draws on a multi-disciplinary investigation to provide unique insight into the status of killer whales and evaluate potential threats and sources of mortality.

On 11 February 2012, a juvenile female Southern Resident killer whale, L-112, stranded just north of Long Beach, Washington. The whale appeared to be in good nutritional condition, with advanced postmortem decomposition. Gross examination revealed extensive subcutaneous bruising on the dorsolateral aspects of the head, tracking to the throat and anterior insertion of the right pectoral fin. Microscopic assessment of sampled tissues was hindered because of advanced postmortem autolysis; at the time of the postmortem examination, generalized gas accumulation in most major organs was noted, and based on this observation, gas was collected and shipped to Woods Hole Oceanographic Institution for analysis. Chemical analysis results indicated that the gas composition was most consistent with putrefaction (bacterial degradation) rather than gas bubble-like disease or blast injury. The lack of associated bleeding, inflammatory infiltrate, or fat embolization with the microcavitations in examined tissues further substantiated the interpretation of postmortem decomposition.

During skeletal preparation, a congenital defect or anomaly of the dorsal aspect of cervical vertebra C7 (the lamina) was observed, with incomplete fusion of the lamina and incomplete formation of the spinous process (Figure 10). The cervical vertebrae were examined by CT scan; it was determined that this malformation was not due to a traumatic fracture (Appendix E-3).

Head imaging studies (CT scans; Appendix E-1) and gross dissection of the skull and brain showed disruption of the right cerebral hemispheres, with marked accumulation of clear fluid, variably extensive fluid that suggests potential hemorrhage, and collapse of the dura. Microscopic examination of brain sections disclosed tissue fragmentation and breakdown with no associated hemorrhage, fluid accumulation, or protein loss. These changes were more consistent with freeze artifact and tissue breakdown because of postmortem decomposition (autolysis) than traumatic insult, and do not support gross and imaging observations of potential hemorrhage. Imaging studies also detected multiple ossified bodies.

CT imaging was conducted to further investigate possible lesions associated with blast or other intensive acoustic injury. The scans were evaluated for calvarial or aural fractures, bleeding within the skull, and dislocation of the auditory ossicles. None of these features were evident in the examined scans. The ossified bodies (bony fragments) detected by imaging studies are chronic features that are frequently seen in the peribullous spaces on CT scans of cetaceans. These bony elements are not considered fractures of ear structures, nor do they support the argument for trauma. Conclusions from the CT scan of the right and left bullae at 1-mm slices did not show any evidence of fractures, dislocation, or crushing of the auditory ossicles, as could occur from intense acoustic exposure. The soft tissue or fluid attenuating material in the cochleae could be either ante- or postmortem. There was no definitive evidence of acoustic damage to the bony ear structures of this whale identified from the CT study.

Histopathology of the right peribullar tissue disclosed chronic inflammatory infiltrate with associated *Crassicauda* spp. nematodes. Parasite infections of the inner ears have previously been documented in a number of cetaceans, and the contributions of these worms to impaired sound perception or disequilibrium is unknown. In this case, the inflammation and parasitic burden would likely not have resulted in clinical disease. We could not determine the etiology for the right bulla to have been less firmly attached than the left. *Crassicauda* (nematode) infection was found in association with the right peribullar area, and imaging revealed small mineralized depositions dorsal to the bulla that could have been dystrophic mineralization or parasitic granulomas; these could have been responsible.

Additional microscopic findings included low- to intermediate-grade accumulation of scar tissue within the heart, liver, and kidney, which cumulatively would not have contributed significantly to antemortem morbidity. There was suggestion of an ascending infection from the gastrointestinal tract, possibly because of hepatobiliary trematodiasis or toxic exposure. Thickening of the stomach lining was also apparent (gastric hyperkeratosis), and suggested inappetence or anorexia. *Anisakis* sp. cf. *A. simplex* was also identified in the stomach but was not considered pathologically significant. The fluid accumulation within the left chest cavity and lung most likely was due to autolysis, although bleeding and edema fluid related to trauma, impaired heart function, or active inflammation may also be considerations (Appendix D-2). Acute inflammation was noted in a small number of lymph nodes and was consistent with a low-grade localized bacterial infection.

Aerobic culture of the sampled tissues yielded variable light-to-heavy growth of *Edwardsiella tarda* from the lung, lymph node, brain, liver, kidney, and blowhole. This bacterium composes part of the normal intestinal flora of killer whales, with secondary invasion and septicemia typically diagnosed in animals with generalized debilitation or immunosuppression. Because of the extent of postmortem decomposition and lack of associated inflammatory infiltrate, this isolate was likely due to postmortem tissue invasion and proliferation. Selective culture of the colon did not recover *Salmonella* spp., *Campylobacter* spp., or *Yersinia* spp. Similarly, the large numbers of *Clostridium perfringens*, *C. sordelli*, and *C. difficile* recovered from the colon by enrichment broth likely represent postmortem overgrowth. Because of the gross appearance of the subcutaneous bruising and gas accumulation, immunofluorescence was undertaken to screen for clostridial toxins (blackleg), and results proved negative for *C. septicum*, *C. sordellii*, *C. noyvi*, and *C. chauvoei*.

Molecular studies were undertaken to screen tissues for potential pathogen exposure, including *Brucella* spp., canine distemper virus (morbillivirus), influenza virus, West Nile Virus, and herpesvirus. Results were negative for these pathogens; however, because of the extent of postmortem decomposition and DNA/RNA degradation, the possibility of false negatives cannot be entirely discounted. PCR of the heart proved positive for Apicomplexa (M. Grigg, NIH); however, close evaluation of the myocardium did not reveal any discernible protozoa. This parasite has previously been detected in stranded killer whales and is associated with massive die-offs of southern sea otters in California and harbor seals and California sea lions in the Pacific Northwest. There was no indication of active protozoal infection or inflammation in L-112.

Metal concentrations in liver and kidney were compared to compiled ranges of published values for odontocetes and mysticetes (Varnassi et al. 1994, Sanpera et al. 1996, Holsbeek et al. 1999, Ruelas et al. 2000, Mendez et al. 2002, Das et al. 2004a, Das et al. 2004b, Endo et al. 2007). All elements were within the ranges of other cetaceans, and most compared to what has been recorded in other odontocetes. Nickel, however, was most comparable to mysticete values, which are higher than those of odontocetes. Some difference in derived levels between labs may be attributed to more labile or volatile trace minerals, which may be lost during transport and processing. In some laboratories, total mercury and total arsenic are analyzed, rather than inorganic and organic quantification. Acid extraction of the tissues in the processing phase may artifactually increase select trace mineral concentrations. The anatomic sample location within an organ may also contribute to variations in heavy metal levels, such as copper levels throughout the liver, or lead partitioning in the renal cortex relative to medulla. None of the heavy metal findings are considered significant to the cause of L-112's stranding. For the vitamin A level, only retinol levels were measured, because postmortem degradation can rapidly deteriorate levels.

Overall, ranked concentrations of POPs in the blubber of L-112 (Table 2) were: ∑DDTs > ∑PCBs > ∑ chlordanes > ∑PBDEs > HCB > ∑HCHs. On a lipid basis (measured in nanograms per gram lipid weight, *ng/g lw*), concentrations of HCB in the 0–2 cm depth (most similar to a biopsy sample) of blubber of L-112 (1,200 ng/g lw) were in the same range as those from other juvenile SRKWs (average range: 440–2,000 ng/g lw). Concentrations of PCBs in the blubber of L-112 (39,000 ng/g lw) were also comparable (average range: 17,000–60,000 ng/g lw). PCB concentrations were also comparable to the mean of ∑PCBs in biopsy samples collected between 1993 and 1996 from adult male Northern Resident killer whales, as reported by Ross et al. (2000): a mean of 37,400 ng/g lw (n = 8).

Total DDTs measured in the 0–2-cm blubber layer of L-112 (Table 2) were at the high end of the range for juvenile SRKWs (60,000 ng/g lw; average range: 20,000–93,000 ng/g lw). The ∑DDT/∑PCB ratio in blubber from L-112 (~1.6) was similar to those from other juveniles from K and L Pods (average range: 1.2–1.8), and higher than those from juveniles from J Pod (average range: 0.6–0.7), reflecting differences in seasonal movements between these groups.

Total PBDEs measured in the 0–2-cm blubber layer of L-112 (Table 2) were on the lower end of the range compared to those of other juvenile SRKWs (4,600 ng/g lw; average range: 3,500–15,000 ng/g lw), but more than an order of magnitude higher than PBDE concentrations measured in biopsies of male Northern Residents reported by Rayne et al. (2004): a mean of 203 ng/g lw (n = 9).

Although PBDEs are an emerging concern in marine and terrestrial biota, few recent measurements have been made in killer whales or other species. Most published measurements have been made on archived samples, as was true of the samples reported in Rayne et al. (2004), which were collected between 1993 and 1996. PBDEs are still used in North America, meaning that environmental PBDE levels may continue to rise. Because of this and biological factors such as maternal offloading of contaminants during gestation and lactation, juvenile killer whales may be particularly at risk. Higher average levels of PBDEs than in adults have been measured recently in juvenile SRKWs (Krahn et al. 2009), as well as in insular Hawaiian Island false killer whales (*Pseudorca crassidens*; Ylitalo et al. 2009).

The levels of persistent organic pollutants in the blubber of L-112 exceeded the thresholds for some biological effects, particularly immunosuppression and thyroid hormone and retinol disruption (AMAP 2004). The levels also exceeded thresholds associated with reproductive success (AMAP 2004). One caution is that these thresholds are determined mostly from studies with captive animals (e.g., harbor seal [*Phoca vitulina*] and mink [*Neovison vison*]), and how these thresholds compare to effects thresholds in wild populations of other species is somewhat speculative. There is no evidence in the literature to suggest that the levels of POPs found in the blubber of L-112 would make an animal more susceptible to death by trauma.

This multi-disciplinary investigation could not determine the source of the blunt trauma, despite gathering and evaluating all available information on the whales, the environment, and human activities. We evaluated the sighting history of the whales to provide insight into the circumstances of the stranding. Autonomous passive acoustic recorders off the coasts of Washington, Oregon, and California indicated that the main group of L Pod, possibly including L-112, was off California and heading north in late January, was possibly off Westport, Washington, in the first week of February, and was detected near Newport, Oregon, after the stranding. Members of the L4s, and L-112, were not seen with the group of K and L Pod whales observed in Discovery Bay on 7 February. This information does not conflict with the theory that L-112 was more likely to have been at sea off southern Washington or Oregon at the time of her death, rather than in the inland waters of the Salish Sea.

There was no indication of a mass stranding event underway when L-112 was discovered, and no additional dead marine mammals, birds, or fish were recovered or reported in the area at the time. A major source of trauma from sonar, explosives, or a seismic event would likely have affected multiple individuals traveling together, as killer whales are known to do. All other members of L-112's family group were sighted following L-112's stranding. No other members of the L4 sub-group were reported missing, injured, or stranded between the time of the L-112 stranding and the summer of 2012. This observation leads us to believe that the trauma suffered by L-112 was likely borne individually and was not an event that covered a large area or that directly impacted the young whale's most likely traveling companions in the L4 sub-group. For these reasons, we do not believe that L-112 succumbed to blast injuries or exposure to other high-intensity sound.

The flow models and drift card studies indicate that current conditions off the Long Beach Peninsula are largely influenced by eddies created by flows from the mouth of the Columbia River (Figure 13). In the days prior to the stranding, eddies would have flowed northward under the influence of the prevailing wind and currents, allowing floating debris trapped in eddies to be deposited on Long Beach. Floating debris arriving from the open sea to the west or north of

Long Beach would have been carried northward by the current to be deposited elsewhere on the Washington or British Columbia coasts. Because of prevailing currents and eddies, it is unlikely that L-112 died in Canadian waters or the Strait of Juan de Fuca and drifted south. Instead, she likely died in the Columbia River plume or farther to the south along the coast of Oregon. Given the state of decomposition at the time of stranding, the body was either carried by eddies for several days, or may have drifted a substantial distance from the south before being trapped by the eddies and cast ashore on the Long Beach Peninsula.

Epimeletic (care giving) behaviors are well documented among cetaceans (Caldwell and Caldwell 1966). Such behaviors, including standing by (remaining near or approaching an injured companion), have been documented among killer whales. Supporting an injured or deceased animal at the surface has also been documented for delphinids, including killer whales in the wild (Visser 1999). Most accounts of delphinid standing by or supporting behaviors in the wild are short-duration, opportunistic events. Some observations, however, indicate that supporting behavior can persist for hours or days, based on the postmortem condition of the supported carcass or repeated encounters of the same individuals supporting a dead juvenile in the same general area over several days (Caldwell and Caldwell 1966, Ritter 2007). Supporting behavior has been documented among SRKW, including L Pod. On 10 September 2010, an adult female, L-72, was observed supporting a dead neonate killer whale on her rostrum for approximately six hours as she swam in the vicinity of other foraging killer whales. L-72 was sighted again on the following day, but the neonate was not seen (Emmons[4]). Although the stranding location and current patterns lead us to believe the death of L-112 occurred in or south of the Columbia River plume, it is unknown whether epimeletic behavior on the part of the young whale's mother or other closely related whales could have influenced the final deposition of the body.

Sea surface temperatures along the central Oregon to southern Washington coast in early February, where and when L-112 likely died, did not appear to differ markedly from the same time frame in the previous or following year. Temperature does not appear to be a contributing factor to this stranding event.

As a result of inquiries for information on military exercises, we learned that no U.S. or Canadian military activities involving sonar or explosives, except those reported from Canada in the Strait of Juan de Fuca, were undertaken off the coast of Oregon or Washington (where L-112 appears most likely to have been at the estimated time of her death). Similarly, there were no in-water construction or seismic activities using explosives either permitted or reported in the area of the stranding, nor were any explosive events detected on the hydrophones deployed near Westport, Washington or Newport, Oregon at the time. The CT results showed no evidence of bone fractures or damage to L-112's middle or inner ear bones. While these results do not conflict with gross observations and the proposed cause of acute or peracute death by blunt force trauma, blast- or seismic-related injuries cannot be entirely discounted. We acknowledge that postmortem decomposition may have obscured some lesions and hindered mass-spectroscopy gas analysis.

Little information is available on the response of odontocetes to earthquakes. In general, earthquakes are low frequency, under 100 Hz, which is outside of the hearing range of killer whales, although they may cause disorientation for species with low-frequency hearing sensitivity. Killer whales are considered to have mid-frequency hearing and ranges from 1 to at least 120 kHz,

[4] Emmons, C. 2010. Pers. commun. NWFSC, Seattle, WA.

but tend to be most sensitive in the range of 18–42 kHz (Szymanski et al. 1999). The investigative team concluded that small-magnitude earthquakes were unlikely to have caused the traumatic injuries noted during the L-112 postmortem exam. We ruled out an earthquake as a causative factor because the earthquakes recorded off southern Oregon in early February were low-magnitude (≤4), and a larger-magnitude quake (4.8-5.6) off Vancouver Island was to the north and therefore downwind from the stranding. There was no evidence of wide-spread damage or disturbance of other wildlife in the area of the stranding.

There was no gross indication of fisheries interaction, such as external markings from nets, hooks, or lines, and there were no reports of interactions from the fishing community. No vessel strikes were reported; however, we could not rule out a vessel strike. Gaydos and Raverty (2010) summarized killer whale strandings from 2005–2010, and human interactions, including fishery interactions and vessel strikes, were implicated in the deaths of three whales (possibly two others):

- A female calf died of complications secondary to fishing interaction (Glacier Bay, Alaska, 2005)
- An adult female featured a subcutaneous abscess and possible vertebral fractures (Humboldt County, California, 2005: Transient N-18)
- A juvenile male was drawn through the propeller of a tug boat (Gold River, British Columbia, 2006: Southern Resident L-98)
- An adult female presented with hemothorax and a subcutaneous hematoma in the neck (Prince Rupert, British Columbia, 2006: Northern Resident C-21)
- An adult female was suspected to have been hit by a large propeller (Westport, Washington, 2007: Transient T-086)

The presentation of L-112 is not consistent with other killer whale vessel strike cases that included more definitive clues, such as propeller marks or broken bones.

Inter-species and intra-species aggression is documented for a variety of odontocete cetaceans, including bottlenose dolphins (*Tursiops truncatus*; Patterson et al. 1998, Kaplan et al. 2009, Robinson 2013), Pacific white sided dolphins (*Lagenorhynchus obliquidens*; Baird 1998), harbor porpoises (*Phocoena phocoena*; Jepson and Baker 1998, Patterson et al. 1998, Barnett et al. 2009), Atlantic spotted dolphins (*Stenella frontalis*; Herzing and Johnson 1997, Herzing et al. 2003), costeros (*Sotalia guianensis*; Wedekin et al. 2004), Commerson's dolphins (*Cephalorhynchus commersonii*; Coscarella and Crespo 2010), and killer whales (Jefferson et al. 1991, Ford and Ellis 1999). In many cases, the targets of aggression are calves or neonates. The primary signs of injury reported from aggressive attacks are rake marks and musculoskeletal and/or intra-tissue trauma (bruising and tearing) attributed to ramming. Sometimes the inflicted injuries result in death. Contrary to the cases reported in the literature, L-112 was a juvenile animal (older and larger than a calf or neonate), and the examiners did not document tooth rake marks associated with the signs of hemorrhage they observed during the gross examination. Nevertheless, we cannot rule out the possibility that the blunt trauma injuries could have resulted from an aggressive attack, such as ramming, by a larger animal.

While the extensive evaluations were all consistent with blunt force trauma from a collision or blow being the cause of death, the exact type or source of the traumatic injuries (what struck the animal) remains unknown. Blast injury cannot be ruled out, but appears unlikely based on the gross and microscopic findings, gas analysis, advanced diagnostic imaging, and remoteness of naval activities to the proposed area where death may have occurred.

Although we could not identify a definitive source of the trauma or cause of death, the thorough investigation provides a unique look into the threats facing SRKWs. A recent review of killer whale strandings in the North Pacific Ocean (Barbieri et al. 2013) highlights the value of stranding investigations as an integral component of a comprehensive population health assessment program, as they yield data on mortality, life history, and threats to conservation. The measurement, prey, and contaminants data from this investigation of L-112 are valuable and provide additional data for ongoing studies and efforts to recover endangered SRKWs.

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Appendix A-1:
Level A Stranding Report, 12 February 2012

Marine Mammal Stranding Report - Level A Data

Field Number :PSU12-02-11-Oo **Nmfs Regional Number** :12NWR02005 **National Database Number** :NW-2012-1139605

Common Name : Whale, killer **Genus** : Orcinus **Species** : orca

Examiner Name : Debbie Duffield **Affiliation** : Portland State University

Address : 630 SW Mill Portland, OR 97201 **Phone** : 503-725-4078

Stranding Agreement or Authority : Portland State University

Location

Country :

State : WA **County** : Pacific

City : Oceanside

Body of Water Pacific

Locality Details :

0.9 mile north of Cranberry Road access

Lat : 46.40939 N

dd.dddd

Long : -124.06134 W

dd.dddd

How Lat/Long Determined GPS

Occurrence Details

Group Event : N **If yes, type :**

N **Restrand** **GE# :**

N **Cow/Calf Pair** N **Mass Stranding** ☐ **UME** **# Animals** 1 actual

Findings of Human Interaction:

CBD

N **1. Boat Collision** N **2. Shot** N **3. Fishery Interaction** N **4. Other Human Interaction**

How Determined Y **1. External Exam** N **2. Internal Exam** Y **3. Necropsy** N **4. Other**

Gear Collected ?

N

Gear Disposition

Other Findings Upon Level A : Y

N **Illness** Y **Injury** ☐ **Pregnant** Y **Other Findings**

significant trauma/hemorrhage around head, chest, right side, and some internal organs; animal had good body condition

Describe how determined :

Y **External Exam** N **Internal Exam** Y **Necropsy** N **Other**

Initial Observation :

Year : 2012 **Month :** FEB **Day :** 11

First Observed : Beach or Land

Condition at Initial Observation :

ADVANCED DECOMPOSITION

Level A Examination :

N **Not Able to Examine**

Year : 2012 **Month :** FEB **Day :** 11

Condition at Examination

ADVANCED DECOMPOSITION

Initial Live Animal Disposition

N **1. Left at Site**
N **2. Immediate Release at Site**
N **3. Relocated**
N **4. Disentangled**
N **5. Died at Site**
N **6. Euthanized at Site**
N **7. Transferred to Rehab.**
N **8. Died During Transport**
N **9. Euthanized During Transport**
N **10. Other**

Morphological Data

Sex FEMALE

Age Class SUBADULT

Whole Carcass : Y **Partial Carcass :** N

Straight Length : 375 cm actual

Weight : 0 kg estimate

Condition/Determination

N **1. Sick**
N **2. Injured**
N **3. Out of Habitat**
N **8. Unknown/CBD**
☐ **4. Deemed Releasable**
N **5. Abandoned/Orphaned**
N **6. Inaccessible**
N **9. Other**

7. Location Hazardous :
N **a. To animal**
N **b. To public**

Comment :

Photos/Videos Taken : Y

Disposition : PSU archive

Tag Data :

Tags Were :

Present at time of Stranding (pre-existing) : N

Applied during Stranding Response : N

ID	Color	Type	*Placement	Applied	Present
				N	N
				N	N
				N	N
				N	N

* D=Dorsal; DF= Dorsal Fin; L= Lateral Body; LF= Left Front; LR=Left Rear; RF=Right Front; RR= Right Rear;

Whole Carcass Disposal (Check one or more)

N **1. Left at Site**
N **2. Buried**
N **3. Rendered**
N **7. Landfill**
Y **9. Other**
N **4. Towed** Lat Long
N **5. Sunk** Lat Long
N **6. Frozen for Later Exam**
N **8. Unknown**

Specimen Disposition

Y **1. Scientific Collection** Y **2. Educational Collection**
3. Other Y

Comments : stomach, repro, and selected tissue samples archived at PSU; various tissue samples collected for CRC, WDFW, NMFS, Raverty, etc.; histo samples taken

Necropsied : Y Complete Fresh

Necropsied by : Duffield, Lambourn, Huggins et al.

Date : 2012-FEB-12

NOAA Form 89-864 (rev. 2007)
OMB No. 0648-0178; Expires 1/31/2014

Additional Identifiers : L112
Additional Remarks : Animal was identified by NMFS as L112, born in 2009.

DISCLAIMER

THESE DATA SHOULD NOT BE USED OUT OF CONTEXT OR WITHOUT VERIFICATION. THIS SHOULD BE STRICTLY ENFORCED WHEN REPORTING SIGNS OF HUMAN INTERACTION DATA.

DATA ACCESS FOR LEVEL A DATA

UPON WRITTEN REQUEST, CERTAIN FIELDS OF THE LEVEL A DATA SHEET WILL BE RELEASED TO THE REQUESTOR PROVIDED THAT THE REQUESTOR CREDIT THE STRANDING NETWORK AND THE NATIONAL MARINE FISHERIES SERVICE. THE NATIONAL MARINE FISHERIES SERVICE WILL NOTIFY THE CONTRIBUTING STRANDING NETWORK MEMBERS THAT THESE DATA HAVE BEEN REQUESTED AND THE INTENT OF USE. ALL OTHER DATA WILL BE RELEASED TO THE REQUESTOR PROVIDED THAT THE REQUESTOR OBTAIN PERMISSION FROM THE CONTRIBUTING STRANDING NETWORK AND THE NATIONAL MARINE FISHERIES SERVICE.

PAPERWORK REDUCTION ACT INFORMATION

PUBLIC REPORTING BURDEN FOR THE COLLECTION OF INFORMATION IS ESTIMATED TO AVERAGE 30 MINUTES PER RESPONSE, INCLUDING THE TIME FOR REVIEWING INSTRUCTIONS, SEARCHING EXISTING DATA SOURCES, GATHERING AND MAINTAINING THE DATA NEEDED, AND COMPLETING AND REVIEWING THE COLLECTION OF INFORMATION. SEND COMMENTS REGARDING THIS BURDEN ESTIMATE OR ANY OTHER ASPECT OF THE COLLECTION INFORMATION, INCLUDING SUGGESTIONS FOR REDUCING THE BURDEN TO: CHIEF, MARINE MAMMAL AND SEA TURTLE CONSERVATION DIVISION, OFFICE OF PROTECTED RESOURCES, NOAA FISHERIES, 1315 EAST-WEST HIGHWAY, SILVER SPRING, MARYLAND 20910. NOT WITHSTANDING ANY OTHER PROVISION OF THE LAW, NO PERSON IS REQUIRED TO RESPOND, NOR SHALL ANY PERSON BE SUBJECTED TO A PENALTY FOR FAILURE TO COMPLY WITH, A COLLECTION OF INFORMATION SUBJECT TO THE REQUIREMENTS OF THE PAPERWORK REDUCTION ACT, UNLESS THE COLLECTION OF INFORMATION DISPLAYS A CURRENTLY VALID OFFICE OF MANAGEMENT AND BUDGET (OMB) CONTROL NUMBER.

NOAA Form 89-864 (rev. 2007)

OMB No. 0648-0178 : Expires 1/31/2014

Appendix A-2:
Initial Necropsy Report, 12 February 2012

PSU 12-02-11 Oo
Killer whale (*Orcinus orca*)
Necropsied at Cape Disappointment State Park on 12 February 2012
Duffield, Lambourn, Huggins *et al*.

Stranding Location: Oceanside, Pacific county, WA
Date of Stranding: 11 February 2012
Sex: Female (subadult)
Decomposition code: Code 3, moderate decomposition

Selected measurements:
TOTAL LENGTH: rostrum–fluke: 375.0 cm
Girth at axilla: 234.6 cm
Mid-dorsal blubber thickness: 4.5 cm
Mid-lateral blubber thickness: 3.7 cm
Mid-ventral blubber thickness: 3.8 cm

Histo samples collected (Batch 1 delivered to OSU on 29 March 2012)

Blubber	Thymus
Heart	Thyroid
Lung	Bladder
Liver	Muscle
Kidney	Lymph nodes (suprascapular, subscapular, mediastinal, and repro)
Spleen	Mammary tissue
Spinal cord	Tongue
Intestines	Spongy hemorrhaged tissue around spinal column
Colon	Stomach (in separate container)
Brain	

Histo samples collected (Batch 2 delivered to OSU on 04 April 2012)
Brain (left side)
Misc. samples #1
Misc. samples #2
Left bulla surrounding tissue
Right bulla surrounding tissue
Left eye and surrounding tissue
Right eye and surrounding tissue

Necropsy notes:
The animal had good body condition. At time of necropsy, carcass was slightly distended, and bruising was noted around throat and chest (visible through skin). Left eye a little bulgy. Genital slit and blowhole swollen, Tongue swollen and darkened. Significant soft tissue trauma in head, chest, down right lateral side of body, lungs, and heart. Other hemorrhagic tissues/organs included thyroid; subscapular, suprascapular, mediastinal, and repro lymph nodes; tongue; epidural rete; esophagus; oropharynx; pharyngeal musculature; liver; and around spinal cord at skull and rib cage bases. Marked red serous fluid in calvarium (~2 L). Brain poured out of foramen magnum in chunks. Red serous fluid (~3 L) in thoracic cavity, mostly on right side. Both lungs congested. Upper lung lobes had marked red serous edema around pleural lining (more on right than left).

Approximately 50 cc of brick red pericardial fluid present. Outer heart lining had red serous edema. Good subcutaneous fat around heart. Internal heart tissue green, possibly due to oxidation after removal from animal. Thymus unremarkable. Liver friable and slightly brownish. Spleen soft and mushy. Kidneys very friable. Unable to locate adrenals. Bladder empty. Much feces present in colon. Forestomach lining sloughed in one piece and was balled up inside main stomach. A roughened area with some erosion (8 cm x 6 cm) in posterior end of forestomach lining. Approximately 30 non-embedded nematodes were found within balled up forestomach lining. Stomach contents included four small fish eye lenses.

Report Printed: 1/2/2013 9:47:03 PM ***Primary Stranding Number*** PSU 12-02-11 Oo

Cascadia Research Stranding and Necropsy Report for: PSU 12-02-11 Oo

Killer Whale ***Orcinus*** ***orca*** **Other#** L112

Response? Yes **Live or Dead?** Dead **Reponse Date** 11-Feb-12 **ResponseTime** 8:00

Responding Agency PSU/Seaside A **Lead Responder** Deb Duffield **Field Photos?** Yes **Frames** Seaside Aquarium/PSU

Other Responders

City Long Beach **Locality** N of Cranberry Beach approach **Dec Lat** 46.4093 **Dec Long** -124.0613

Initial Sigthing Date 11-Feb-12 **Contact Name** **Contact #**

Response Comments

Reported dead on beach at 0700 11-Feb-12. Animal wasn't on beach overnight (report of people attempting to remove teeth when responder arrived). Moved to Cape Disappointment same day for necropsy. Identified by photo id as L112.

External Exam Details-Dead

Sex Female **Age Class** Sub-adult/Juve **Estimated Age** 2-3 years **Cohort** 2009 **Cohort Status** Confirmed

Body Condition Good **Carcass Condition** 3 **Time Dead** 3-5 days **Scavenging** Minimal **External Injuries** Yes

(For Pups/Calves) **Dentition** **Umbilicus** **Percent Lanugo**

External Comments

Carcass slightly distended, bruising around throat and chest (visible through the skin). Eyes intact, left eye slightly bulgy. Was found on beach laying on rt side. 2 Small linear scars on dorsal right, one just behind dorsal fin, the other at same level as anus, both appear healed. Skin just barely starting to slough, intact except for small patch on mid L flank. Swelling at genital slitand blowhole. Tongue swollen and darkened. No abrasions/lesions/lacerations seen externally.

Tag Present? No **Tag #** **Tag Color** **Tag Type** **Tag Location**

Human Interaction Unable to Determine Boat Collision? ☐ Shot? ☐ Fishery interaction? ☐ Other, Describe ☐

HI Detail Severe bruising around head, chest, scapulae, and down right flank to just anterior of dorsal fin. Brusing more severe on R side of animal. Origin of injuries unknown. HI form completed: Detailed external/internal, CT scan and bones examined. Final determination pending.

Carcass Disposition Collected for Necropsy **Disposition Comments** skeletal material and entire head retained. Soft tissues disposed of by Deb Duffield and State Parks

Basic Measurements-Dead

Standard Length 375 **Length Units** cm **Length Type** Actual **Sternal Blubber Depth (cm)** 3.8

Weight **Weight Units** **Weight Type**

Measurement Comments:

External Exam Details and Measurements-Live

Condition Determination

Sex	**Age Class**	**Percent Lanugo**	**Body Condition**	**Injuries?**	No
Standard Length	**Length Units**	**Length Type**	**Weight**	**Wt Units**	**Wt Type**

Physical Exam Notes

Initial Temp	**Initial Heart Rate**	**Initial Respirations**	**Initial MM Color**	**Initial Cap Refill**

Diagnosis **Treatment**

Post-treat Temp	**Heart Rate**	**Respirations**	**MM Color**	**Cap Refill**

Action Taken

Rehab Date **Rehab Facility**

Live Disposition Comments

Tag Present? 0	**Tag Applied?** 0	**Tag #**	**Tag Color**	**Tag Type**	**Tag Location**	

Detailed Measurements (cetaceans Only)

Length Unit	Centimeter	Length of Throat Grooves		Tooth/Baleen Count (Upper Right)	12		
Standard Length	375	Number of Throat Grooves		Tooth/Baleen Count (Upper Left)	12		
Snout to Anus	526.9	Flipper Length, Anterior	48.7	Tooth Count (Lower Right)			
Snout to Genital Slit	243.9	Flipper Length, Posterior	37.7	Tooth Count (Lower Left)			
Snout to Umbilicus	172.2	Flipper Width (Maximum)	30.8	Diameter Largest Tooth/Length Longest Plate			
Snout to Throat Grooves		Dorsal Fin Height	41.2	Baleen Color			
Snout to Dorsal Fin Tip	192	Dorsal Fin Length at Base	55.5	Blubber Thickness (Dorsal)	4.5		
Snout to Dorsal Fin Insertion	155	Fluke Width	90	Blubber Thickness (Lateral)	3.7		
Snout to Pectoral Fin Insertion	80	Fluke Depth (Lobe)	26.1	Blubber Thickness (Ventral)	3.8		
Snout to Ear	59.2	Fluke Depth (Notch)	30.6	Ovary Dimensions (LxWxD) (Right)			
Snout to Eye	42.5	Fluke Notch Depth	5	Ovary Dimensions (LxWxD) (Left)			
Snout to Gape	37.5	Length of Mammary Slits (Right)	2.2	Testis Dimensions (LxWxD) (Right)			
Snout to Blowhole	55.1	Length of Mammary Slits (Left)	2	Testis Dimensions (LxWxD) (Left)			
Eye to Ear	18.6	Length of Genital Slit	23	Penis Length			
Eye to Gape	6.3	Length of Anal Slit	3.5	Weight Units:			
Eye to Blowhole Edge (Right)	37.7	Repro Opening to Anus		Testis Weight with Epididymis (Right)			
Eye to Blowhole Edge (Left)	35.1	Girth at Eye	177.8	Testis Weight with Epididymis (Left)			
Head Diameter at Eyes	48.4	Girth at Axilla	234.6	Testis Weight without Epididymis (Right)			
Length of Eye Opening	48.4	Girth, Maximum	246.4	Testis Weight without Epididymis (Left)			
Rostral Width at Melon Apex		Girth at Anus	146.1	Ovary Weight (Right)			
Projection Upper/Lower Jaw	9.3	Girth, Midway Anus to Notch	37.9	Ovary Weight (Left)			

Measurement Comments: some bloating. Other measurements: Blowhole length=11.8, tailstock to notch=5.8

Necropsy Summary

Necropsy Date 12-Feb-12 **Necropsy Agency** PSU/CRC/WDFW **Necropsy Location** Field

Lead Scientist Deb Duffield **Necropsy Assistants** Dyanna Lambourn, Jessie Huggins, Dalin D'Alessandro, CRC interns, WDFW interns, Seaside Aquarium, Adrianne Akmajian, Seattle Seal Sitters

General Necropsy Notes

Entire head retained intact for CT scan. No broken bones seen on gross but bones will be cleaned and examined in detail for fractures. Significant trauma in blubber and muscle on head, chest and around scapulae, down rt lateral side to just forward of dorsal fin. Marked hemorrhage and edema, marked red serous fluid in cranium and chest cavity (mostly on R side in chest cavity). Bleeding into muscle/blubber layers, blood absent in arteries and veins. Crepitus between blubber and mucle on L flank. Air bubbles in various tissues (see specifics below)--unsure if due to decomp or other factor. Crepitis and air bubbles aslong dorsal L adbdominal cavity. All organs intact with exception of kidneys and pancreas (presumed). Cranial exam conducted on 06 and 07 Mar by Joe Gaydos and Dyanna Lambourn, notes in tissue table below. Head had been frozen and was partially thawed at time of exam. Left side head bruising extended ~2 inches above eye to tooth # 6 on lower jaw and distending back ~8 inches toward the shoulder. Right side head bruising was noted ~2 inches above eye tooth #4 on right lower jaw and extends back past the insertion of right pectoral fin and across ventral lower jaw almost all the way to inside of the left lower mandible. The tongue is dark gray to black, swollen and edematous. There are 12 teeth erupted from the right and left mandible as well as from the right and left maxillae. On the left mandible, the 10th tooth caudal is angled more medially than the other teeth; it appears to have erupted in this direction as the tooth is firmly held by the periosteal ligament and there is no associated bruising or signs of trauma. Dissection reveals bruising in the subcutaneous tissue over the left and right eyes, with that over the left eye being more significant. On cut surface the melon has an almost clear appearance at its ventro-medial aspect, but the tissue surrounding that (dorsally and laterally) is diffusely pink to red, especially from the area just in front of the blowhole and lateral diverticulae or multiple sacs associated with the blowhole extending cranially to about 27cm towards the beak (Image 13). The pink to red color is darker on the right side than it is on the left. The rostral muscles adjacent to the melon on the right side just above the maxillae is dark red with apparent hemorrhage as is the connective tissue on the right side at the junction of the blowhole's rostral vestibular sac and the melon. Approximately 5-10cc of serosanguinous fluid is present frozen in the left side of the paired nares within the blowhole. The right side is clear.

Ventrally, a triangular section of tissue just medial to the right mandible and below the tongue measuring approximately 7cm at its base with a 16cm height is dark brown and green and aerated (Image 14). A smaller area on the medial to the left mandible (~3cm long) is noted as well. Similar colored tracks extend caudally in towards the ramus of the mandible and pharyngeal area. The mandibular or pan-bone fat of the left mandible is dark red (Image 15). The fat in the right mandible appears more autolyzed and darker. Removal of the mandibles and the hyoid reveals an air-filled sponge-like brown material just rostral to the tympanic bulla on the right side. Less of this material is present on the left side.

Frozen serosanguinous fluid suspected to be blood is evident in the cranial esophagus / pharyngeal region as well as at the junction of the larynx / narial passage. The left-side pharyngeal muscles are red and appear hemorrhagic.

Dissection of the tympanic bulla reveals that the right bulla is less adherent to the skull or at least significantly looser leaving easier visualization of what we presume is the fibro-venous plexus than is the left (Images 16 and 17). Post-dissection of the tympanic bulla, 1 small (1-2cm) nematode and approximately 12 slightly longer (2-4cm) worms that are more flat, are present in the area of the skull that was adjacent to the tympanic bulla, including peribullar sinus, fibro-venous plexus and surrounding peri-bulla soft tissue of both bulla. Concomitant with the parasites is a brown, sponge like material that appears to extend into the bulla. Approximately 6cc of red serous fluid is present in both peribullar sinuses. Two small bony fragments dorsal to the right and left bulla are present. On the left side they measure approximately 2.5cm x 2cm and 1.5cm x 1.5 cm and they appear to not be displaced but are easily removed. On the right side they measure 4cm x 2cm and 1.5 cm x 1.5 cm and are displaced into the calvarium The edges of all four pieces are irregular and well rounded and don't appear to be freshly fractured (Image 18).

Removal of a large triangular section of the occipital bone revealed slightly frozen brain material on the left side cerebrum encapsulated by meninges and a brain free meninges that was adherent to the calvarium in 3-4 places. Cerebellum was mostly gone and portion were leaking into the left bulla area. Roughly 20cc of dark red to brown frozen serosanguinous fluid is visible between the dura and the calvarium (Image 19). This fluid was consistent with the fluid that was noted during the initial necropsy. The sutures on the right side calvarium appear to be looser then on left and red serous fluid is leaking around suture area.

Radiographs:

CT conducted on 23-Feb. Findings written by Tori McKlveen DVM, MS, Diplomate, American College of Veterinary Radiology, Sophie Dennison, BVM&S, MRCVS, Diplomate ACVR)

CT Findings: This patient is positioned with the left side down and the right side up.On the scan window overlay, A= Left and P=Right.

There is extensive gas accumulation in the soft tissues and fat throughout the head Including intracranially (the head has been disarticulated for imaging).

There is loss of brain matter. The right side of the calvarium is almost completely devoid of brain tissue- the majority of the right side of the brain is missing including the right cerebral hemisphere and the right side of the cerebellum. Soft-tissue attenuating striations suspended within the calvarium are suggestive of residual meninges on the right. Brain tissue is present on the left side. On the sequences with the head positioned as straight as possible, no asymmetry to the large included bones of the skull is noted. No large displaced fractures of the calvarium are seen. There are a few small, smoothly marginated mineral attenuating densities (small bones and/or otoliths and/or dystrophic mineralization) and also somewhat thin linear area of mineralization at the level of, but separate from, the osseous bulla. Most of these structures are dorsal to the bulla. Some of these are quite small and are present bilateral, but are asymmetric. There is displacement of some of these very small mineralized bodies on the right side through the calvarial

foramina. Image 1. This is interpreted with caution because in this area, especially on the right side, there is loss of normal soft tissue structures (brain, fat etc) that may previously have held these in place outside of the calvarium. The peribullous sinus contains a mixture of air and soft tissue attenuating material bilaterally. There is accumulation of soft tissue or fluid attenuating material and gas in the pterygoid sinuses with the left side having more soft-tissue or fluid-attenuating material than the right. There is soft-tissue or fluid-attenuating material in the majority of the left osseous bulla. There is soft-tissue or fluid-attenuating material and air in the rostral aspect of the right osseous bulla with air in the mid to caudal right osseous bulla. The right bulla has more air in it than the left. The left is almost completely filled with fluid and/or soft tissue. Considerations for this fluids/soft tissue attenuating material are blood, infectious or inflammatory debris, polyp like material-chronic inflammation or parasites and/or post-mortem accumulation of fluid or engorged mucous membranes. See image 2 and 3. The tympano-periotic complexes are intact bilaterally. The auditory ossicles cannot be fully evaluated due to limitations of resolution.
CT summary:
-Extensive gas accumulation in the soft tissues and fat. Disarticulation prevents further comment.
-Absence of right cerebral hemisphere and right cerebellum of the brain secondary to loss of tissue during disarticulation. Significance is uncertain based on imaging alone but is an atypical observation.
-Bilateral small mineral (i.e. bone) attenuating structures dorsal to the bulla (Ddx: otoliths, dystrophic mineralization, parasitic granulomas) with right-sided displacement through the foramina into the calvarium due to loss of supporting tissues (presumptive).
-Sinusitis, likely parasitic.
-Fluid and/or soft tissue in both osseous bullae (middle ears) worse on the left. Possibilities include: blood, infectious or inflammatory debris, polyp like material and chronic inflammation, parasites (including worms) and/or post mortem accumulation of fluid or engorged mucous membranes.

Necropsy Case Summary and Significant Findings

Trauma, unknown origin. This Animal was in good body condition and moderate post-mortem condition. Significant soft tissue trauma was present in the head, chest and down the right lateral side of the body as evidenced by marked hemorrhage and edema present in the skin, blubber, subcutaneous tissues and muscles (described above), as well as in lung and heart. Marked red serous fluid was present in the calvarium (~ 2 L) and the brain poured out of foraman magnum in chunks. Red serous fluid (~ 3 L) also was present in the thoracic cavity, mostly on the right side. Blood was absent in arteries and veins examined and air bubbles were closely associated with vessels. No broken bones were noted on the initial necropsy, CT scan or head dissection or during further flensing of the carcass except for the noted two small bony fragments displaced dorsal to the right and left bulla (seen on CT and head dissection).

Case Summary Lab Results

Primary COD Pending **Secondary COD** Pending

Tissue Observation Table

Blubber	good lipid content, non-hemorrhaged areas appear normal. See general comments for location of hemorrhage
Muscle	hemorrhage deep into muscle tissue around head, chest and scapulae with dark red serous edema. Backstrap muscle slightly friable, pulls easily from vertebrae. More friable on R than L
Heart	Outer lining has red serous edema. Air bubbles in vessels and surround coronary veins/vessels (gases sampled). Good subcu fat around heart. Tissue green in color on inside (possibly due to oxidation after removal from chest cavity)
Lung	Both dark, congested and hemorrhagic. Upper lung lobes have marked red serous edema around pleural lining (more on R than L)
Liver	friable but intact, crepitus throughout, slightly brownish in color.
Spleen	soft and mushy but intact
Kidney	severe crepitis and decomp behind kidney, soupy and falling apart when removed.
Pancreas	unable to locate.
Stomach	forestomach tore on dorsal aspect when moved (decomp). Forestomach lining sloughed in one piece and was balled up inside the main stomach. A roughened area with some erosion (8 cm x 6 cm) was present in the posterior end of the forestomach lining. Approximately 30 non-embedded nematodes were found within the balled up forestomach lining.
Intestines	air bubbles in mesenteric arteries and vessels (gases sampled), sm amt of feces in colon.
Urinary Bladder	empty, full of air
Reproductive Tract	not reproductively active, collected whole by PSU

Adrenal Glands	Unable to locate.
Brain	poured freely out of skull. Medium red serous fluid w/firm, intact chunks of brain tissue. Small chunks at first, larger chunks after head detached. Fluid got darker as chunks got larger. No frank blood or blood clots. ~2 L red serous fluid total from skull, fluid slightly thickened.
Spinal Cord	significant hemorrhage around cord both at base of skull and base of ribcage.
Tonsils	not examined (removed with intact head)
Thyroid	Hemorrhaged, with edema around tissue
Thymus	NSF
Suprascapular L.N.	Edema and hemorrhage
Subscapular L.N.	Edema and hemorrhage
Mediastinal L.N.	significant hemorrhage
Mesenteric L.N.	not examined
Inguinal L.N.	not examined
Reproductive L.N.	NSF
Pericardial Fluid	~50cc present, brick red
Vitreous Humor	clear but straw colored
Stomach Contents	Main:17 mL slightly mucousy, brown colored; Connecting:14 mL pasty, salmon pinkish tan colored; Pyloric:20 mL pasty, salmon pinkish tan colored. 4 small fish eye lenses, 2 from connecting/2 from pyloric.
Tongue	swollen and bluging from mouth, edema and hemorrhage
Mammary Tissue	NSF
Air bubble (mesenteric artery)	1 sample, collected with sterile 3cc monojet syringe and 20g needle, placed into 2ml glass bd vacutainer conventional red top tube (photo Matt DSC6694)
Air bubble (mesenteric vessels)	2 samples, collected with sterile vacutainer adapters into 5ml glass bd vacutainer conventional red top tubes (photo Matt DSC6670)
Air bubble (off coronary artery)	1 sample, collected with sterile 3cc monojet syringe and 20g needle, placed into 2ml glass bd vacutainer conventional red top tube
Epidural rete	hemorrhaged, dark red and edematous
Melon	distal to point 27cm. Entire melon is red to pink. mostly red centrally, bruising significant over L eye more than R eye. Caudal melon more bruisin on muscle on R (temporalis)
Mandible, R	15cm long at base x 7.5cm
Muscle, Mandible,R medial aspect to central tongue	brown green, aerated, obvious hemorrhage distributed through tissues, lateral bruising tracks extend distally into submandibular tissue. 0.5-4.5cm diameter
temporalis muscle, L	no hemorrhage apparent
temporalis muscle, R	no hemorrhage apparent
Mandible, Inside L	mild hemorrhage medial aspect
Blowhole, L	serosanguinous fluid, 5-10cc
Blowhole, R	looks clear, small amt of blood
Junction of melon/phonic lips, R side	reddened/darkened
Underneath Melon, R side	dark connective tissue
Junction, hyoid, posterior to bulla, R side	appears airfilled with weblike brown material

Junction, hyoid, posterior to bulla, L side	mostly clear, no brown material like right side
Mandible, L	10th tooth back angled toward the tongue (medially)
Mandible, Caudal L	appears to have some hemorrhage and edema in pan fat
Mandible, Caudal R	appears to have more hemorrhage and edema in pan fat than L
Esophagus, cranial	hemorrhage
Oropharynx	appears to be some hemorrhaging
Pharyngeal musculature, L	hemorrhage
Mandibles, L and R	no obvious fractures
Bullae	space around R earbone (~2cm around dorsal surface), on L tissue is (lightly opposed?). R ear bone also significantly looser than L ear bone
Upper Jaw	12 teeth each side upper jaw. Most caudal teeth both sides are angled slightly medially
Peribullar area, L	1cm nematode, just external to bulla. ~4-6" flatworm also recovered
Bulla, R	0.25cm layer of thick brown material around caudal and dorsal aspect of R bulla, 20cc brown red fluid dorsal/cranial aspect R bulla
Ear, R	8cm worm recovered from R ear, plus 6 additional flatworms in R peribullar area. No nematodes seen.
Skull, R side	fluid in space between dura mater and calvarium. roughly 20-60 cc dark red to brown serosanguinous fluid between skull and meninges
Meninges	bleeding inside
Brain, L side	no brain architecture (fell apart when meninges removed). Most of cerebellum gone-presumed to have leaked into bullae area.
Bullae, behind cranial hiatus	2 free-floating pieces behind both ear bones (R and L). L#1=2.5x2cm, L#2=1.5cmx1.5cm, R#1=4x2cm, R#2=1.5x1.5cm

Samples Collected for: Andrew Allison **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:** 29-Feb-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☑	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

tissues contributed to his research project at Cornell University (NOAA permission granted)

Samples Collected for: Bact Archive **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:**

Blood	☐	Kidney	☐	Brain	☑	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☑	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☑	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☑
Heart	☑	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☑			Stomach Contents	☐
Liver	☐	Colon	☑	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☑			Other, Desc in Comments	☑

Sample Comments

Other: tongue, mammary tissue, repro LN. Comment: This set of tissues not sent with other tissues to UC Davis (per Stephen Raverty instructions)

Samples Collected for: Bact Archive **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:**

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☑
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Other sample: thoracic cavity fluid. Comment: This set of tissues not sent with other tissues to UC Davis (per Stephen Raverty instructions)

Samples Collected for: Brad Hanson-2 **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:** 10-May-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Dorsal fin

Samples Collected for: Brad Hanson **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:** 10-May-12

Blood	☐	Kidney	☑	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☑
Heart	☐	Stomach	☑	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☑	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

Brad picked up stomach tissue and contents from Deb on 28-Mar. The rest of the tissues transferred on 10-May.

Samples Collected for: Brad Hanson **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:** 10-May-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☑	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

Samples Collected for: CAS **Stored in: Glass Jar** **and Preserved by: Frozen** **Date Sent:** 22-Oct-12

Blood	☐	Kidney	☑	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☑	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

for metals analysis

Samples Collected for: CRC **Stored in: Glass Jar** **and Preserved by: Frozen** **Date Sent:**

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

Samples Collected for: Dawn Noren **Stored in: Foil** **and Preserved by: Frozen** **Date Sent:** 10-May-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

tissue contributed to her research at NMML.

Samples Collected for: Ken Balcomb **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:** 06-Mar-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☑	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

from head exam on 06 March, he collected tissues himself. Lower jaw fat taken as well?

Samples Collected for: Mike Grigg **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:** 01-Oct-12

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☑	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☑	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☑	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☑		Other, Desc in Comments ☑

Sample Comments

Other: tongue

Samples Collected for: Mike Grigg **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:** 01-Oct-12

Blood ☐	Kidney ☐	Brain ☑	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☑

Sample Comments

thoracic cavity fluid

Samples Collected for: NMFS-Cont **Stored in: Foil** **and Preserved by: Frozen** **Date Sent:** 10-May-12

Blood ☐	Kidney ☑	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☑	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☑	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☑	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☐

Sample Comments

Samples Collected for: NMFS-DA **Stored in: Falcon tube** **and Preserved by: Frozen** **Date Sent:** 30-Apr-12

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☑
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☑
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☐

Sample Comments

Samples Collected for: NVSL (Archive) **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:**

Blood ☐	Kidney ☑	Brain ☑	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☑	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☑	Spleen ☐	Thymus ☑	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☑
Heart ☑	Stomach ☐	Thyroid ☑	Ovary ☐	Muscle ☑	Suprascapular LN ☑	Pituitary Gland ☐	Parasites ☐
Lung ☑	Intestines ☑	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☑		Stomach Contents ☐
Liver ☑	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☑		Other, Desc in Comments ☑

Sample Comments

Other Samples: mammary tissue, tongue, spongy homorrhaged tissue around spinal column, repro L.N. Comment: brain collected in a 500ml plastic container.

Samples Collected for: NVSL (Archive) **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:**

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☑
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☑

Sample Comments

Other Samples: thoracic cavity fluid

Report Printed: 1/2/2013 9:47:03 PM ***Primary Stranding Number*** PSU 12-02-11 Oo

Samples Collected for: PNNL? or UCSC? Stored in: Bag and Preserved by: Frozen Date Sent:

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☑	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

from head exam on 07 March.

Samples Collected for: PSU Stored in: Bag and Preserved by: Frozen Date Sent:

Blood	☑	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☐	Uterus	☑	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☑
Heart	☑	Stomach	☐	Thyroid	☐	Ovary	☑	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☑	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☑	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Other: tongue

Samples Collected for: Raverty (OSU) Stored in: Plastic Cont and Preserved by: Formalin Date Sent: 27-Mar-12

Blood	☐	Kidney	☑	Brain	☑	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☑	Urinary Bladder	☑	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☑	Thymus	☑	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☑	Stomach	☑	Thyroid	☑	Ovary	☐	Muscle	☑	Suprascapular LN	☑	Pituitary Gland	☐	Parasites	☐
Lung	☑	Intestines	☑	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☑			Stomach Contents	☐
Liver	☑	Colon	☑	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☑			Other, Desc in Comments	☑

Sample Comments

Other Samples: mammary tissue, tongue, spongy homorrhaged tissue around spinal column, repro L.N. Comment: Stomach samples collected at PSU by Deb Duffield. Samples transferred by Dyanna Lambourn to Deb Duffield (to go to OSU) on 27-Mar-2012

Samples Collected for: Raverty-2 (OSU) Stored in: Plastic Cont and Preserved by: Formalin Date Sent: 03-Apr-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Big container, from head exam on 06-07 March. Contains melon, muscle from lower jaw, blubber and muscle on top of head.
Transferred to Deb Duffield on 03-Apr to go to OSU

Samples Collected for: Raverty-2 Stored in: Bag and Preserved by: Frozen Date Sent: 16-Apr-12

Blood	☐	Kidney	☐	Brain	☑	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

From head exam on 07 March. R side lower jaw, melon slice, brain, meninges, lower R jaw pan fat (x2), lower L jaw pan fat (x2), pharyngeal musculature hemorrhage L side.Tranferred to Dyanna Lambourn on 09-Apr-12 for transfer to border.

Samples Collected for: Raverty-2 Stored in: Vial and Preserved by: Frozen Date Sent: 16-Apr-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

from head exam on 07 March. Blowhole/pharynx junction (oropharynx), meningeal fluid, peribullar fluid R side, Cranial esophagus, blowhole fluid. Tranferred to Dyanna Lambourn on 09-Apr-12 for transfer to border.

Samples Collected for: Raverty-3 (OSU) Stored in: Plastic Cont and Preserved by: Formalin Date Sent: 03-Apr-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☑	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Big container, from head exam on 06-07 March. Contains blowhole tissue, lower jaw, pan fat
Transferred to Deb Duffield on 03-Apr to go to OSU

Samples Collected for: Raverty-4 (OSU) Stored in: Plastic Cont and Preserved by: Formalin Date Sent: 03-Apr-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☑	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Small container, from head exam on 07 March. Contains L side eye and tissues around L side head that were abnormal.
Transferred to Deb Duffield on 03-Apr to go to OSU

Samples Collected for: Raverty-5 (OSU) Stored in: Plastic Cont and Preserved by: Formalin Date Sent: 03-Apr-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☑	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Small container, from head exam on 07 March. Contains R side eye and tissues around R side head that were abnormal.
Transferred to Deb Duffield on 03-Apr to go to OSU

Samples Collected for: Raverty-6 (OSU) Stored in: Plastic Cont and Preserved by: Formalin Date Sent: 03-Apr-12

Blood ☐	Kidney ☐	Brain ☑	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☐

Sample Comments

large container, from head exam on 07 March. Contains brain and meninges from L side of head
Transferred to Deb Duffield on 03-Apr to go to OSU

Samples Collected for: Raverty Stored in: Bag and Preserved by: Frozen Date Sent: 16-Apr-12

Blood ☐	Kidney ☑	Brain ☑	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☑	Urinary Bladder ☑	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☑	Spleen ☑	Thymus ☑	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☑
Heart ☑	Stomach ☐	Thyroid ☑	Ovary ☐	Muscle ☑	Suprascapular LN ☑	Pituitary Gland ☐	Parasites ☐
Lung ☑	Intestines ☑	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☑		Stomach Contents ☐
Liver ☑	Colon ☑	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☑		Other, Desc in Comments ☑

Sample Comments

Other Samples: mammary tissue, tongue, spongy homorrhaged tissue around spinal column, repro L.N. Comment: brain frozen in 500ml plastic container.

Samples Collected for: Raverty Stored in: Dry Swab and Preserved by: Frozen Date Sent: 16-Apr-12

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☑

Sample Comments

from blowhole

Samples Collected for: Raverty **Stored in: Plastic Cont** **and Preserved by: Formalin** **Date Sent:**

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Two Large and 2 Small containers, from head exam on 07 March. Large containers have bull and two small accompanying bones (L and R in separate containers). 2 Small containers have tissues surrounding the bulla (L and R in separate containers).

Samples Collected for: Raverty **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:** 16-Apr-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☑
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☑	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Other: thoracic cavity fluid

Samples Collected for: SWFSC **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:** 20-Feb-12

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☑	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☑	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☐

Sample Comments

Samples Collected for: U of Fl, Toni McIntos Stored in: Vial and Preserved by: Ethanol Date Sent: 20-Mar-12

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☑
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☐

Sample Comments

from head exam on 07 March. Contains parasites from bullae.

Samples Collected for: UC Davis-Bact Stored in: Bag and Preserved by: Other Date Sent: 13-Feb-12

Blood ☐	Kidney ☑	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☑	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☑	Pituitary Gland ☐	Parasites ☐
Lung ☑	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☑	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☐

Sample Comments

Refrigerated

Samples Collected for: UC Davis-Bact Stored in: Culture and Preserved by: Other Date Sent: 13-Feb-12

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☑

Sample Comments

Culture swab from blowhole

Report Printed: 1/2/2013 9:47:03 PM ***Primary Stranding Number*** PSU 12-02-11 Oo

Samples Collected for: UC Davis-Vir **Stored in: Bag** **and Preserved by: Frozen** **Date Sent:**

Blood	☐	Kidney	☑	Brain	☑	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☑	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☑	Spleen	☑	Thymus	☑	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☑	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☑	Suprascapular LN	☑	Pituitary Gland	☐	Parasites	☐
Lung	☑	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☑			Stomach Contents	☐
Liver	☑	Colon	☑	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

ARCHIVED. Other Samples: tongue, mammary tissue, repro LN

Samples Collected for: UC Davis-Vir **Stored in: Vial** **and Preserved by: Frozen** **Date Sent:**

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

ARCHIVED. Other: thoracic cavity fluid

Samples Collected for: Whale Museum **Stored in:** **and Preserved by: Frozen** **Date Sent:**

Blood	☐	Kidney	☐	Brain	☐	Adrenal Glands	☐	Skin	☐	Salivary Gland	☐	Mesenteric LN	☐	Pericardial Fluid	☐
Serum	☐	Pancreas	☐	Spinal Cord	☐	Urinary Bladder	☐	Eye	☐	Sublingual LN	☐	Pulmonary LN	☐	Urine	☐
Blubber	☐	Spleen	☐	Thymus	☐	Uterus	☐	Vitreous Humor	☐	Submandibular LN	☐	Inguinal LN	☐	Feces	☐
Heart	☐	Stomach	☐	Thyroid	☐	Ovary	☐	Muscle	☐	Suprascapular LN	☐	Pituitary Gland	☐	Parasites	☐
Lung	☐	Intestines	☐	Tonsils	☐	Testis	☐	Umbilicus	☐	Subscapular LN	☐			Stomach Contents	☐
Liver	☐	Colon	☐	Trachea	☐	Gall Bladder	☐	Lower Jaw	☐	Mediastinal LN	☐			Other, Desc in Comments	☑

Sample Comments

Entire head (for CT scan and preservation), all other skeletal material.

Report Printed: 1/2/2013 9:47:03 PM ***Primary Stranding Number*** PSU 12-02-11 Oo

Samples Collected for: WHOI **Stored in: Blood Tube** **and Preserved by: Other** **Date Sent:** 20-Feb-12

Blood ☐	Kidney ☐	Brain ☐	Adrenal Glands ☐	Skin ☐	Salivary Gland ☐	Mesenteric LN ☐	Pericardial Fluid ☐
Serum ☐	Pancreas ☐	Spinal Cord ☐	Urinary Bladder ☐	Eye ☐	Sublingual LN ☐	Pulmonary LN ☐	Urine ☐
Blubber ☐	Spleen ☐	Thymus ☐	Uterus ☐	Vitreous Humor ☐	Submandibular LN ☐	Inguinal LN ☐	Feces ☐
Heart ☐	Stomach ☐	Thyroid ☐	Ovary ☐	Muscle ☐	Suprascapular LN ☐	Pituitary Gland ☐	Parasites ☐
Lung ☐	Intestines ☐	Tonsils ☐	Testis ☐	Umbilicus ☐	Subscapular LN ☐		Stomach Contents ☐
Liver ☐	Colon ☐	Trachea ☐	Gall Bladder ☐	Lower Jaw ☐	Mediastinal LN ☐		Other, Desc in Comments ☑

Sample Comments

Gas samples from mesentery and heart

Appendix A-4:
Gross Report & Findings, 19 March 2012

MARINE MAMMAL MORTALITY INVESTIGATION GROSS REPORT

STRANDING CASE NUMBER: **PSU 12-02-11 Oo (identified as SRKW L112)**
DATE FOUND: **February 11, 2012**
DATE OF REPORT: **March 19, 2012**

LOCATION FROM WHICH CARCASS WAS RECOVERED **N. of Cranberry Beach Approach (WA Coast)**

(46.40939/-124.06134)

SPECIES (NO.) Killer whale (1) **SEX** Female **AGE** 3 years **WEIGHT** N/A

STRANDING HISTORY and GROSS FINDINGS (D. Duffield, D. Lambourn, J. Huggins and others)

This female juvenile killer whale, later identified as southern resident killer whale L112, was found on morning of February 11, 2012 dead in the beach lying on its right sided, it remained on its right side as it was winched on a flat bed truck and moved to a secure location. It was necropsied by Debbie Duffield (PSU), Dyanna Lambourn (WDFW), Jessie Huggins (CRC) and a supporting team the following day. Gross examination revealed a moderately distended carcass that measured 375 cm straight from the tip of the nose to the deepest notch in the tail fluke. Scavenging was minimal on left side of body (superficial mostly from birds). It was in fresh-moderate postmortem condition and the skin just barely starting to slough. Estimated time dead was 2-4 days. It was in good body condition based on 3.4 cm blubber thickness and fat noted on heart. Bruising was seen around the head and chest (visible through the skin). Left side head bruising extended ~2 inches above eye to tooth # 6 on lower jaw and distending back ~8 inches toward the shoulder. A couple other smaller areas of hemorrhage were observed mid patch and in front of the pectoral fin insertion (Image 1). Right side head bruising was noted ~2 inches above eye tooth #4 on right lower jaw and extends back past the insertion of right pectoral fin and across ventral lower jaw almost all the way to inside of the left lower mandible (Image 2 and 3). The eyes were intact but appeared to be slightly bulging. Two small linear scars were present on the dorsal right, one just behind dorsal fin, the other at same level as anus; both appear healed. Swelling was present at genital slit and the anterior blowhole was raised and swollen. The tongue was markedly swollen, darkened and edematous, and a portion of the right side appeared deflated.

The entire head was excised and retained intact for CT scanning at a later date. When cut, copious dark red serous fluid (~2 liters) and chunks of brain poured from the foramen magnum (Image 4). Spinal cord and epidural rete taken at and between C1 and base of skull; tissues were dark red with red serous fluid surrounding (Image 5). No broken bones were seen on gross necropsy and the bones will be cleaned and examined in detail for fractures. The blubber was dark red on the head, chest and around both scapulae, and down right lateral side to just forward of the dorsal fin. Blubber appeared normal along the back and left ventral side where measurements are normally taken (Image 6). Chest cavity (mostly right side) had ~3 liters of clear red serous fluid. Blood was absent in most examined arteries and veins. Marked hemorrhage and edema was seen in muscle and subcutaneous tissue in same area noted with abnormal blubber. Crepitus was palpated between blubber and muscle on the left flank. Air bubbles were present in various tissues that could have been due to decomposition or other factors. Crepitus and air bubbles also were palpable and visible along the dorsal left abdominal

cavity. All organs were intact with exception of the kidneys (which were severely autolyzed) and the pancreas. With respect to the heart: air bubbles were detected coronary veins/vessels, and red serous edema was obvious on the external layer. The tissue on the inside of the heart was green and no significant amount of blood was noted. (Image 7 and 8) Lungs were both dark and congested. Pleural lining on both lungs had red serous edema, markedly more pronounced in upper lobes and more on right than left (Image 9). Stomachs were mostly empty with 4 fish lenses, and ~30 non-embedded nematodes were found within the balled up sloughed forestomach lining. The forestomach contained 17cc of brown mucous fluid; other stomachs contained ~35cc pinkish salmon colored fluid. Intestines were air-filled with air bubbles tracking throughout mesenteries. They were mostly empty with a small amount of feces in colon (Image 10).

CT FINDINGS (Tori McKlveen DVM, MS, Diplomate, American College of Veterinary Radiology)
Date: 2/23/2012 Head only, frozen.
This patient is positioned with the left side down and the right side up.
On the scan window overlay, A= Left and P=Right.

There is extensive gas accumulation in the soft tissues and fat.

There is loss of brain matter. The right side of the calvarium is almost completely devoid of brain tissue- the majority of the right side of the brain is missing. Some brain tissue is noted on the left side.

On the sequences with the head positioned as straight as possible, no asymmetry to the large included bones of the skull is noted. No large displaced fractures are seen. The brain case/calvarium and large bones of the skull such as the parietal bone do not appear to be crushed or broken. There are a few small, smoothly marginated bones at the level of the osseous bulla (separate from the bulla), mostly dorsal to the bulla. Some of these are quite small. These are probably part of normal anatomy as they are bilateral. However, there does appear to be displacement of some of these very small bones on the right side. See image 11. This is interpreted with caution because in this area, especially on the right side, there is loss of normal soft tissue structures (brain, fat etc.) that would normally hold these bones in place. On the right side (see image 11) two or three of the small bones are displaced into the calvarium and the similar bone (s) on the left are outside the calvarium, closer to the bulla. Also, there is heterogenous/irregular tissue surrounding and dorsal to both bulla and the other small bony structures dorsal to the bulla are not aligned. I compared these images to the 3 day old Orca scan from last fall and there is what appears to be a normal void of bone tissue dorsal to the bulla so the bilateral, symmetrical space in the bone there seemed consistent with the other whale anatomy I've seen.

There is mixed accumulation of soft tissue, fluid and gas in the sinuses rostral to the brain, with the left side having more soft-tissue or fluid-attenuating material than the right.

There is soft-tissue or fluid-attenuating material in the majority of the left osseous bulla. There is soft-tissue or fluid-attenuating material and air in the rostral aspect of the right osseous bulla with air in the mid to caudal right osseous bulla. The right bulla has more air in it than the left. The left is almost completely filled with fluid or tissue. Considerations for this fluids/soft tissue attenuating material are blood, infectious or inflammatory debris, polyp like material-chronic inflammation or parasites (See image 12).

CT summary:
-Extensive gas accumulation in the soft tissues and fat could be secondary to trauma, post-mortem change, and disarticulation (or all the above).
-No large skull fractures seen.

-Displacement of some of the small bones dorsal to the bulla on the right into the calvarium. Additional small bones dorsal to the bulla on both sides that not in alignment with each other. Possibilities include secondary to trauma or post-mortem as surrounding supportive tissues are gone.
-Fluid or soft tissue in sinuses.
-Fluid or soft tissue in both osseous bulla (middle ears) worse on the left. Possibilities include: blood, infectious or inflammatory debris, polyp like material and chronic inflammation or parasites (including worms).

HEAD DISSECTION (March 6 & 7, 2012; D. Lambourn & J. Gaydos)

The entire head, severed between C1 and the foramen magnum was preserved frozen for CT scan and thawed in air for 48 hours prior to dissection on March 6. The head was partially frozen at time of dissection. The tongue is dark gray to black, swollen and edematous. There are 12 teeth erupted from the right and left mandible as well as from the right and left maxillae. On the left mandible, the 10^{th} tooth caudal is angled more medially than the other teeth; it appears to have erupted in this direction as the tooth is firmly held by the periosteal ligament and there is no associated bruising or signs of trauma. Dissection reveals bruising in the subcutaneous tissue over the left and right eyes, with that over the left eye being more significant. On cut surface the melon has an almost clear appearance at its ventro-medial aspect, but the tissue surrounding that (dorsally and laterally) is diffusely pink to red, especially from the area just in front of the blowhole and lateral diverticulae or multiple sacs associated with the blowhole extending cranially to about 27cm towards the beak (Image 13). The pink to red color is darker on the right side than it is on the left. The rostral muscles adjacent to the melon on the right side just above the maxillae is dark red with apparent hemorrhage as is the connective tissue on the right side at the junction of the blowhole's rostral vestibular sac and the melon. Approximately 5-10cc of serosanguinous fluid is present frozen in the left side of the paired nares within the blowhole. The right side is clear.

Ventrally, a triangular section of tissue just medial to the right mandible and below the tongue measuring approximately 7cm at its base with a 16cm height is dark brown and green and aerated (Image 14). A smaller area on the medial to the left mandible (~3cm long) is noted as well. Similar colored tracks extend caudally in towards the ramus of the mandible and pharyngeal area. The mandibular or pan-bone fat of the left mandible is dark red (Image 15). The fat in the right mandible appears more autolyzed and darker. Removal of the mandibles and the hyoid reveals an air-filled sponge-like brown material just rostral to the tympanic bulla on the right side. Less of this material is present on the left side.

Frozen serosanguinous fluid suspected to be blood is evident in the cranial esophagus / pharyngeal region as well as at the junction of the larynx / narial passage. The left-side pharyngeal muscles are red and appear hemorrhagic.

Dissection of the tympanic bulla reveals that the right bulla is less adherent to the skull or at least significantly looser leaving easier visualization of what we presume is the fibro-venous plexus than is the left (Images 16 and 17). Post-dissection of the tympanic bulla, 1 small (1-2cm) nematode and approximately 12 slightly longer (2-4cm) worms that are more flat, are present in the area of the skull that was adjacent to the tympanic bulla, including peribullar sinus, fibro-venous plexus and surrounding peri-bulla soft tissue of both bulla. Concomitant with the parasites is a brown, sponge like material that appears to extend into the bulla. Approximately 6cc of red serous fluid is present in both peribullar sinuses. Two small bony fragments dorsal to the right and left bulla are present. On the left side they measure approximately 2.5cm x 2cm and 1.5cm x 1.5 cm and they appear to not be displaced but are easily removed. On the right side they measure 4cm x 2cm and 1.5 cm x 1.5 cm and are

displaced into the calvarium The edges of all four pieces are irregular and well rounded and don't appear to be freshly fractured (Image 18).

Removal of a large triangular section of the occipital bone revealed slightly frozen brain material on the left side cerebrum encapsulated by meninges and a brain free meninges that was adherent to the calvarium in 3-4 places. Cerebellum was mostly gone and portion were leaking into the left bulla area. Roughly 20cc of dark red to brown frozen serosanguinous fluid is visible between the dura and the calvarium (Image 19). This fluid was consistent with the fluid that was noted during the initial necropsy. The sutures on the right side calvarium appear to be looser then on left and red serous fluid is leaking around suture area.

Gross dissection of the head was conducted and this report was written and approved of by:

Dyanna Lambourn, Washington Department of Fish and Wildlife
Joseph K. Gaydos, SeaDoc Society / UC Davis Wildlife Health Center / San Juan County MM Stranding Network
Debbie Duffield, Portland State University
Jessie Huggins, Cascadia Research
Tori McKlveen, VCA Veterinary Specialty Center of Seattle

Executive Case Summary

This Animal was in good body condition and fresh-moderate post-mortem condition. Significant soft tissue trauma was present in the head, chest and down the right lateral side of the body as evidenced by marked hemorrhage and edema present in the skin, blubber, subcutaneous tissues and muscles (described above), as well as in lung and heart. Marked red serous fluid was present in the calvarium (~ 2 L) and the brain poured out of foraman magnum in chunks. Red serous fluid (~ 3 L) also was present in the thoracic cavity, mostly on the right side. Blood was absent in arteries and veins examined and air bubbles were closely associated with vessels. No broken bones were noted on the initial necropsy, CT scan or head dissection or during further flensing of the carcass except for the noted two small bony fragments displaced dorsal to the right and left bulla (seen on CT and head dissection).

Images

Image1. Left side head to pectoral fin

Image 2. Right side head to dorsal fin

Image3. Ventral head and chest from inside skull

Image 4. Brain and dark red serous fluild

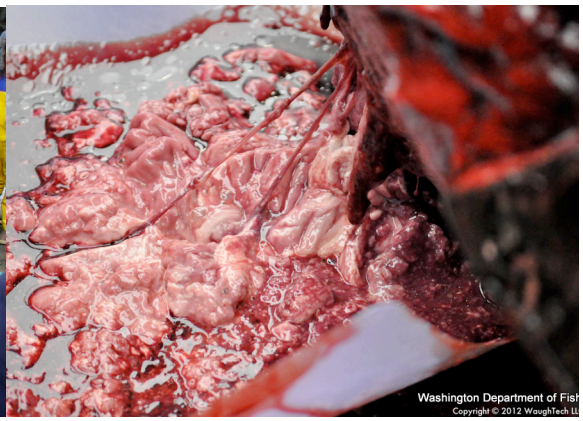

Image 5. Cervical vertebrae 1 and epidural rete muscle and blubber

Image 6. Left side behind head-

Image 7. Heart external

Image 8. Heart left ventricle

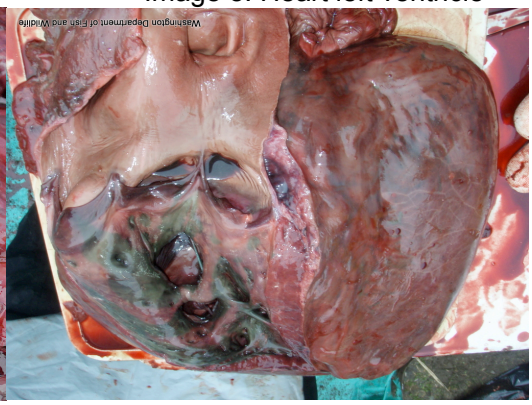

Image 9. Right lung

Image10. Intestines, mesentery

Image 11. Small bones displaced into calvarium on the right side. The side with the missing brain matter.

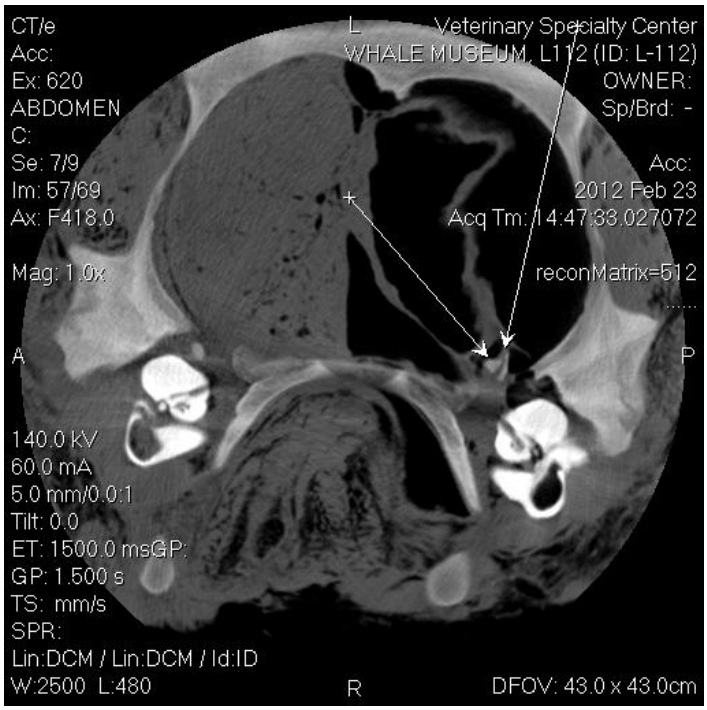

Image 12. Caudal aspect of the bulla. Fluid or soft tissue in left osseous bulla. Air in the right.

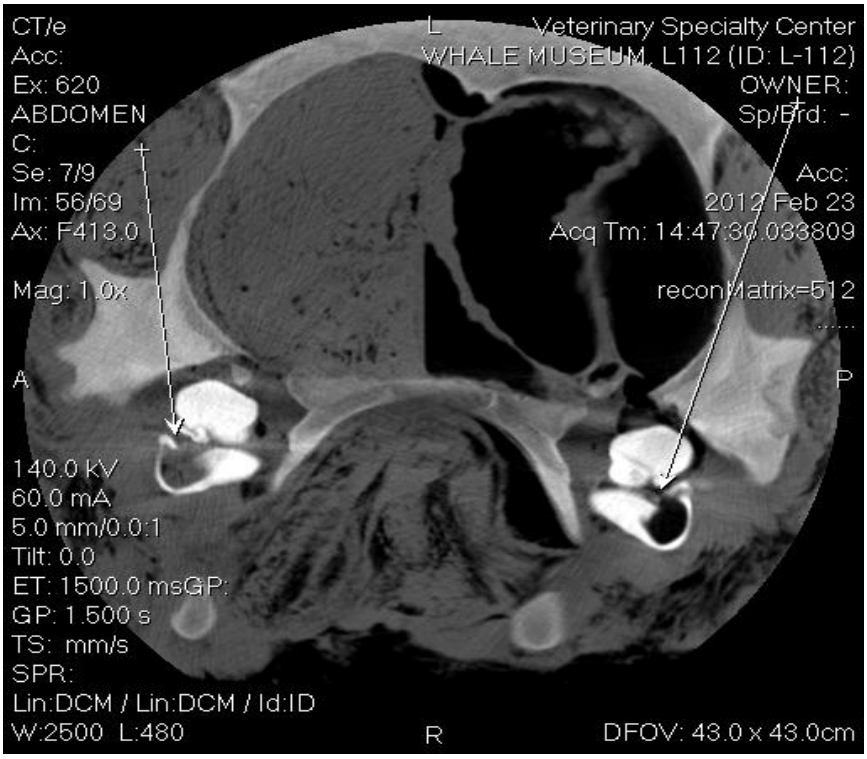

Image 13: Pink to red staining of melon tongue

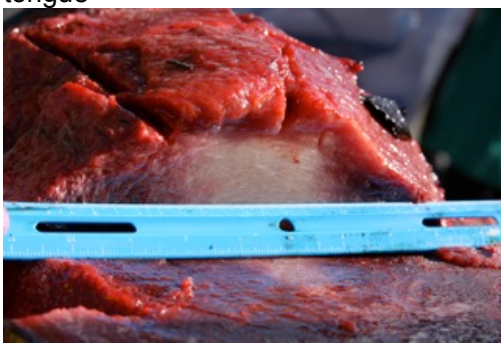

Image 14: Lesions ventral to the

Image 15: Mandibular fat

Image 16: Left bulla in-situ

Image 17: Right bulla in-situ

Image 18: Bulla and associated bone fragments dura and calvarium

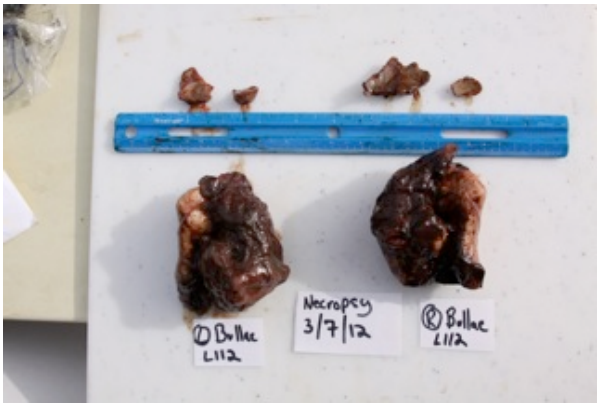

Image 19: Hemorrhage between

Appendix B-1:
First Progress Report, 2 April 2012

Killer Whale Stranding Progress Report

April 2, 2012

The Northwest Region Marine Mammal Stranding Network, administered by NOAA Fisheries, Protected Resources Division in Seattle, WA is investigating the death of a juvenile killer whale that stranded on the Long Beach peninsula on February 11, 2012. The whale, was tentatively identified as L-112 based on a comparison of its external markings with a photographic catalogue of known whales. L112 typically travels with a family group of whales from the "L" pod of the Southern Resident killer whale population, a species listed as endangered under the Endangered Species Act.

Several organizations belonging to the Stranding Network are participating in the stranding investigation including Dr. Deborah Duffield, Portland State University, Jessie Huggins, Cascadia Research Collective, Dyanna Lambourn, Washington Department of Fish and Wildlife Marine Mammal Investigations, Amy Traxler, The Whale Museum, Dr. Joe Gaydos, University of California SeaDoc Society, and Dr. Stephen Raverty, Animal Health Center in British Columbia.

Initial Examinations

Dr. Duffield, Portland State University, is the primary responder for the Long Beach area and led the team that conducted the post-mortem examination of the whale in the field on February 12, 2012. The team collected morphometric data, photographs and tissues for analysis. Samples were submitted for genetic analysis to confirm the whale's identification as a Southern Resident. Observations indicate the animal was moderately decomposed but likely dead for less than a week when found. The investigative team has not yet determined a cause for the loss of this animal but examiners found extensive hemorrhage in the soft tissues of the chest, head and right side of the body. Photographs from the examination and a preliminary report of observations by the field team have been posted online at:
http://www.cascadiaresearch.org/examination_of_dead_killer_whale-12Feb2012.htm

The head was collected, frozen, and later scanned at the VCA Veterinary Specialty Center of Seattle. The computer tomography data collected by the scanner are being analyzed by veterinary radiologists, Dr. Tori Mcklveen, VCA and Dr. Sophie Dennison-Gibby, NOAA Fisheries. After scanning, Dr. Gaydos lead a team that performed a forensic dissection of the head at the Friday Harbor Laboratory on March 6-7th, 2012.

Environmental Conditions

Based on the estimated time of death, NOAA Fisheries and the NOAA Hazardous Materials Response Division reviewed environmental data from early February and found that prevailing wind and currents, between February 1 and February 11 were predominantly from the south. In addition, local current conditions are largely influenced by eddies flowing northward from the

mouth of the Columbia River. This indicates that the animal likely died in the Columbia River plume or to the south and may have drifted a substantial distance before being cast ashore on Long Beach. Other environmental factors that are being researched include; earthquakes and if they could cause trauma or disorientation and sea surface temperature. Since little is known about the winter distribution of L pod the investigative team has been researching the availability of prey resources (Chinook salmon) off the coast that may have drawn L112 and her group to the area.

Human Activities

We are seeking information from a variety of sources in an attempt to identify whether human activities may have contributed to the injuries we observed. Communication with the United States Navy, Canadian Navy, United States Coast Guard, United States Air Force, Pacific Fisheries Management Council and state fisheries managers is on-going or being initiated. NOAA Fisheries has reviewed reports received by the Marine Mammal Authorization Program from commercial fishing vessels between January and February 2012 and found that no incidental mortality or injuries involving killer whale(s) was reported anywhere on the west coast during this timeframe.

Sample Analysis

Cascadia Research Collective is managing distribution of samples, sample data, and the dissemination of results to the investigation team. Parasites, bacteriology, and food habit samples have been sent to several labs for analysis and results are pending. Histopathology samples collected during the post mortem examination and head dissection will be analyzed by the Oregon State University School of Veterinary Medicine and Dr. Raverty. The results of these analyses, which are likely to take several weeks to compile, will be used to supplement the preliminary findings from the field examinations and compiled into a report, possibly for publication. Submission of contaminant, virology, and biotoxin samples are also pending.

Information collected by the Stranding Network and NOAA Fisheries Protected Resources is being shared with the NOAA Fisheries Office for Law Enforcement which is conducting an independent enforcement investigation of the event. Media inquiries for this case can be directed to NOAA Public Affairs, Brian Gorman at 206-526-6613 or Brian.Gorman@noaa.gov who can provide updates as information becomes available.

Appendix B-2:
Second Progress Report, 15 May 2012

Southern Resident Killer Whale L112 Stranding Progress Report

May 15, 2012

The Northwest Region Marine Mammal Stranding Network, administered by NOAA Fisheries, Protected Resources Division in Seattle, Wash., continues to investigate the death of a juvenile killer whale that stranded on the Long Beach peninsula on Feb. 11, 2012 and identified as Southern Resident, L112.

Investigative Team: Dr. Deborah Duffield, Portland State University; Jessie Huggins, Cascadia Research Collective; Dyanna Lambourn, Washington Department of Fish and Wildlife Marine Mammal Investigations; Amy Traxler, The Whale Museum; Dr. Joe Gaydos, University of California SeaDoc Society; Dr. Stephen Raverty, Animal Health Center in British Columbia; Tori McKlveen, VCA Veterinary Specialty Center of Seattle; and Brad Hanson, NOAA Northwest Fisheries Science Center. The Investigative Team met at NOAA on May 10, 2012, to review environmental and diagnostic findings to date and to discuss the case.

Gross Examination: Gross examination disclosed extensive bruising and swelling on both sides of the head and neck, more pronounced on the right, and continuing down the right side of the body. Although no skull fractures were seen during examination of the head, there was fragmentation of the brain and increased fluid in the right side of the skull. The significance of this finding is under investigation.

Sample Analysis:

> **Microscopic Examination:** Due to advanced tissue degradation, the presence of hemorrhage (blood outside of vessels) couldn't be confirmed by microscopic evaluation. Further tests are pending that might assist with our ability to confirm hemorrhage microscopically and gain further insights as to the time of injury and subsequent death of the animal, as well as the detection of possible fat embolization (dislodged blubber fat cells can be transported by blood to internal organs). The latter has been associated with traumatic injuries in cetaceans and would indicate antemortem trauma.
>
> **Bacteria and Viruses:** A complete screen for infectious agents did not detect any significant disease-causing organisms. The advanced decomposition may have hindered detection or recovery of some agents; however, there was no indication of significant inflammation or infection within the examined tissues.
>
> **Additional pending studies**: Review of environmental conditions and possible presence of algal blooms at the time and in the vicinity of the stranding have been requested. Sources of acoustic data have been identified and the analysis of this

data will not be available until late summer 2012. Laboratory analysis to determine the presence of algal toxins, contaminant loads, and heavy metal burdens in tissues are underway. A closer examination of the skeleton for evidence of blunt force trauma will be conducted after cleaning is complete.

CT Scan: The head was collected, frozen, and scanned at the VCA Veterinary Speciality Center of Seattle. The computed tomography (CT) data has been collected and reviewed by veterinary radiologists Dr. Tori Mcklveen, VCA, and Dr. Sophie Dennison-Gibby, NOAA Fisheries. The scan of the head was completed and did not show any fractures of the skull. A recent secondary CT scan of ear bones confirmed findings consistent with the presence of parasites observed during the gross examination. Parasites are common in cetaceans and their presence in this case are considered incidental and unlikely related to the cause of stranding.

Environmental Conditions: Ocean current conditions at the time this animal died were largely influenced by eddies flowing northward from the mouth of the Columbia River. This indicates that the animal likely died near the Columbia River or to the south and drifted before being cast ashore on Long Beach.

Requests for Information on Human Activities: NOAA Fisheries has contacted a variety of government agencies and other sources in an attempt to identify whether human activities may have contributed to the injuries that were observed. The United States Navy responded to our request for information, and has no records indicating that Navy units used sonar or explosives between Feb. 1 and Feb. 11 within the Northwest Training Range Complex, which includes the coastal area between Newport, Ore., and Cape Flattery, Wash. The Royal Canadian Navy confirmed the use of sonar and two small under water charges by HMCS Ottawa on Feb. 6, 2012, as part of an anti-submarine warfare exercise near Constance Bank and in the Straight of Juan de Fuca. HMCS Ottawa activities included following a Marine Mammal Mitigation Policy prior to and during the period when they were using ships' sonar and prior to deploying the charges. Whales were not observed during that time. The Department of the Army confirmed with all military organizations resident on Joint Base Lewis-McChord (JBLM) that no military training involving JBLM units took place during the timeframe of the stranding. The Fishing Vessel Owners' Association responded that vessels are not typically on the water and fishing in February, and reported no interactions between whales and fishing vessels. Responses are pending from the United States Coast Guard and the United States Army Corp of Engineers.

Preliminary Conclusion: The grossly noted hemorrhage around the head and neck is consistent with physical trauma, which would have been sufficiently severe to account for the loss of this animal. The cause of this injury remains undetermined and investigations are ongoing.

Media Requests: Information collected by the Stranding Network and NOAA Fisheries Protected Resources Division is being shared with the NOAA Fisheries Office for Law Enforcement, which is conducting an independent enforcement investigation of the event. Media inquiries for this case can be directed to NOAA Public Affairs, Brian Gorman, at 206-526-6613, who can provide updates as information becomes available.

Appendix C-1:
Histopathology Report, 17 December 2012

CASE NUMBER: 12/01426, PSU 12-02-11Oo
OWNER: PSU-DFO
VETERINARIAN: Dr. Stephen Raverty
DATE: Dec 17, 2012

MORPHOLOGIC DIAGNOSES:
1). Heart: Fibrosis, perivascular and interstitial, moderate, multifocal with occasional entrapment and replacement of myocardial fibers
2). Kidneys: Fibrosis, interstitial and perivascular, mild to moderate, multifocal with effacement and occasional peripheral entrapment of tubules
3). Liver: Biliary ductular hyperplasia, moderate, multifocal and occasionally bridging with periductular fibrosis and scattered cholestasis
4). Liver: Fibrosis, capsular, moderate, multifocal with projections into the parenchyma
5). Lymph nodes, multiple: Microcavitations, moderate, multifocal to coalescing with scattered mineralized precipitate
6). Lymph node, 1 of multiple: Lymphadenitis, moderate, multifocal to coalescing, suppurative with numerous microcavitations
7). Brain: Fragmentation, moderate, multifocal with occasional nodular aggregates of acicular clefts interspersed within a proteinaceous background
8). Stomach, junction of glandular and nonglandular compartments: Hyperplasia, squamous epithelia, moderate, multifocal with ortho and parakeratotic hyperkeratosis and transverse clefts and rare superficial, luminal nematode parasites
9). Stomach, glandular compartment, submucosa: Gastritis, mild to moderate, multifocal, nonsuppurative
10). Skeletal muscle, multiple sites: Proteinaceous fluid, endo and epimysial, moderate, variably extensive with numerous microcavitations and occasional myocellular hyalinization, fragmentation, vacuolation, central migration of nuclei and scattered endomysial lymphohistiocytic infiltrates and fibroplasia
11). Lung: Proteinaceous fluid, bronchoalveolar, moderate, multifocal (autolysis)
12). Spleen: Mineralized precipitate, moderate, multifocal, random with occasional serpiginous margins and tan brown marginal deposits
13). Skeletal muscle: Sarcocystosis, mild, random, multifocal
14). Dermis: Proteinaceous fluid, moderate, multifocal, perivascular and interstitial
15). Fascia, presumptive: Nematodiasis, encapsulated, moderate, multifocal to coalescing

There are no overt lesions within the brain, spinal cord, peripheral nerves, peripheral vasculature, heart, brain, spinal cord, vascular rete, pancreas, oropharynx, tongue, eye, optic nerve or adipose tissue.

COMMENTS:
Post mortem change hampered microscopic review of the sectioned tissues and precluded evaluation of multiple levels of bowel; a precise cause of death could not be determined by histopathology. Throughout multiple sections of lymph nodes, skeletal muscle, and other tissues, there are numerous microcavitations (emphysema) with occasional acellular to hypocellular proteinaceous fluid. Based on the lack of attendant hemorrhage, associated clostridial overgrowth, degree of autolysis and aspirated gas mass spectrometry findings from WHOI, the

emphysema is most likely associated with putrefaction, rather gas bubble disease. Based on the distribution and nature of the cavities, another differential may include fat embolization and results from special stains of the lung, brain and skeletal muscle are pending and to follow. The myocardial, hepatic and renal fibrosis are chronic and low to intermediate grade. Without antemortem clinical chemistries it is difficult to assess the impact of these changes to normal homeostasis. Myocardial fibrosis has previously been documented in adult stranded killer whales and may be attributed to long past toxic (domoic acid), heavy metal (mercury), infectious and other disease processes. PCR of the heart proved positive for Apicomplexa (NIH, Dr M Grigg); however, close evaluation of the myocardium did not reveal any discernible protozoa. In this case, the lack of grossly noted hydrothorax and ascites tends to discount cardiopulmonary compromise. The biliary ductular hyperplasia and periductular fibrosis is suggestive of an ascending infection from the gastrointestinal tract. Hepatobiliary trematodiasis and possible toxic insults may also be considerations. The fibrous connective tissue bands emanating from the liver capsule and projective to varying levels of the underlying parenchyma is unusual; this may represent a normal anatomic variation for the species, possible parasite migration tracts or some other entity. The overall impact on hepatobiliary function would be minimal. The reniculi appear small relative to the overall size of this animal; in regions, there are bands of fibrous connective tissue extending from the corticomedullary junction to capsule; randomly throughout the parenchyma, there are cords and trabeculae of moderately cellular fibrous connective tissue which efface and occasionally entrap individual tubules and glomeruli. Due to the chronicity of the lesions and lack of discernible pathogens, a specific etiology or pathogenesis could not be resolved. In 1 of multiple sections of brain, there is extensive fragmentation of the neuropil with scattered poorly delineated nodular aggregates of acicular clefts interspersed within varying amounts of proteinaceous material throughout the brain fragments; it is possible that this section may represent the grossly noted liquifactive change and fragmentation of the brain. The lack of associated hemorrhage, neuronolysis, edema fluid, fibrin deposition or inflammatory infiltrate suggests that this change may be due to freeze artefact and autolysis, rather than a distinct pathologic (or traumatic) process. The light growth of Edwardsiella tarda from the lung with moderate yield of alpha Streptococcus spp from the colon likely represent post mortem invasion and overgrowth as well as normal flora, respectively. Enrichment and selective culture for Salmonella and Yersinia did not feature any isolates and the lack of microbial growth from the brain, fluid, lung nodes, and spleen is likely related to putrefaction. Polymerase chain reaction (PCR) of pooled tissues proved negative for herpesvirus, Apicomplexa, Brucella, canine distemper virus, West Nile virus and influenza virus and trace mineral and vitamin A analysis of the liver proved largely within acceptable reference limits; the reduced calcium may be associated with the multisystemic fat saponification and mineral deposition. Due to the grossly noted hemorrhage and emphysema within the neck and head regions, immunoflorescence for Clostridial toxins was pursued and proved negative for C chauvoei, C noyvi, and C sordelli. The encapsulated nematodes are suggestive of Crassicauda spp and more precise speciation may entail consultation with a parasitologist. The gastric hyperkeratosis may suggest inappetance or anorexia and the luminal parasite is considered incidental. The chronic gastritis may suggest a source for the ascending cholangiohepatitis. Although the fluid accumulation within the lung was most likely due to autolysis, aspirated sea water, hemorrhage and erythrolysis and pulmonary edema may also be considerations. Variable amounts of debris were noted within the proteinaceous material and there were discernible heart failure cells. The suppurative lymphadenitis is consistent with a bacterial infection; involvement of 1 of multiple examined

lymph nodes suggests a localized process, either due to direct seeding of bacteria or draining infection. Imaging studies disclosed lesions consistent with a congenital anomaly in the cervical vertebrae; based on the condition of this animal, ability to swim and lack of asymmetric atrophy, it is likely that these changes were incidental to the immediate cause of death.

FINAL REPORT

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Erin Zabek on 04/22/12 @ 9:06 AM

Specimen	ID	Isolate	Result	Level
Brain			No Bacteria Isolated	
Fluid	CSF		No Bacteria Isolated	
Tissue	Meninges		No Bacteria Isolated	
Lung		Edwardsiella tarda	Positive	1+
Lymph Node			No Bacteria Isolated	
Tissue	mammary gland		No Bacteria Isolated	
Swab	Blowhole		No Bacteria Isolated	
Spleen			No Bacteria Isolated	
Colon		Streptococcus sp. (alpha)	Positive	2+

Anaerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 04/26/12 @ 11:42 AM

Specimen	ID	Isolate	Result	Level
Skin		Clostridium septicum	Positive	4+

Culture - Campylobacter Resulted by: Sean Byrne Verified by: Sean Byrne on 05/07/12 @ 4:02 PM

Specimen	ID	Isolate	Result	Level
Colon			Negative	

Culture - Yersinia Resulted by: Erin Zabek Verified by: Sean Byrne on 05/07/12 @ 3:38 PM

Specimen	ID	Isolate	Result	Level
Colon			No Yersinia sp. Isolated	

Culture - Salmonella Resulted by: Erin Zabek Verified by: Sean Byrne on 05/07/12 @ 3:38 PM

Specimen	ID	Isolate	Result	Level
Colon			No Salmonella sp. Isolated	

FA - C. chauvoei Resulted by: Erin Zabek Verified by: Sean Byrne on 04/19/12 @ 3:17 PM

Specimen	ID	Test	Result
Skin		FA - C. chauvoei	Negative

FA - C. novyi Resulted by: Erin Zabek Verified by: Sean Byrne on 04/19/12 @ 3:17 PM

Specimen	ID	Test	Result
Skin		FA - C. novyi	Negative

FA - C. septicum Resulted by: Erin Zabek Verified by: Sean Byrne on 04/19/12 @ 3:17 PM

Specimen	ID	Test	Result
Skin		FA - C. septicum	Negative

FA - Clostridium sordelli Resulted by: Erin Zabek Verified by: Sean Byrne on 04/19/12 @ 3:18 PM

Specimen	ID	Test	Result
Skin		FA - Clostridium sordellii	Negative

Molecular Diagnostics

Apicomplexa Resulted by: Ken Sojonky Verified by: Sean Byrne on 04/19/12 @ 4:07 PM

Specimen	ID	Test	Result
Tissue	brain & skin	Apicomplexa	Negative
**: Test validation in progress.			

Brucella spp. Resulted by: Julie Bidulka Verified by: Sean Byrne on 04/23/12 @ 3:46 PM

Specimen	ID	Test	Result
Tissue	sp.cord,lv,ln,sp,thy,mam.gland	Brucella spp.	Negative
**: Test validation in progress.			

Canine Distemper virus Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/24/12 @ 3:51 PM

Specimen	ID	Test	Result
Tissue	sp.cord,lv,ln,sp,thy,mam.gland	Canine Distemper virus	Negative

Influenza Virus-Consensus Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/23/12 @ 3:44 PM

Specimen	ID	Test	Result
Tissue	sp.cord,lv,ln,sp,thy,mam.gland	Influenza Virus-Consensus	Negative

West Nile virus Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/24/12 @ 10:26 AM

Specimen	ID	Test	Result
Tissue	sp.cord,lv,ln,sp,thy,mam.gland	West Nile virus	Negative

Herpesvirus-Consensus Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/23/12 @ 4:38 PM

Specimen	ID	Test	Result
Tissue	sp.cord,lv,ln,sp,thy,mam.gland	Herpesvirus-Consensus	Negative
**: Test validation in progress.			

Toxicology

Phosphorus-Inorganic(Alcyon) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		P	2.7	mg/dl			

Calcium-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Ca-t	2	ppm	50	200	<rang

Copper-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Cu-t	9	ppm	3.0	50.0	in range

Iron-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Fe-t	175	ppm	100	400	in range

Mercury-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Hg-t	12.4	ppm	0.1	30.0	in range

Manganese-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Mn-t	2.0	ppm	2.0	6.0	in range

Molybdenum - MO Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		mo	.28	ppm			

Selenium-Tissue(Flour.) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Se-t	5.18	ppm	0.30	20.0	in range

Cobalt - Tissue Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Co-T	.009	ppm			

Magnesium-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Mg-t	213	ppm	100	250	in range

Zinc-Tissue(AA) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		Zn-t	98	ppm	20	120	in range

Vitamin Package (Liver) Resulted by:Shawnee Landsiedel Verified by:Stephen Raverty on 06/08/12 @ 2:40 PM

Specimen	ID	Test	Level	Units	Range Low	Range High	Result
Liver		VitA-l	1844.4	ug/g			
Liver		VitE-t	829.2	ug/dl			

Staff Comments:
Toxicology testing performed by Prairie Diagnostic Services.

Appendix C-2:
Final Histopathology Report (OSU-VDL), 6 June 2012

Oregon State University Veterinary Diagnostic Laboratory

PO Box 429
Corvallis, OR 97339-0429
Phone(541) 737-3261
FAX (541) 737-6817

Addendum
Version 2
This report supersedes all previous reports for this case

VDL Accession #: 12V10771
Referral #:
VTHCase #:
Date Collected:
Date Received: 04/04/2012
Case Coordinator: Rob Bildfell, DVM, Diplomate ACVP
Electronically Signed and Authorized By: Chantelle Onderko on behalf of Rob Bildfell, DVM, Diplomate ACVP on 6/6/2012 3:29:12PM

C101638

Email To:
Prescott Grant NA229A
jim.rice@oregonstate.edu

Collection Site:
Hatfield Marine Science Center
NEWPORT, OR 97365
Phone: 5418670446

Specimens Received: 24 blocks; 1 Tissue - Fixed;

Submitter	Prescott Grant	NA229A	5418670446	Hatfield Marine Science Center, 2030 SE Marine Science Drive, Newport, OR 97365-5296

ID	ID Type	Other IDs	Taxonomy	Gender	Age/DOB
PSU 12-02-11 Oo	Facility ID		Whale	Female	Adolescent

Parasitic cellulitis and sinusitis, right bullae tissues.

These comments pasted from 12-10511, an earlier tissue submission from same animal so overall case picture is retained. That case # will be finalized once more gastric sections are cut.

As you anticipated, these tissues are frequently too decomposed for useful analysis. It sounds like a traumatic event is suspected but I cannot positively confirm that at the histologic level. At this point in tissue decomposition process the erythrocytes have generally lysed, so even identifying hemorrhage is difficult. Fluid leaks freely from decomposing blood vessels and this effect is magnified on the down-side of the animal (hypostatic congestion). In order to be certain that the fluid was accumulating as a "bruise" antemortem I need to see some leukocytes (especially macrophages) coming into the tissue. This is not seen here; one interpretation is that the trauma resulted in rapid death - no time for a cellular response. The other is that the fluid is merely post mortem change. The extensive emphysema in these tissues can also be better interpreted as a post mortem change vs antemortem bacterial infection due to the lack of a cellular response.

In terms of potentially useful findings, subtle changes in various key tissues such as liver and kidney would be masked by autolysis, so we can't completely discount underlying illness. There is clearly a **suppurative lymphadenitis** in a solitary node - overall significance is unclear; could be a hint of bacterial sepsis but could also merely be a localized and controlled problem of no life-threatening significance.
The nematode infection in area of bullae (suspect *Stenurus sp.)* is not surprising and is so common in cetaceans that it is difficult to ascribe any clincial significance, but there is inflammation here as well (these findings under submission 12-10771).
Finally I find the inclusions in gastric epithelium to be pretty convincing, even though the tissue orientation is suboptimal. There is a gross description of an roughened area of forestomach so perhaps pathology was associated with this infection. It looks like a papillomavirus to me and these have been reported as a cause of cutaneous lesions in killer whales, and of gastric lesions in belugas. I will try check wet tissue and see if I can obtain

a better section before finalizing case. If this viral infection resulted in a site of bleeding then it could be a potential cause for anemia/weakness, as well as a portal of entry for bacteria.

Histopathology

Slides 1 - 6 = Jar 1
1: Skeletal muscle - myocytes lack nuclei but generally retain striations. There is extensive emphsyema. Pockets of loose connective tissue appear hypercellular and bacterial numbers are high in these areas - interpreted as decomposing vascular elements.
Skeletal muscle with dermal interface - interfacing dermal pegs on outer aspect include intravascular eosinophilic densities interpreted as intravenous fibrin thrombi.
2: Dense regular connective tissue - more emphysema.
3: Dense regular connective tissue plus skeletal muscle - more emphysema.
4: Adipose tissue with skeletal muscle - some protein rich fluid accumulation in fascia but no cellular response. Mild emphysema present.
5: Skeletal muscle and associated connective tissue - no new findings
6: Skin - includes some lobular gland formations in deep dermis but cells too autolytic to identify type.
Slides 7 -10 = Jar 2
7: Adipose tissue - No Significant lLsions
8: Adispose tissue - NSL
9: Skin - NSL
10: Skin - NSL
11: Brain - severe autolysis
12 - Choriod plexus - odd crystalline, hypereosinophilic character to portions of adipose tissue - post mortem autolysis change or consequence of hemorrhage in the area?
13, 14 - Left bullae - autolytic tissue with abundant bacteria, some fluid in interstitial planes, emphysema but no cellular infiltrates. A few nerve profiles are seen and these appear normal.
15-17 - Right bullae - Tissue is edematous and includes dilated vascular/sinusoidal spaces that are occupied by several collapsed profiles of nematodes and very large numbers of thick-shelled nematode eggs containing partially developed larvae. Although epithelium has been sloughed from cavity surfaces the stroma contains moderate numbers of round cell "ghosts" interpreted as infiltrating leukocytes. Some of the parasites in lumina are also bathed in exudate. The odd hypereosinophilic foci of saponified fat are again seen in this section.
Slide 17 contains good quality section of a nerve - no changes seen in nerve fibers
18,19 Left -eye - fluid and emphysema in fascial planes of skeletal muscle. No cellular component. Periocular skin normal.
23: Left eye, globe - poor quality section but NSL. Lens fragmentation is interpreted as post-mortem artifact.
20-22: Right eye - larger amounts of interstitial fluid than seen on left side but still no convincing evidence of a cellular response. Myocytes tend to be more fragmented but usually retain striations.
Fluid filled cleft forming along dermoepidermal junction of skin (20) - post mortem change. Emphysema evident in deep dermis.
There is irregular spongiosis of epidermal cells in #22 and a few of the capillaries in dermal papillae here appear to be blocked by fibrin thrombi. No exudation of leukocytes identified.
24: Right globe - poor quality section with NSL

HISTOPATHOLOGY REPORT

Animal/Source	Specimen	Specimen Type	Date Resulted	Results
PSU 12-02-11 Oo		Tissue - Fixed	22-Apr-2012	Report Completed

Administration

6/6/12 Charges re-accessioned to case #12V13146 per request of Jim Rice and Kathy Minta. CO

Appendix D-1:
POPs Report, 21 November 2013

Northwest Fisheries Science Center
Environmental Conservation Division
2725 Montlake Boulevard East
Seattle, Washington 98112-2097

November 21, 2013

MEMORANDUM FOR: F/WRC/WCRO/PRD – Brent Norberg

FROM: F/NWC5 – Jennie Bolton

THROUGH: F/NWC5 – Gina Ylitalo

SUBJECT: Persistent organic pollutant and lipid analyses of blubber from a Southern Resident killer whale (*Orcinus orca*)

We have completed analyses for organochlorines, PBDEs (polybrominated diphenyl ethers) and lipids in a blubber sample collected from a juvenile female Southern Resident killer whale (L112) that was killed by blunt force trauma on or around February 2, 2012.

The report, "Persistent organic pollutant and lipid analyses of blubber of a deceased Southern Resident killer whale (*Orcinus orca*)" by Bolton is attached.

Please feel free to email or call Gina Ylitalo (gina.ylitalo@noaa.gov; 206-860-3325) if you have any questions about the analyses or data.

cc:
Gina Ylitalo – F/NWC5
Teri Rowles – F/PR2
Brad Hanson – F/NWC1
Walter Dickoff – F/NWC1

Persistent organic pollutant and lipid analyses of blubber of a deceased Southern Resident killer whale (*Orcinus orca*)

Jennie L. Bolton

Introduction

Persistent organic pollutants (POPs; e.g., DDTs, PCBs and chlordanes) are lipophilic compounds that have been used widely in the northern hemisphere in agricultural and industrial applications. Many of these compounds have been regulated because exposed wildlife can exhibit toxic effects, including immunosuppression and reproductive impairment (Ross *et al*., 1995; Beckmen *et al*., 2003; AMAP 2004). In addition, these contaminants are transported to the Arctic ecosystem via atmospheric processes, where they circulate and accumulate within the complex marine food web (Barrie *et al*., 1992; Iwata *et al*., 1993; AMAP 1998, Schmidt, 1998; de Wit *et al*., 2004). POPs accumulate to high levels in tissues of marine predators, such as killer whales, due to biomagnification of POPs with increasing trophic level. Because "transient" killer whales feed primarily on other marine mammals (Ford *et al*., 1998; Saulitis *et al*., 2000), they are especially likely to accumulate high levels of POPs.

One group of POPs, the polybrominated diphenyl ethers (PBDEs), has elicited concern because of their recently reported wide geographic distribution in tissues of wildlife and humans (de Wit *et al*., 2002). PBDEs are effective flame retardants, but are also highly persistent and bioaccumulative contaminants, with structures similar to the PCBs (AMAP 1998, Ikonomou *et al*. 2002a,b, AMAP 2004). Exposure to PBDEs has been linked to various effects, including immune suppression, delays in reproductive development and impaired fetal brain development (Beineke *et al*., 2005, Birnbaum and Staskal 2004). Furthermore, PBDE levels are increasing rapidly in marine mammals in the northern hemisphere (Ikonomou *et al*., 2002b, LeBeuf *et al*., 2004, Krahn *et al*., 2009).

A sample of blubber from a juvenile female Southern Resident killer whale (L112) was analyzed for a suite of POPs (e.g., PCBs, DDTs and other pesticides, and PBDEs). The results of these analyses found that the tissues of this killer whale were moderately contaminated with these toxic chemicals.

Analytical Methods

Sample collection

This 3 year-old female Southern Resident killer whale (L112) from L Pod's L4 matriline, was the second surviving calf of L86. The animal was found dead near Long Beach, Washington and the cause of death was determined to be blunt force trauma occurring on or around February 8, 2012. L112 was necropsied on February 12, 2012, and various tissue samples, including blubber, were collected, frozen and transported to NWFSC by Brad Hanson of NWFSC. They were transferred to the analytical lab for analysis on May 10, 2012.

POP analyses by GC/MS
Methods for POP analysis were described in Sloan *et al*. (2005). Briefly, blubber (a 0-2 cm depth from the skin was analyzed as this depth is most comparable to a biopsy sample) was extracted using accelerated solvent extraction (ASE) with methylene chloride. The sample extract was filtered through a column of silica gel and alumina and concentrated for further cleanup to remove interfering lipid compounds. This cleanup step used size exclusion chromatography with high-performance liquid chromatography (HPLC), which separated larger lipid molecules from the compounds of interest and allowed collection of the fraction containing the POPs. The HPLC fraction was analyzed for chlordanes, DDTs and other pesticides, PCBs and PBDEs by high resolution gas chromatography with low resolution mass spectrometry (GC/MS), with the mass spectrometer operated in selected ion monitoring (SIM) mode. Total PCBs (∑PCBs) were calculated by summing the concentrations of 46 PCB congeners present as 40 chromatographic peaks (congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209). PCB and PBDE congeners are numbered according to the scheme in Ballschmiter *et al.* (1992). The total DDTs (∑DDTs) were calculated by summing the concentrations of *o*,*p*'-DDD, *o*,*p*'-DDE, *o*,*p*'-DDT, *p*,*p*'-DDD, *p*,*p*'-DDE, and *p*,*p*'-DDT; ∑chlordanes is the sum of oxychlordane, *gamma*-chlordane, nona-III-chlordane, *alpha*-chlordane, *trans*-nonachlor, and *cis*-nonachlor; ∑HCHs (hexachlorocyclohexanes) is the sum of *alpha*-, *beta*-, and *gamma*-HCH isomers and ∑PBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183, plus 2 pentabrominated, 1 hexabrominated, and 1 heptabrominated congeners whose congener numbers are not known.

Lipid Determination by TLC/FID
Blubber of L112 was analyzed for lipid classes and concentrations by TLC/FID using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan) as described by Ylitalo *et al*., (2005). Five classes of lipids (i.e., wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) were separated based on polarity. The total lipid (total extractable organics) reported was determined gravimetrically.

Results and Discussion

The relative percentages of five lipid classes in blubber are shown in Table 1. The sample had no free fatty acids present, which indicates that the blubber sample was not subject to decomposition prior to analysis.

Concentrations of POPs are shown in Table 2. Overall, ranked concentrations were ∑DDTs > ∑PCBs >> ∑ chlordanes > ∑PBDEs > HCB > ∑HCHs. On a lipid basis, concentrations of HCB in the blubber of L112 (870 ng/g lw) were substantially higher than those in two somewhat older (15 year-old) juvenile males from L Pod (L78, Krahn *et al.* 2007, 600 ng/g lw; L87, Krahn *et al.* 2009, 350 ng/g lw). Concentrations of PCBs in the blubber of L112 (27,000 ng/g lw) were comparable to but somewhat higher than those animals (L78, Krahn *et al.* 2007, 22,000 ng/g lw; L87, Krahn *et al.* 2009, 24,000 ng/g lw). PCB concentrations were somewhat lower than the mean of ∑PCBs in biopsy

samples collected between 1993 and 1996 from adult male northern resident killer whales, as reported by Ross *et al.* (2000) (a mean of 37,400 ng/g lw, n=8).

Total DDTs measured in the 0-2 cm blubber layer of L112 (Table 2) were ~2 times higher than those measured in the same layer of an adult (30 year-old) female southern resident killer whale, L60, that stranded in Washington state in 2002 (Krahn *et al*. 2004) (43,000 ng/g lw vs. 19,400 ng/g lw, the average of five locations). The ∑DDT/∑PCB ratio in blubber from L112 (~1.6) was somewhat higher than the ratio of 1.1 determined from the mean of the ∑DDT and ∑PCB concentrations from the 0-2 cm layers from five locations on L60 reported in Krahn *et al.* (2004). Concentrations of DDTs were very similar to those in two somewhat older (15 year-old) juvenile males from L Pod (L78, Krahn *et al.* 2007, 38,000 ng/g lw; L87, Krahn *et al.* 2009, 44,000 ng/g lw).

Total PBDEs measured in the 0-2 cm blubber layer (most similar to a biopsy sample) of L112 (Table 2) were approximately sixteen times higher on a lipid basis than PBDE concentrations measured in biopsies of male northern residents reported by Rayne *et al*. (2004) (3,300 ng/g lw vs. a mean of 203 ng/g lw, n=9). Concentrations of PBDEs were comparable to but somewhat higher than those in two somewhat older (15 year-old) juvenile males from L Pod (L78, Krahn *et al.* 2007, 2,600 ng/g lw; L87, Krahn *et al.* 2009, 2,600 ng/g lw).

Although PBDEs are an emerging concern in marine and terrestrial biota, few recent measurements have been made in killer whales or other species. Most published measurements have been made on archived samples, as was true of the samples reported in Rayne *et al*. (2004), which were collected between 1993 and 1996. Because PBDEs are still used in North America, environmental PBDE levels may continue to rise. Due to this and biological factors such as maternal offloading of contaminants during gestation and lactation, juvenile killer whales may be particularly at risk, and higher average levels of PBDEs compared to adults have been measured recently in juvenile Southern Resident killer whales (Krahn *et al*. 2009), as well as insular Hawaiian Island false killer whales (Ylitalo *et al*. 2009).

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Table 1. Lipid classes[a] in blubber of a juvenile female Southern Resident killer whale (L112) stranded near Long Beach, Washington, in February 2012.

Depth (cm)	SALE (% of Total)	TG (% of Total)	FFA (% of Total)	CHOL (% of Total)	PL (% of Total)
0-2	19.8	80.2	0	0	0

[a] Lipid classes are measured by TLC-FID to precision of 0.1%.
SALE = stearic acid laurel (wax) esters; TG = triacylglycerols; FFA = free fatty acids; CHOL = cholesterol; PL = phospholipids

Table 2. Concentrations (ng/g, wet wt or ng/g, lipid) of POPs in blubber of a juvenile female Southern Resident killer whale (L112) stranded near Long Beach, Washington, in February 2012.

		ng/g, wet wt						ng/g, lipid wt					
Depth (cm)	Lipid %[a]	HCB	∑HCHs	∑CHLDs	∑DDTs	∑PCBs	∑PBDEs	HCB	∑HCHs	∑CHLDs	∑DDTs	∑PCBs	∑PBDEs
0-2	72.6	630	390	4,100	31,000	20,000	2,400	870	530	5,600	43,000	27,000	3,300

[a] Lipid % was measured gravimetrically as total extractable organics; POPs concentrations are reported to two significant figures.

Appendix D-2:
Table of Laboratory Tests

Category	Pathogen	Lab	Method	Tissues	Result	Comments
Bacterial	*Campylobacter* spp	Animal Health Center	Culture	Colon	Negative	
	Campylobacter spp	UC Davis	Aerobic culture	Colon	Negative	
	Clostridium chauvoei	Animal Health Center	Immunoflorescence	Skin	Negative	
	Clostridium novyi	Animal Health Center	Immunoflorescence	Skin	Negative	
	Clostridium perfringens	UC Davis	Anaerobic culture	Colon	Positive	Large numbers
	Clostridium septicum	Animal Health Center	Anaerobic culture	Skin	Positive	Level 4+
	Clostridium septicum	Animal Health Center	Immunoflorescence	Skin	Negative	
	Clostridium sordellii	Animal Health Center	Immunoflorescence	Skin	Negative	
	Clostridium sordellii	UC Davis	Anaerobic culture	Colon	Positive	Large numbers
	Edwardsiella tarda	Animal Health Center	Aerobic culture	Lung	Positive	Level 1+
	Edwardsiella tarda	UC Davis	Aerobic culture	Lung, Lymph Node, Brain, Liver, Blowhole, Kidney	Positive	Large numbers (lung), small numbers (brain, lymph node, liver, kidney, blowhole)
	Micrococcus spp	UC Davis	Aerobic culture	Spleen	Positive	4 colonies
	Salmonella spp	Animal Health Center	Culture	Colon	Negative	
	Salmonella spp	UC Davis	Aerobic culture	Colon	Negative	
	Streptococcus spp (alpha)	Animal Health Center	Aerobic culture	Colon	Positive	Level 2+
	Yersina sp	Animal Health Center	Culture	Colon	Negative	
	No bacteria isolated	Animal Health Center	Aerobic culture	Brain, CSF, Meninges, Lymph Node, Mammary Gland, Blowhole, Spleen		
Viral	*Brucella* spp	Animal Health Center	PCR	Pooled Tissue: Spinal Cord, Liver, Lung, Spleen, Thymus, Mammary Gland	Negative	
	Brucella spp	UC Davis	Aerobic culture	Unspecified	Negative	
	Brucella spp	UC Davis	PCR	Pulmonary Lymph Node, Ovary	Negative	
	Brucella spp	UC Davis	PCR	Brain, Mesenteric Lymph Node, Umbilical Vein	Suspect Positive	Sequences obtained for the mesenteric lymph node and umbilical during the first round of sequencing were very short and not clean to further identify the brucella strain present. PCR and sequencing was repeated and the short sequence obtained from brain tissue indicated the strain present was *Brucella abortus* .
	Canine Distemper Virus	Animal Health Center	PCR	Pooled Tissue: Spinal Cord, Liver, Lung, Spleen, Thymus, Mammary Gland	Negative	
	Herpesvirus-Concensus	Animal Health Center	PCR	Pooled Tissue: Spinal Cord, Liver, Lung, Spleen, Thymus, Mammary Gland	Negative	
	Influenza A	UC Davis	PCR	Blowhole, Lung, Pulmonary Lymph Node	Negative	
	Influenza-Concensus	Animal Health Center	PCR	Pooled Tissue: Spinal Cord, Liver, Lung, Spleen, Thymus, Mammary Gland	Negative	
	Morbillivirus	UC Davis	PCR	Liver, Brain, Lung, Pulmonary lymph node, Umbilical Vein	Negative	Products of the expected size were amplified with two primer sets to detect the presence of morbilliviral RNA in brain, lung, pulmonary lymph node and umbilical vein. Repeated sequencing attempts have not been successful thus these tissues must be considered negative for morbillivirus.
	West Nile Virus	Animal Health Center	PCR	Pooled Tissue: Spinal Cord, Liver, Lung, Spleen, Thymus, Mammary Gland	Negative	
Fungal	*Cryptococcus* spp	WDFW, in-house	CrAg Assay	Pericardial Fluid	Negative	

Protozoal	Apicomplexa	National Institute of Health Laboratory of Parasitic Diseases	PCR	Brain, Muscle, Lymph Node, Tongue, Heart (1 of 2 sections)	Negative	
	Apicomplexa	Animal Health Center	PCR	Pooled Tissue: Brain, Skin	Negative	
	Toxoplasma gondii	National Institute of Health Laboratory of Parasitic Diseases	PCR	Heart (2 of 2 sections)	Positive	Only detected in one section of heart.
Biotoxin	*Pseudo-nitzschia* spp (domoic acid)	Northwest Fisheries Science Center Biotoxin Lab	ELISA	Stomach Contents, Feces	Negative	
	Saxitoxin (Paralytic Shellfish Poisoning)	Northwest Fisheries Science Center Biotoxin Lab	ELISA	Stomach Contents, Feces	Negative	
Parasitic	*Crassicauda* spp	University of Florida, Department of Infectious Diseases and Pathology		From bullae	Positive	
	Aniskasis spp	National Marine Mammal Laboratory		From Stomach	Positive	26 *Anisakis* sp. cf. *A. simplex* ranging in length from 30 – 42 mm

Appendix D-3:
Bulla Parasite Report, 29 March 2012

UF UNIVERSITY of FLORIDA

College of Veterinary Medicine
Department of Infectious Diseases and Pathology

PO Box 110880
Gainesville, FL 32611-0880
352--294-4125
352-392-9704 Fax
hdstockdale@ufl.edu

March 29, 2012

Amy Traxler
Assistant Research Curator
The Whale Museum
PO Box 945
Friday Harbor, WA 98250
360-378-4710 X27

Final Results: Killer Whale, L-112, bulla, Parasite ID

Parasite identified as *Crassicauda* sp.

Please feel free to contact me with any questions or concerns at the above phone number or email address.

Sincerely,

Heather D. Stockdale Walden, PhD
Research Assistant Professor of Parasitology

Appendix D-4:
Gas Analysis Report, 21 February 2012

CASE REPORT: PSU12-02-11Oo

SPECIES: Killer whale (*Orcinus orca*)

DATE EXAMINED: 12-Feb-12

CASE REPORT BY:
Yara Bernaldo de Quirós Miranda
Biology Department, Woods Hole Oceanographic Institution,
Woods Hole, MA 02543 USA

HISTORY

A killer whale was found dead in Long Beach (WA) on February 12th 2012. The Cascadia Research Stranding group performed a complete necropsy. Gas bubbles were found in different tissues. Four samples from the heart (n=2) and from the mesenteric veins (n=2) were taken. Samples were shipped in a sealed chamber together with a barometer/altimeter to register any changes in pressure during air shipment, and remitted to the Woods Hole Oceanographic Institution. Samples were received on February 21st 2012. Only 30 m altitude equivalent difference was found. This is negligible from a pressure point of view. Thus the samples remained at sea level pressure despite being shipped by air in the unpressurised plane hold.

RESULTS

PSU12-02-11Oo	Remarks	% H_2	% CO_2	% O_2	% N_2	% CH_4	
Heart	monoject	9.5	84.2	0.0	6.3	0.0	CO_2>>> H_2>N_2
Mesenteric v	BD vac	14.6	85.4	0.0	0.0	0.0	CO_2>>>H_2

Heart samples: 81.3±4.1 %CO_2; 10.2±1.0 %H_2 and 8.5±3.2 %N_2
Mesenteric vein samples: 84.0±1.9 %CO_2 and 16.0±1.9 %H_2

DISCUSSION

Gas composition is typical for putrefaction gases. However it is important to remember that the presence of putrefaction gases does not rule out the existence of a previous gas

embolism (Bajanowski et al., 1998; Bernaldo de Quirós et al., 2011; Pierucci and Gherson, 1968; Pierucci and Gherson, 1969). Samples did not suffer changes in pressure during shipping (only a difference in 30 m was recorded by the altimeter). No differences were found between the two types of evacuated tubes used, suggesting that monoject tubes might be as suitable for gas storage as BD vacutainers (Bernaldo de Quirós et al., 2011).

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Appendix E-1:
CT Report, Head, 23 February 2012

VCA Veterinary Specialty Center of Seattle

20115 44th Avenue West Lynnwood, WA 98036
425.697.6106 ▪ 425.697.4746 [FAX]

CT Report

Patient: Female ORCA L-112 **Date of Exam:** 02/23/2012 **Referring doctor:** Whale Museum
Date dictated: 02/29/2012
Study: Majority of the head.

*****Due to patient size and shape, several series of scans were obtained focusing on certain anatomical areas. The entire head could not be included on one scan, and some of the soft tissues are outside of the field of view. Some of the scans are quite rotated as a preliminary scan was done to assess if the plastic coating would at all interfere with the image (which it didn't). There are several scans that are quite straight but this sequence of scans could not include the caudal ventral most aspect of the skull due to patient size restriction. Keep in mind that the scans with the head rotated will distort the image.

NOTE: The patient was lying on her side with the left side down, blowhole facing the workstation for CT, mandible facing away from the workstation and right side up, nose to window. A=left side. P=right side.

CT Findings: This patient is positioned with the left side down and the right side up. On the scan window overlay, A= Left and P=Right.

There is extensive gas accumulation in the soft tissues and fat throughout the head Including intracranially (the head has been disarticulated for imaging).

There is loss of brain matter. The right side of the calvarium is almost completely devoid of brain tissue- the majority of the right side of the brain is missing including the right cerebral hemisphere and the right side of the cerebellum. Soft-tissue attenuating striations suspended within the calvarium are suggestive of residual meninges on the right. Brain tissue is present on the left side.

On the sequences with the head positioned as straight as possible, no asymmetry to the large included bones of the skull is noted. No large displaced fractures of the calvarium are seen. There are a few small, smoothly marginated mineral attenuating densities (small bones and/or otoliths and/or dystrophic mineralization) and also somewhat thin linear area of mineralization at the level of, but separate from, the osseous bulla. Most of these structures are dorsal to the bulla. Some of these are quite small and are present bilateral, but are asymmetric. There is displacement of some of these very small mineralized bodies on the right side through the calvarial foramina. Image 1. This is interpreted with caution because in this area, especially on the right side, there is loss of normal soft tissue structures (brain, fat etc) that may previously have held these in place outside of the calvarium. The peribullous sinus contains a mixture of air and soft tissue attenuating material bilaterally.

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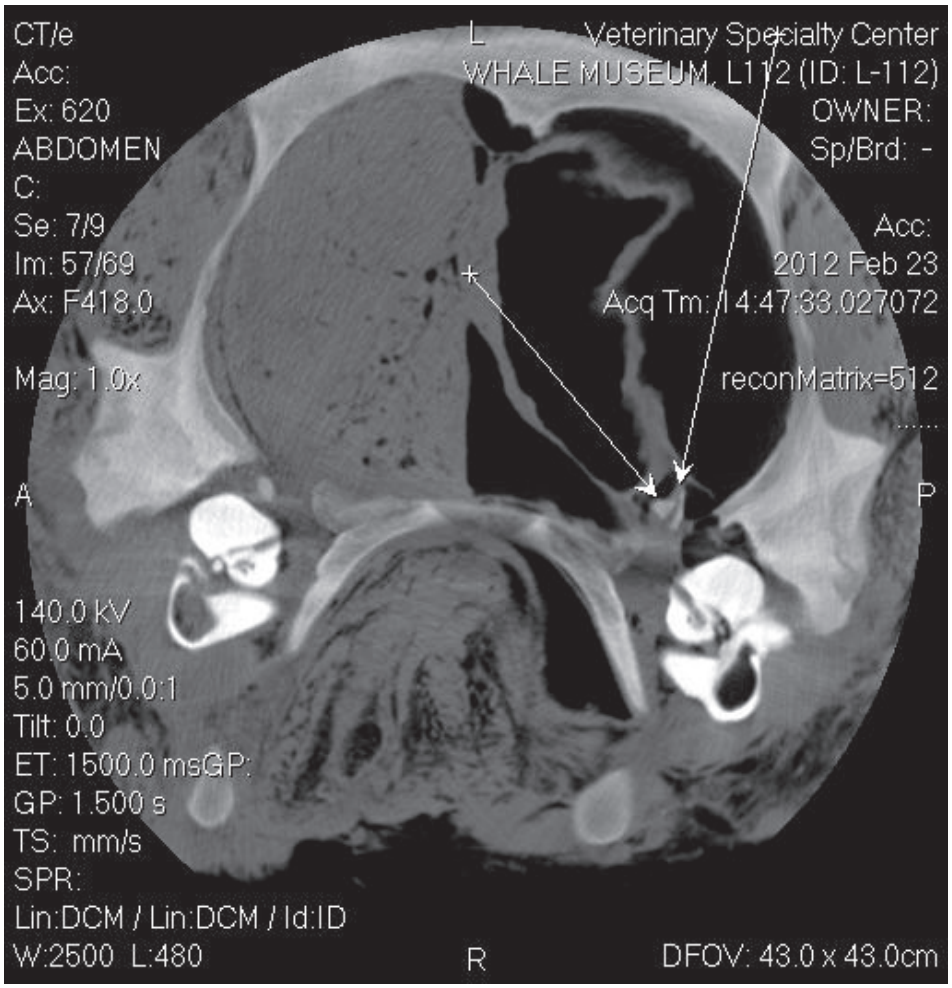


Image 1- small mineral attenuating structures displaced into calvarium on the right side. The side with the missing brain matter.

There is accumulation of soft tissue or fluid attenuating material and gas in the pterygoid sinuses with the left side having more soft-tissue or fluid-attenuating material than the right.

Female Orca L-112
Pt ID No.

There is soft-tissue or fluid-attenuating material in the majority of the left osseous bulla. There is soft-tissue or fluid-attenuating material and air in the rostral aspect of the right osseous bulla with air in the mid to caudal right osseous bulla. The right bulla has more air in it than the left. The left is almost completely filled with fluid and/or soft tissue. Considerations for this fluids/soft tissue attenuating material are blood, infectious or inflammatory debris, polyp like material-chronic inflammation or parasites and/or post-mortem accumulation of fluid or engorged mucous membranes. See image 2 and 3. The tympano-periotic complexes are intact bilaterally. The auditory ossicles cannot be fully evaluated due to limitations of resolution.

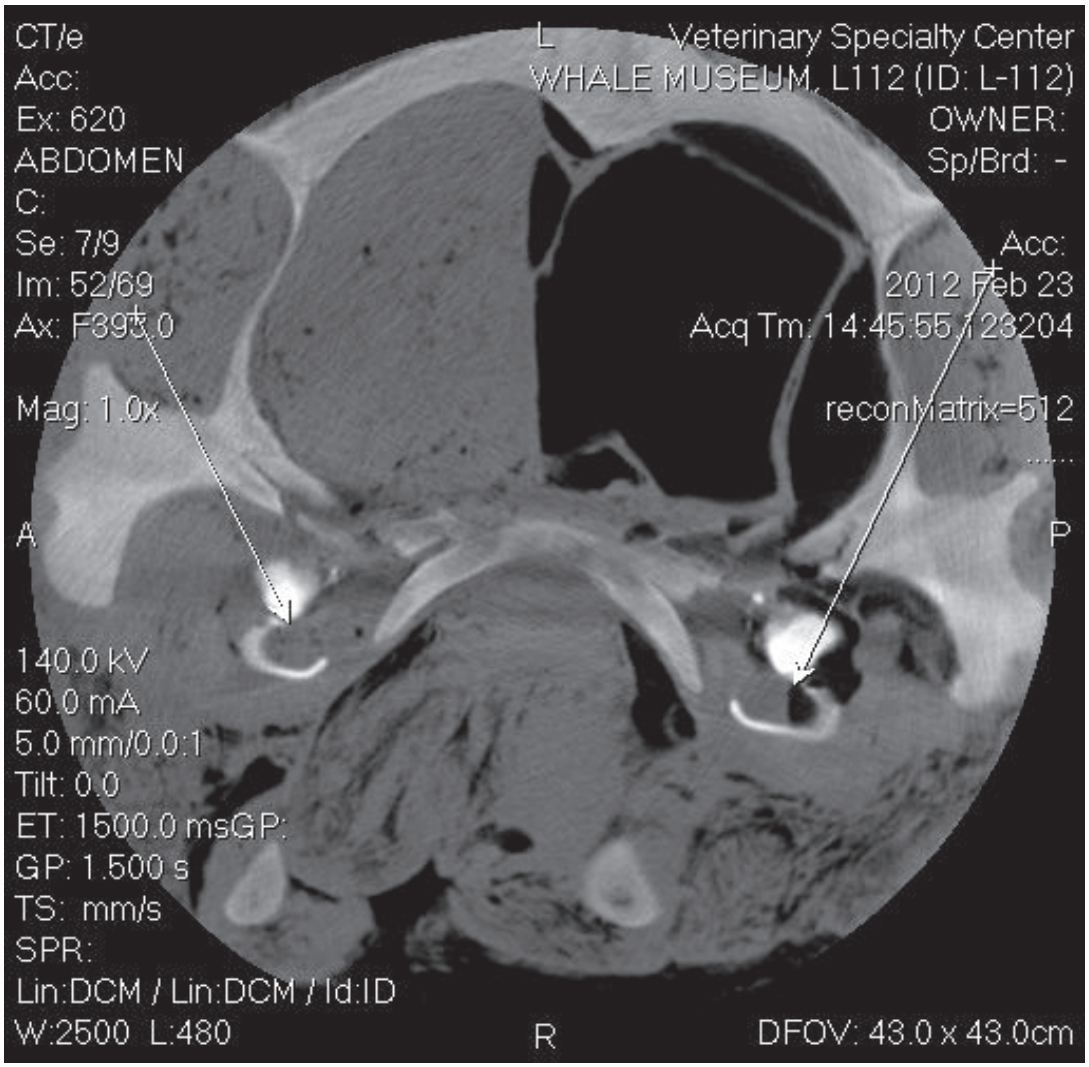


Image 2. The more rostral aspect of the osseous bulla. Arrows pointing to bulla. Fluid or soft tissue attenuating material occluding the left osseous bulla and air and fluid/soft tissue attenuating material in the right.

Female Orca L-112

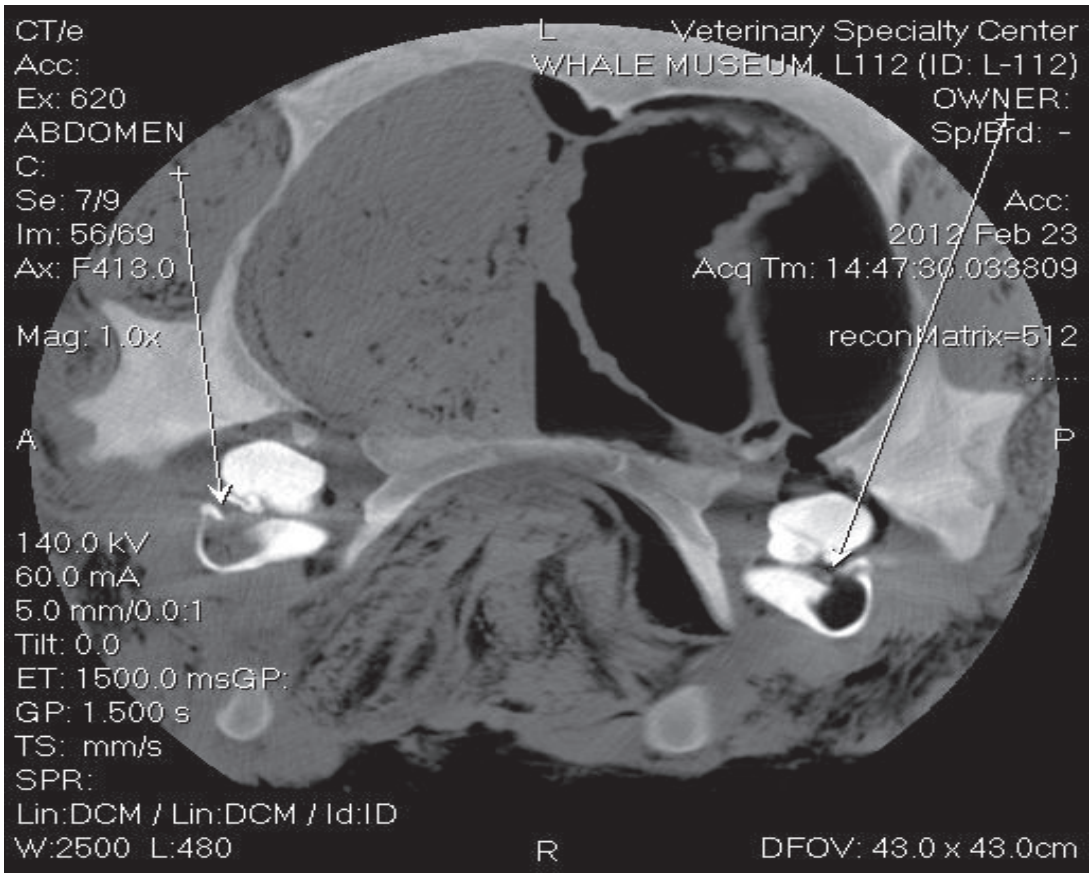


Image 3. Caudal aspect of the bulla. Fluid or soft tissue in left osseous bulla. Air in the right.

CT summary:

-Extensive gas accumulation in the soft tissues and fat. Disarticulation prevents further comment.
-Absence of right cerebral hemisphere and right cerebellum of the brain secondary to loss of tissue during disarticulation. Significance is uncertain based on imaging alone but is an atypical observation.
-Bilateral small mineral (i.e. bone) attenuating structures dorsal to the bulla (Ddx: otoliths, dystrophic mineralization, parasitic granulomas) with right-sided displacement through the foramina into the calvarium due to loss of supporting tissues (presumptive).
-Sinusitis, likely parasitic.
-Fluid and/or soft tissue in both osseous bullae (middle ears) worse on the left. Possibilities include: blood, infectious or inflammatory debris, polyp like material and chronic inflammation, parasites (including worms) and/or post mortem accumulation of fluid or engorged mucous membranes.

Tori McKlveen, DVM, MS, Diplomate ACVR
Sophie Dennison, BVM&S, MRCVS, Diplomate ACVR
Female Orca L-112

Appendix E-2:
CT Report, Bulla, 10 April 2012

VCA Veterinary Specialty Center of Seattle

20115 44th Avenue West Lynnwood, WA 98036
425.697.6106 ▪ 425.697.4746 [FAX]

CT Report

Patient: Female ORCA L-112 – ear bones
Date of Study: April 10, 2012
Referring doctor: Whale Museum
Study: Right and left ear bones including the bullae. Individual scans of the right and left ear bones including the bones of the inner and middle ear were performed. The scans are done at 1-mm slice thickness. Ear bones were indicated right side and left side. Specimens were scanned in liquid medium and in room air.
Technical factors: Standard and bone.
CT Findings:

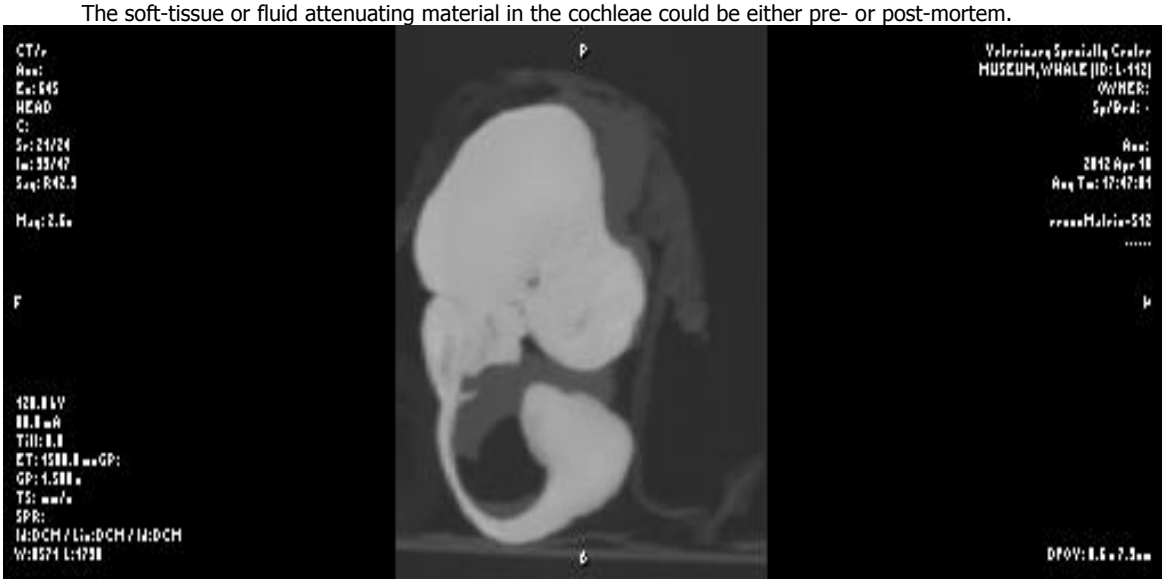
The intact ears were disarticulated from the head and scanned individually.

No fractures were seen. The ossicles of the middle ear are intact and do not appear to be displaced. There was fluid or soft-tissue attenuating material in the cochleae.

CT Conclusions:

CT scan of the right and left ear bones at 1-mm slice thickness did not show any evidence of fractures, dislocation, or crushing.

The soft-tissue or fluid attenuating material in the cochleae could be either pre- or post-mortem.



Bones of the middle and inner ear

Tori McKlveen, DVM, MS, Diplomate ACVR
Sophie Dennison, BVM & S, MRCVS, Diplomate ACVR

Appendix E-3:
CT Report, Cervical Vertebra, 12 December 2012

VCA Veterinary Specialty Center of Seattle

20115 44th Avenue West Lynnwood, WA 98036
425.697.6106 ▪ 425.697.4746 [FAX]

CT Report

Patient: Female ORCA L-112
Date of Study: December 12, 2012
Referring doctor: Whale Museum
Study: Cervical vertebrae and T1
Technical factors: Standard and bone. 1-mm slices

CT Findings: The cervical vertebrae and T1 were scanned as a group attached by a cable tie. There was also an individual scan performed on the fused cranial cervical vertebrae and an individual scan performed on C7.

Cervical vertebrae 1 through 4 are fused (congenital).

There is a small 4-mm in width defect in the right side of the lamina of C7 (the dorsal aspect of the vertebra). The ends are blunted and smooth, and the bony margins of this defect are sclerotic. The spinous process is lacking.

The end plate of C7 is lifting from the cranial and caudal aspect of the vertebral body. There is a small fissure in the endplate affiliated with the elevation.

No fractures of the cervical or 1st thoracic vertebrae are appreciated.

The malalignment is because these are individual bones.

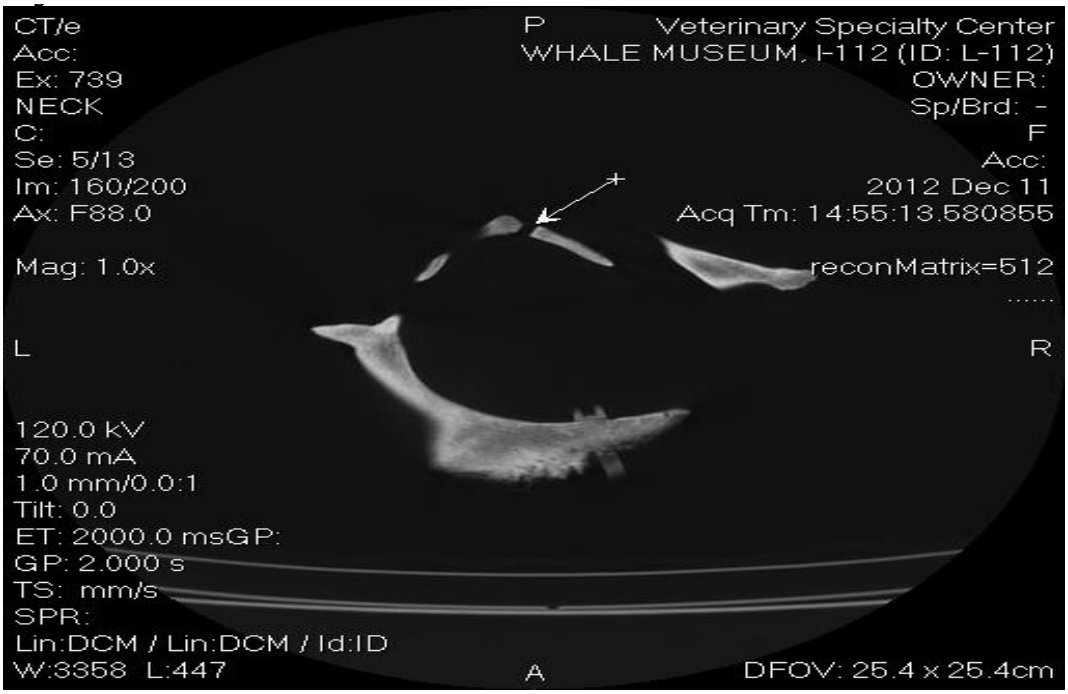
CT Conclusions: Congenital defect or anomaly of the dorsal aspect of vertebra C7 (the lamina of C7) with incomplete fusion of the lamina and incomplete formation of the spinous process. This does not appear to be an acute or traumatic fracture.

Fusion of the cranial cervical vertebrae; this has been noted in other orca specimens as well.

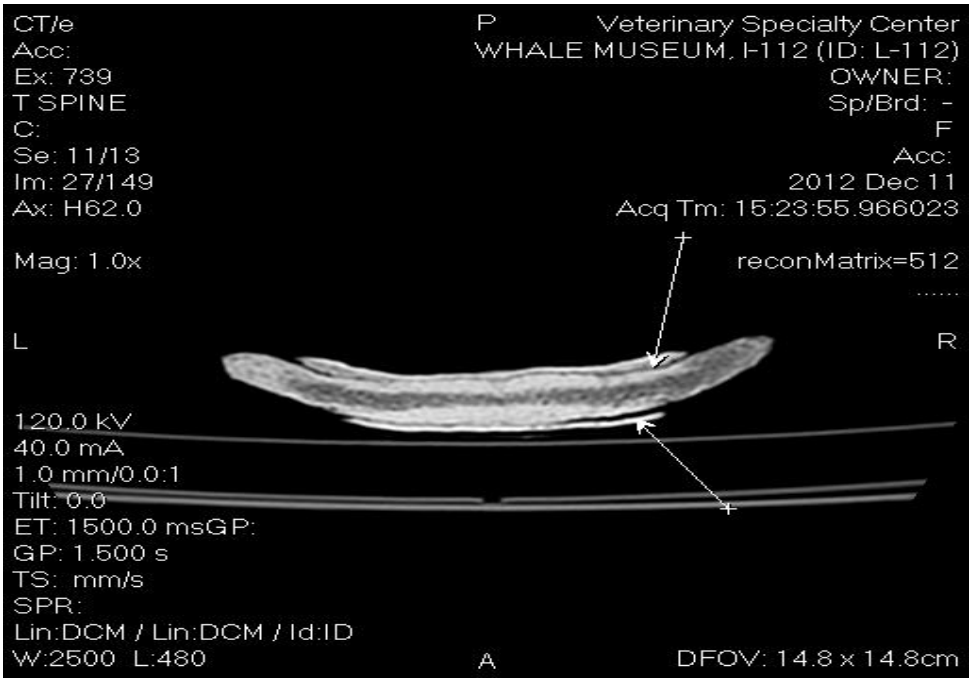
The lifting and fissure of the end plates of C7 is believed to be post mortem and likely a result of skeletal preparation and drying and not evidence of a traumatic injury.

Comments: Skeletal specimens were also reviewed by Karen Kline, DACVIM- Neurology, and Courtney Watkins, DACVS (surgery), and they concur with these findings.

Female ORCA L-112



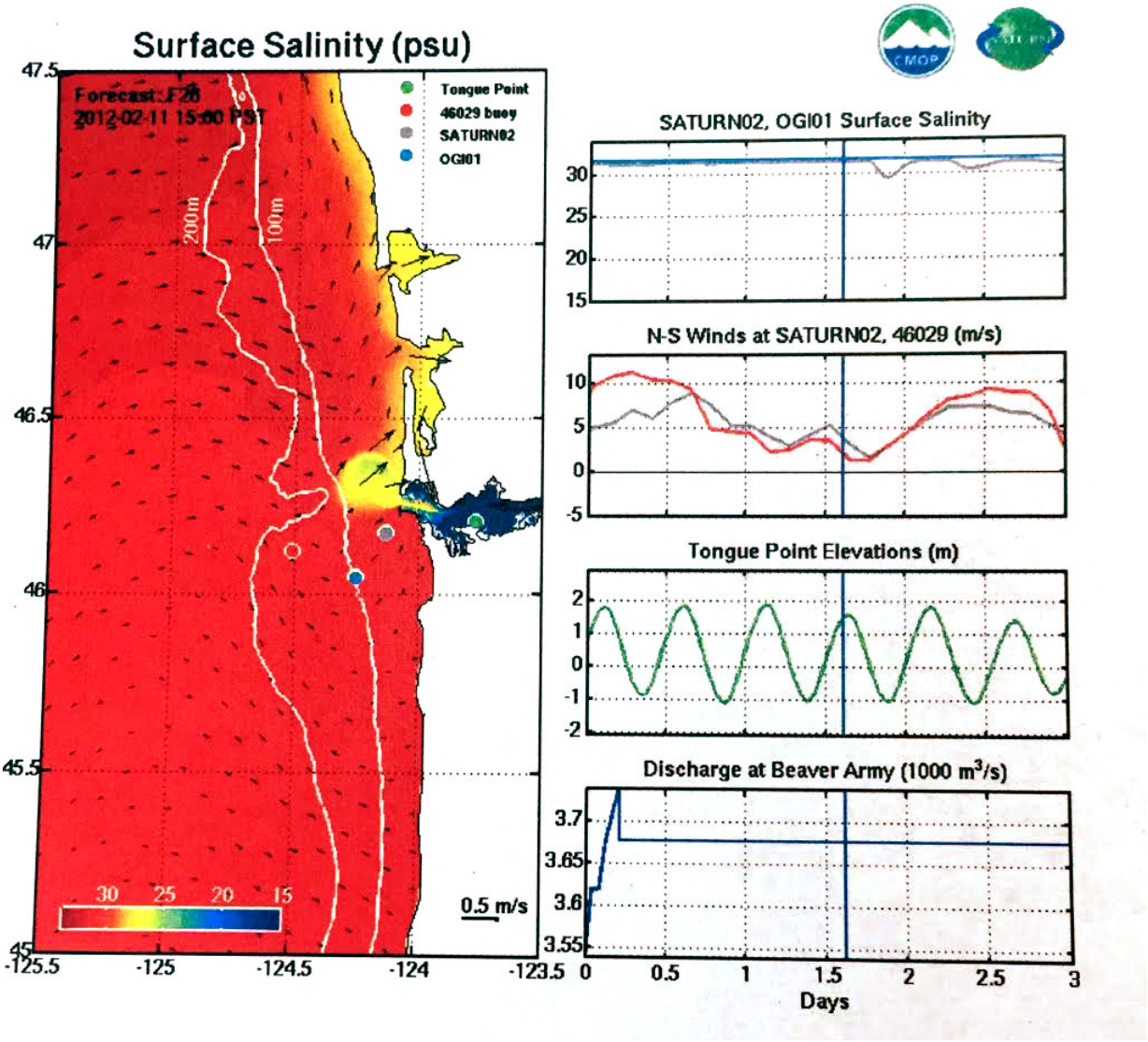
Incomplete fusion of the right side of the lamina of cervical vertebra 7 (arrow)



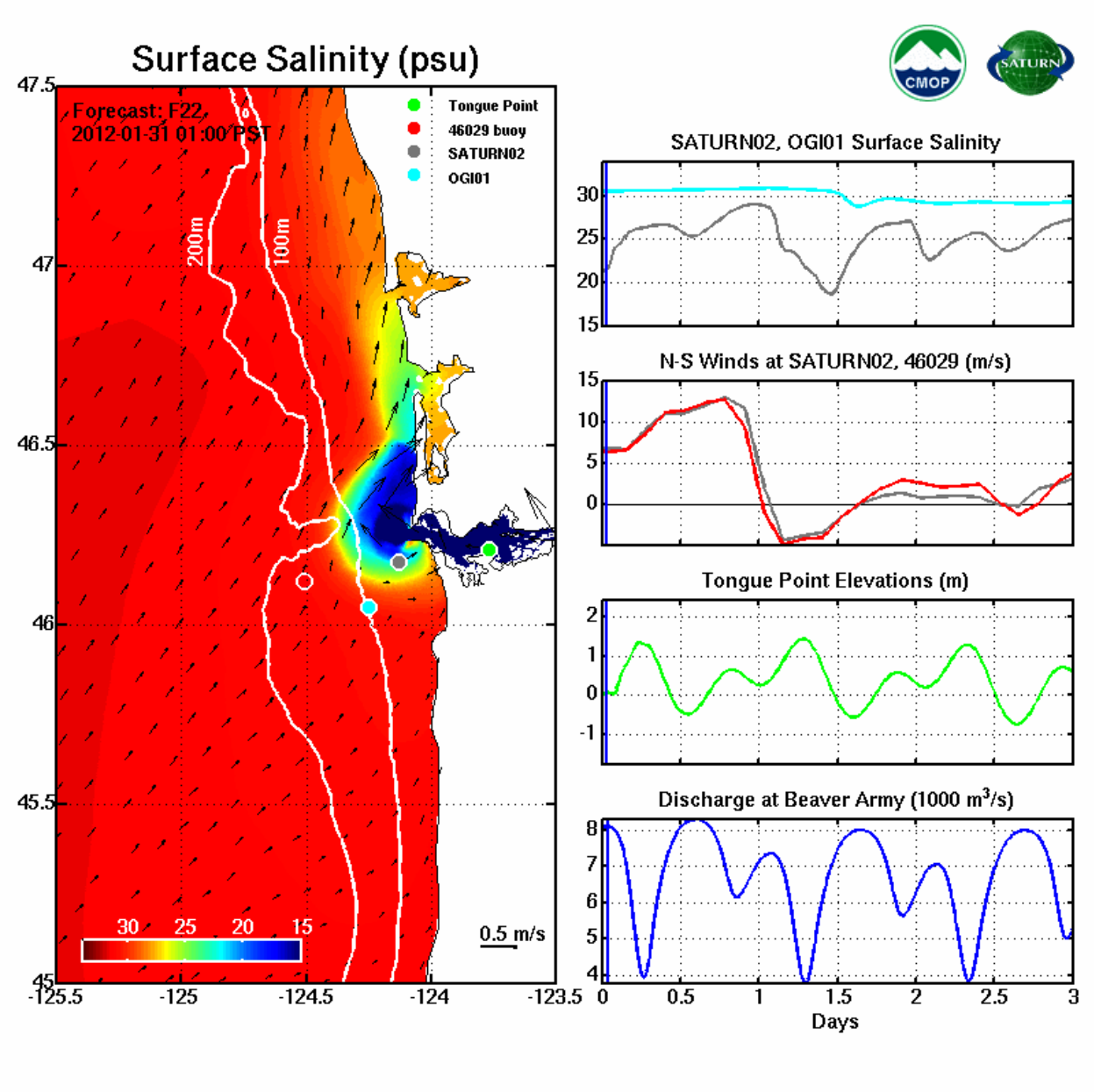
Lifting of the endplates of cervical vertebra 7 (arrows)

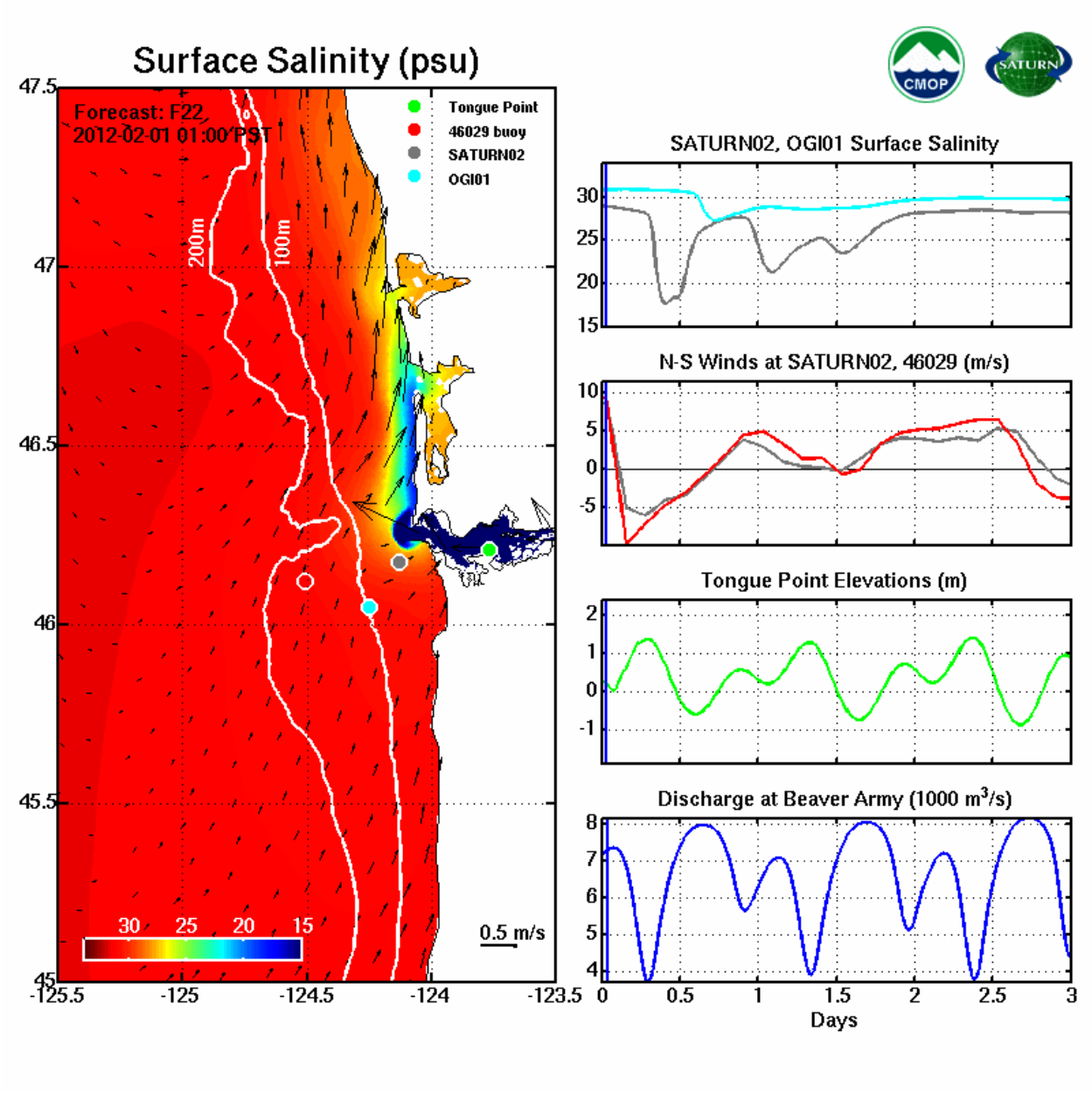
Tori McKlveen, DVM, MS, Diplomate ACVR (Radiology)
Karen Kline, DVM, MS, Diplomate ACVIM (Neurology)
Courtney Watkins, DVM, MS, Diplomate ACVS (Surgery)

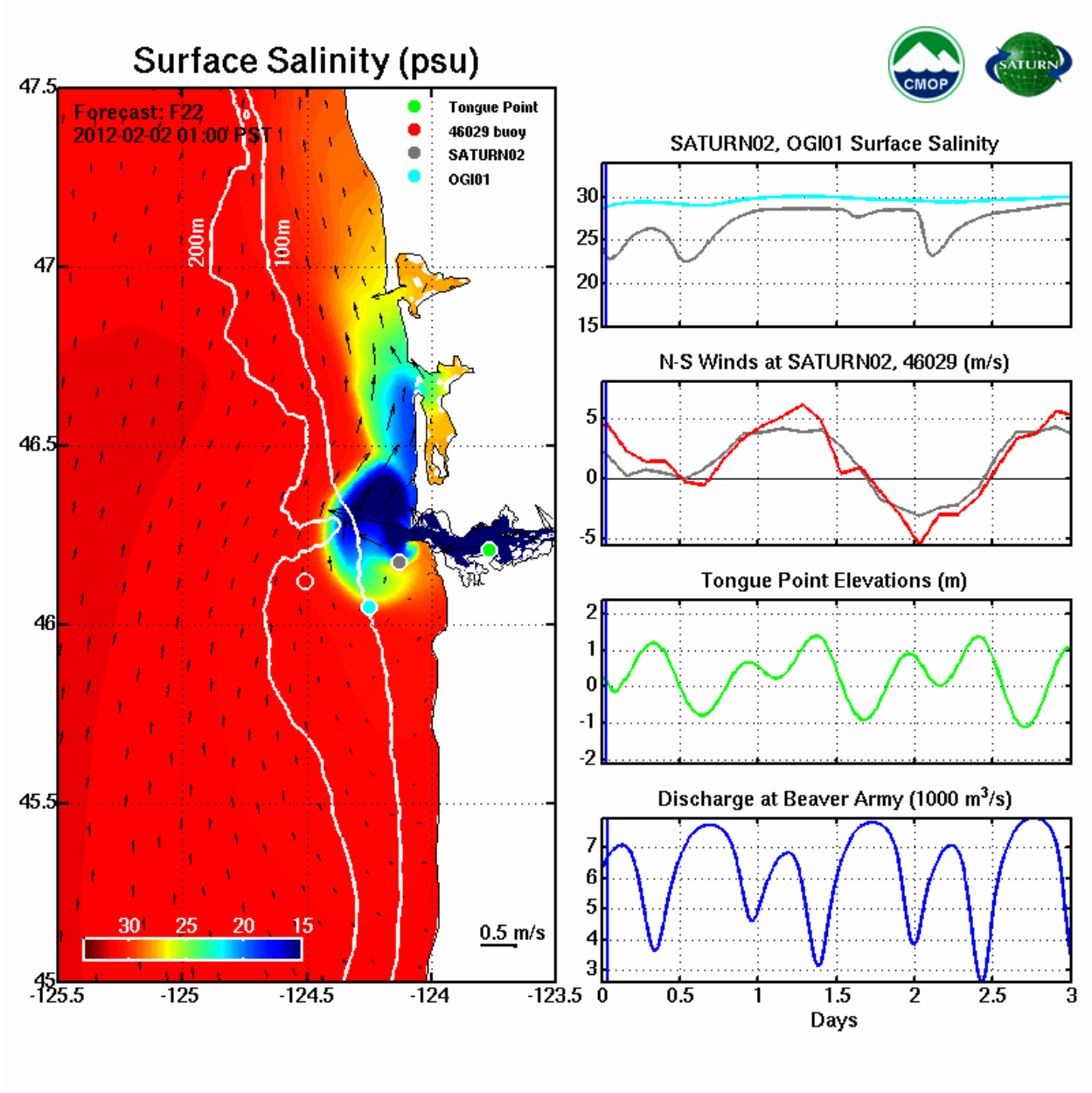
Appendix F-1:
Current Forecast, 11 February 2012



Appendix F-2:
Current Maps, 31 January–10 February 2012




Surface Salinity (psu)
Forecast: F22
2012-02-01 01:00 PST
Tongue Point
46029 buoy
SATURN02
OGI01
200m
100m
30 25 20 15
0.5 m/s
47.5
47
46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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46029 buoy
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OGI01
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100m
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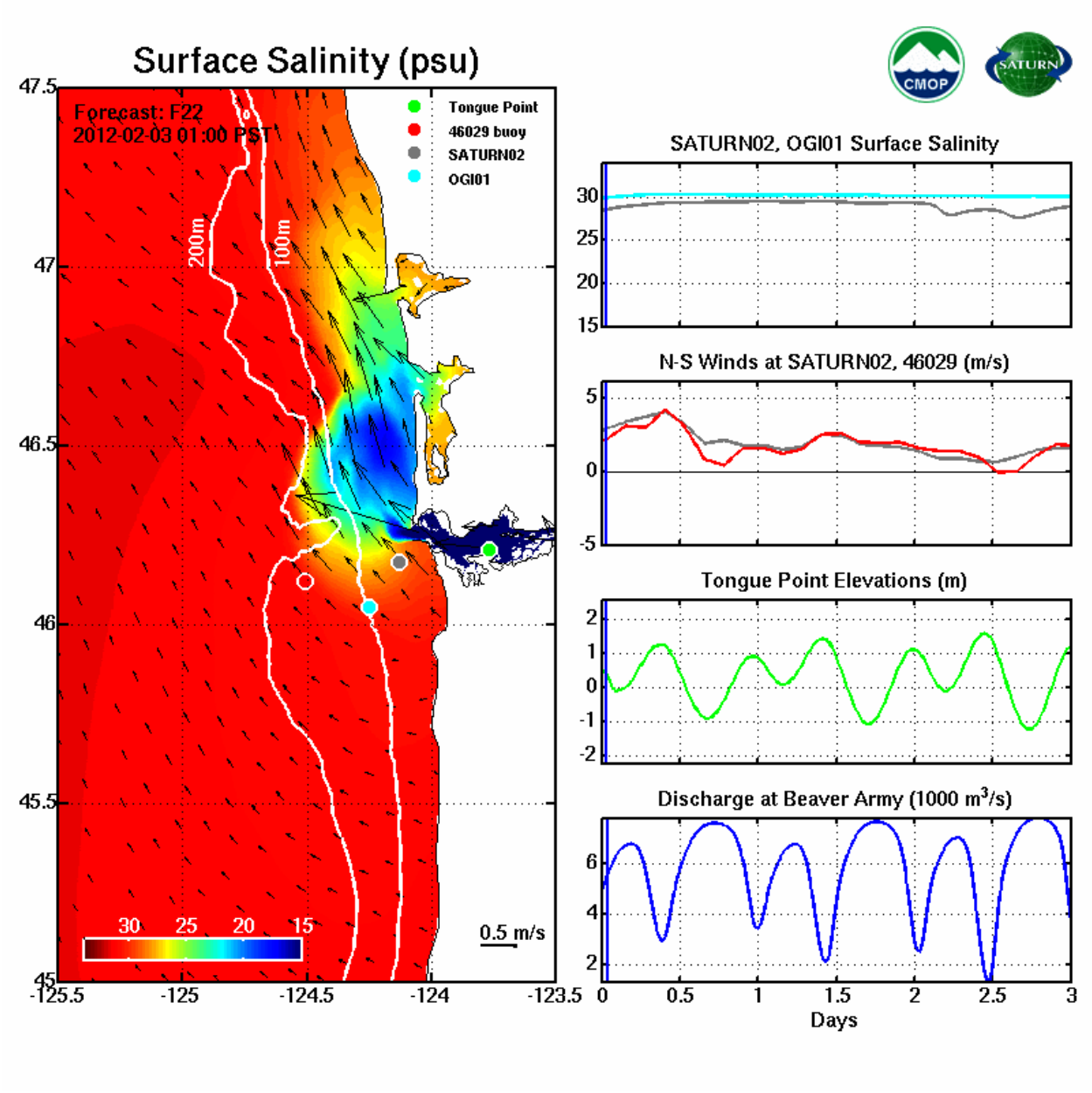
0.5 m/s
47.5
47
46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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OGI01
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100m
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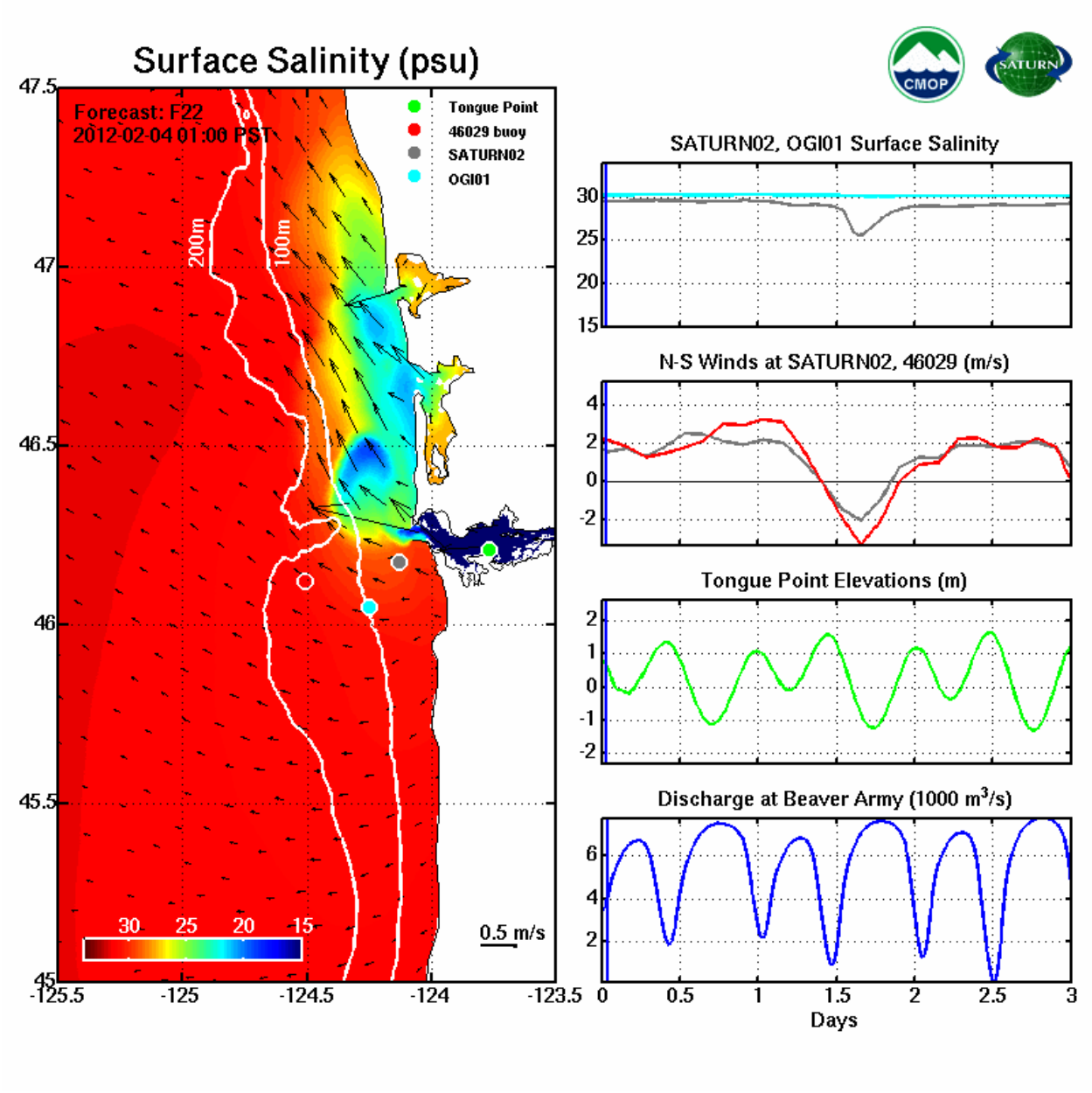
47
46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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OGI01
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0.5 m/s
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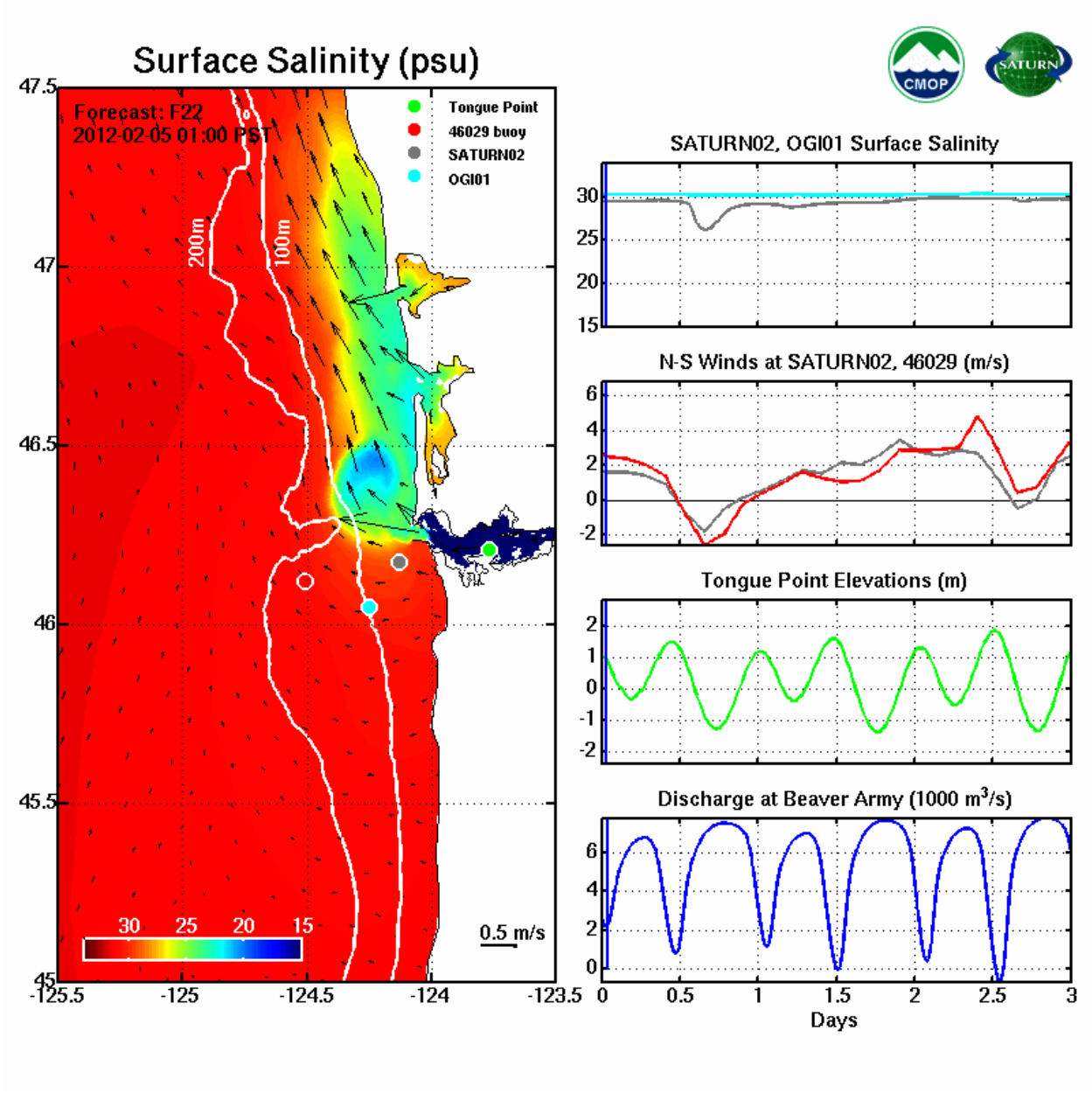
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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OGI01
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100m
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0.5 m/s
47.5
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46.5
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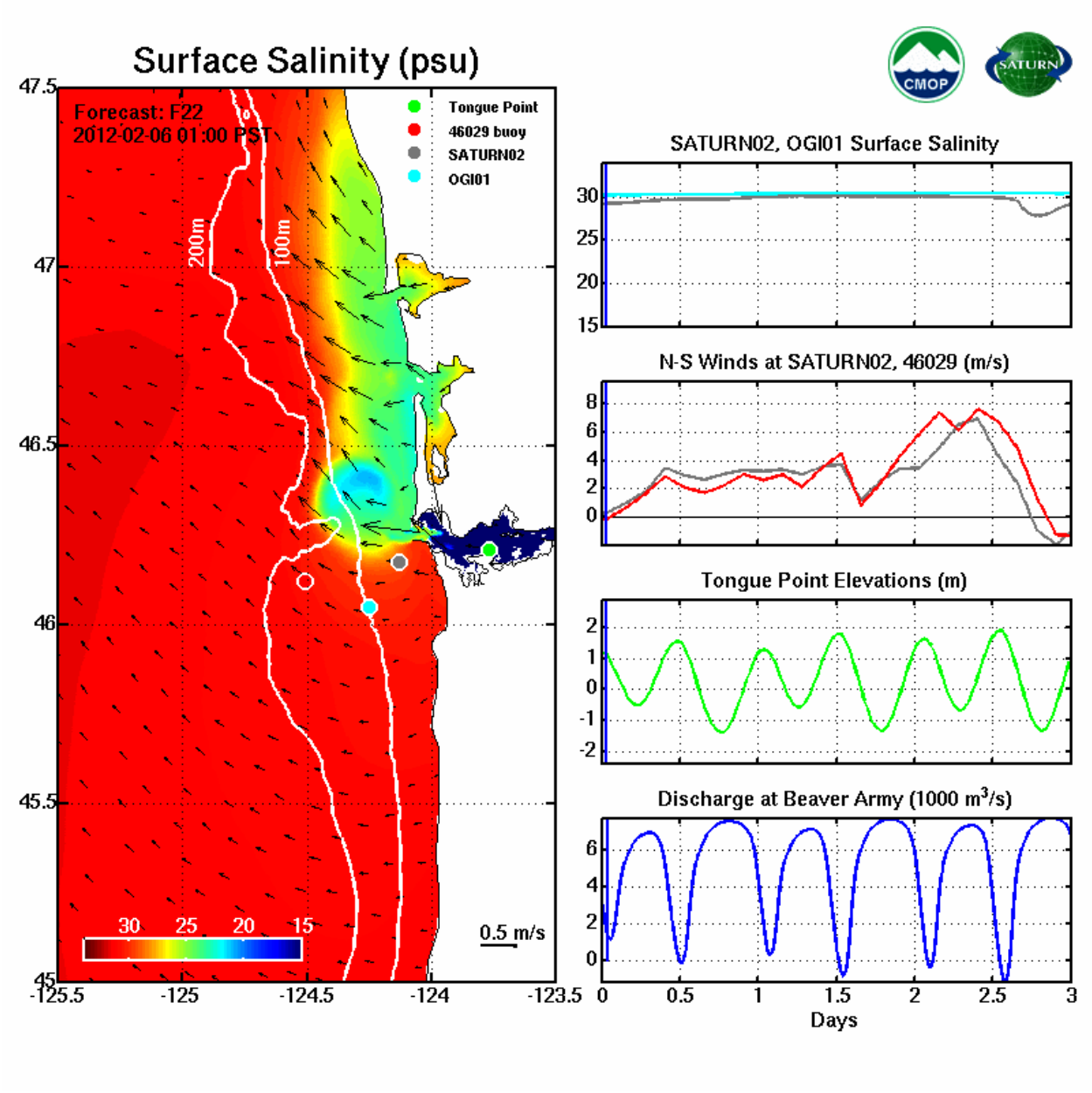
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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OGI01
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100m
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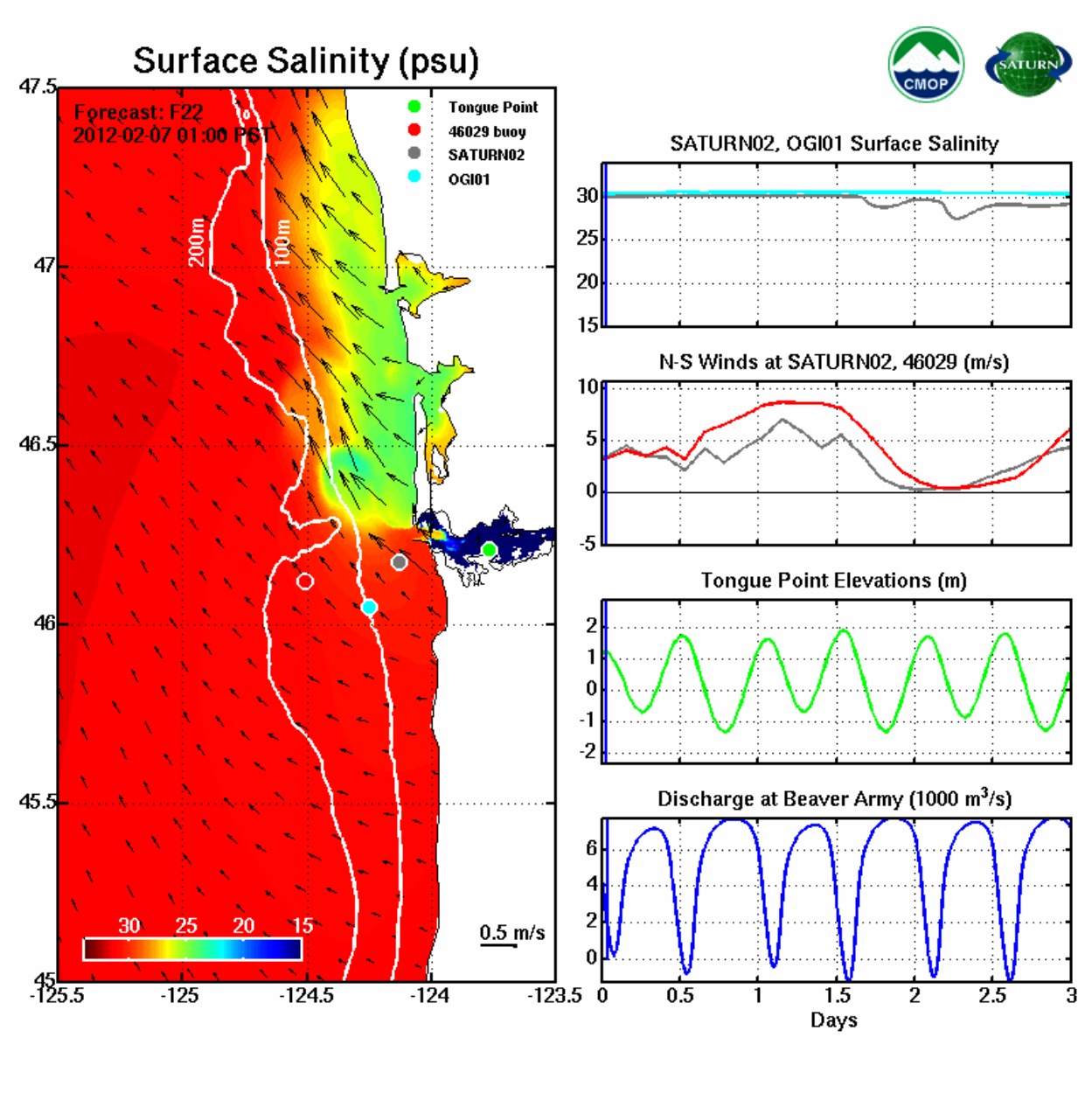
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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0.5 m/s
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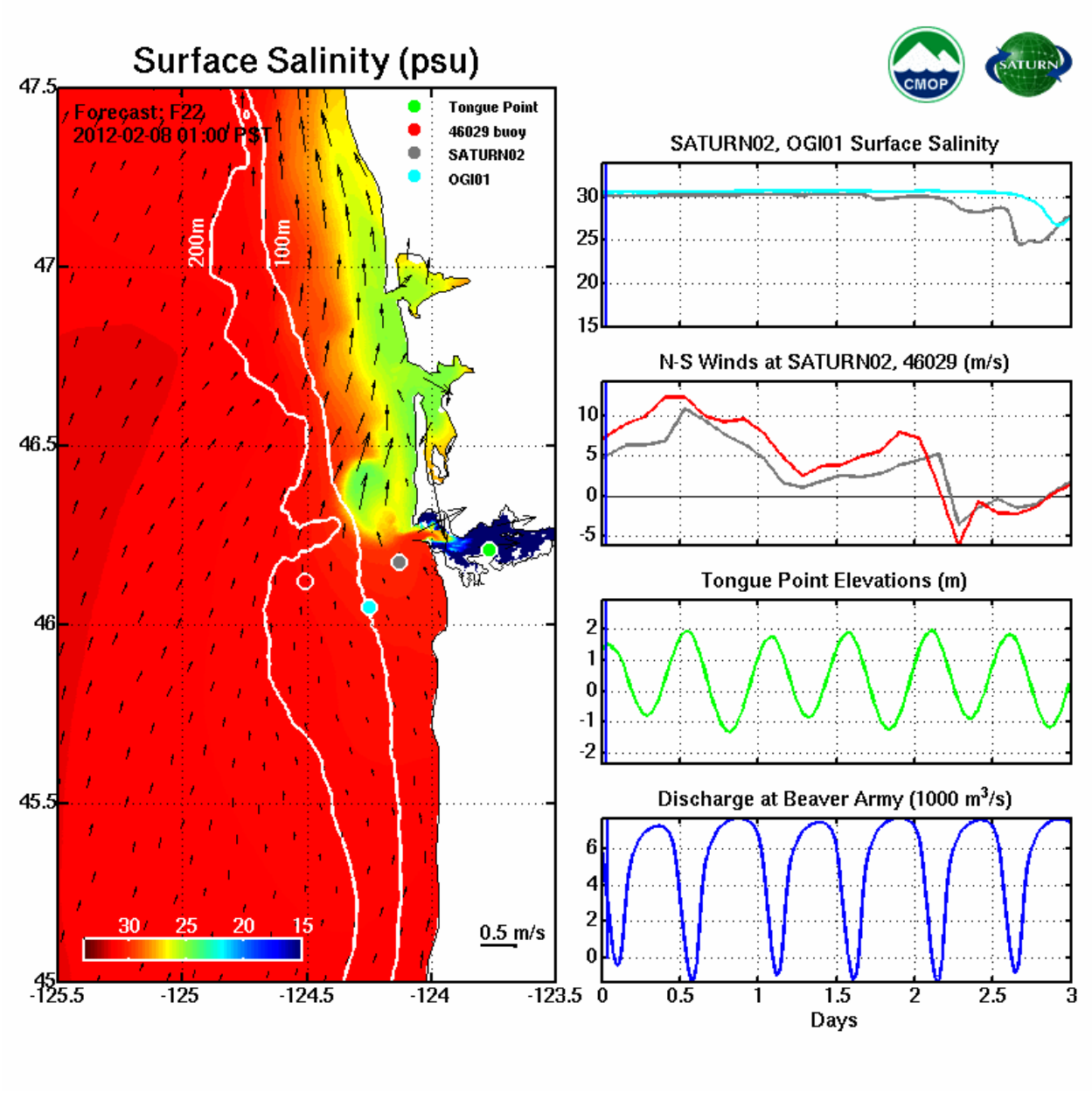
46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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OGI01
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100m
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0.5 m/s
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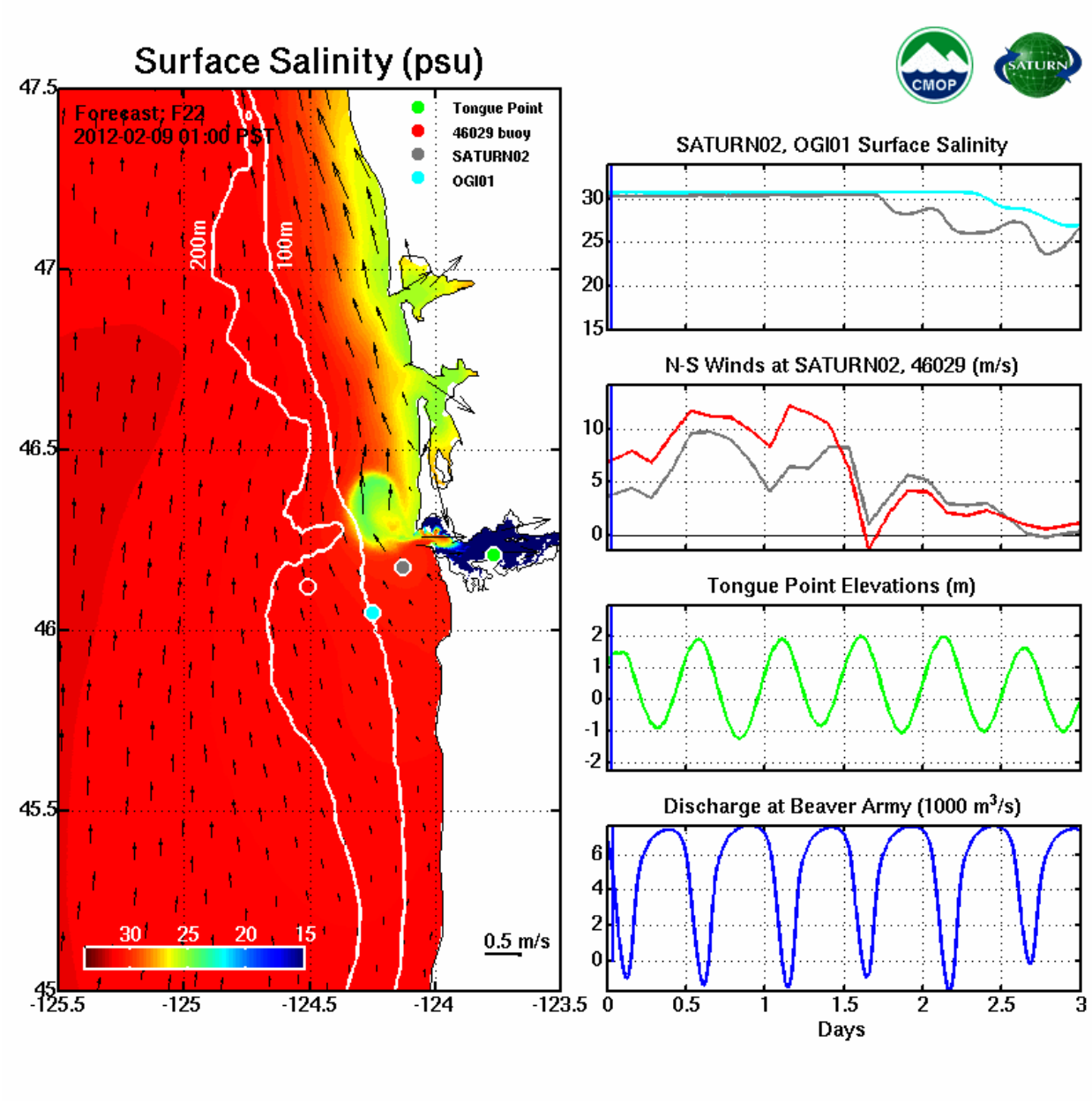
47
46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days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OGI01
200m
100m
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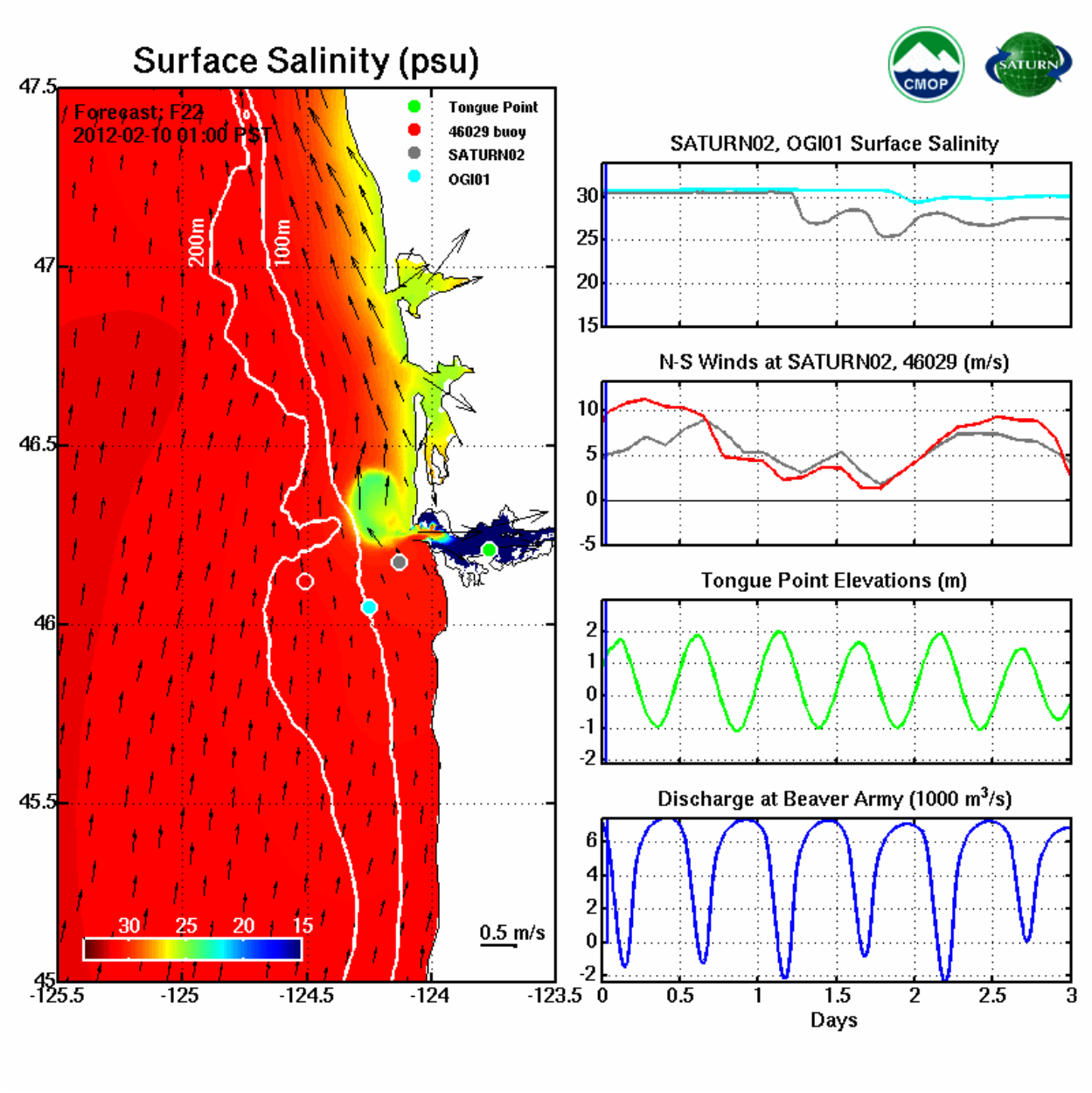
20
15
0.5 m/s
47.5
47
46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days
CMOP
SATURN


Surface Salinity (psu)
Forecast; F22
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46029 buoy
SATURN02
OGI01
200m
100m
30
25
20
15
0.5 m/s
47.5
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46.5
46
45.5
45
-125.5
-125
-124.5
-124
-123.5
SATURN02, OGI01 Surface Salinity
N-S Winds at SATURN02, 46029 (m/s)
Tongue Point Elevations (m)
Discharge at Beaver Army (1000 m3/s)
Days

Appendix F-3:
Sample Request for Information, 18 April 2012

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Northwest Region
7600 Sand Point Way N.E., Bldg. 1
Seattle, WA 98115

April 18, 2012

Mr. Kevin Moynahan
USACE, Portland District Regulatory Office
333 SW First Ave., P.O. Box 2946
Portland, OR 97204-3495

Dear Mr. Moynahan:

We request your assistance in obtaining any information in possession of the U. S. Army Corps of Engineers which may contribute to our understanding of a stranding event involving a dead juvenile killer whale that was discovered in the surf 0.9 miles north of the Cranberry approach on the Long Beach Peninsula, Pacific County, Washington on February 11, 2012. Representatives from the Northwest Marine Mammal Stranding Network responded to the stranding event and transported the carcass to a secure area at Cape Disappointment, Washington for post-mortem examination.

The animal was tentatively identified from external examination as L112, a member of the "L" pod of the Southern Resident killer whale population, a species listed as endangered under the Endangered Species Act. Under the direction of Dr. Deborah Duffield, Portland State University, the primary responder for the Long Beach area, a post-mortem examination was conducted on February 12, 2012 with assistance from Cascadia Research Collective and the Washington Department of Fish and Wildlife. The team collected morphometric data, photographs and tissues for analysis. The head was collected, frozen and later scanned at a diagnostic imaging service in Washington. Further forensic examination of the head was conducted by Dr. Joe Gaydos, University of California Sea-Doc Society, and the Whale Museum, March 6 and 7th.

We have yet to determine a cause for the loss of this animal but examiners found extensive hemorrhage in the soft tissues of the chest, head and right side of the body. Observations indicate the animal was moderately decomposed, but likely dead for less than a week when found.

We have reviewed environmental data between February 1 and February 11and found that prevailing wind and currents, in the days prior to the stranding, were predominantly from the south. In addition, local current conditions are largely influenced by eddies flowing northward

from the mouth of the Columbia River. This would indicate that the animal likely died in the Columbia River plume or to the south and may have drifted a substantial distance before being cast ashore on Long Beach.

We are seeking information from a variety of sources in an attempt to identify whether human activities may have contributed to the injuries we observed. We do not have any information linking the L112 stranding to a specific event, but we are seeking information on civilian activities such as in-water construction, offshore surveys, or resource extraction along the Oregon coast to as far north as Ledbetter Point, Washington that may have been permitted by or reported to your agency between February 1 and 11, 2012 prior to the stranding. Of particular interest are any activities involving explosives (ordinance, blasting, dynamite) or intense impulsive sounds (air guns, pile driving), but we have not ruled out other factors, such as a vessel strike, as a contributing factor. We would appreciate your assistance in compiling any such reports.

Please contact Brent Norberg of my staff at (206) 526-6550 or brent.norberg@noaa.gov if you would like additional information from the analysis of the stranding.

Sincerely,

Lynne Barre, Chief
Puget Sound Ecosystem
and Marine Mammals Branch

Appendix F-4:
Response to Inquiry (DOD), 7 May 2012

DEPARTMENT OF THE ARMY
JOINT BASE GARRISON
BOX 339500, MAIL STOP 1AA
JOINT BASE LEWIS-MCCHORD, WA 98433-9500

REPLY TO
ATTENTION OF

MAY - 7 2012

Office of the Chief of Staff

NOAA - NMFS
RECEIVED
MAY 10 2012
NORTHWEST REGIONAL OFFICE

Ms. Lynne Barre
Chief, Puget Sound Ecosystem and Marine Mammals Branch
United States Department of Commerce National Oceanic and
Administration National Marine Fisheries Service Northwest Region
7600 Sand Point Way NE, Bldg 1, Seattle, WA 98115

Dear Ms. Barre:

Thank you for your letter of 18 April 2012 regarding queries as to military training in the Long Beach area in February 2012. We have confirmed with all military organizations resident on Joint Base Lewis-McChord that no military training involving JBLM units took place in that area during that timeframe. To confirm, we did assess whether any resident units were involved in any training activities during that timeframe and in that general area (and particularly any which may have involved shipping, resource exploration, fisheries, in-water construction, or explosive events).

Please let me know if you need any more information from JBLM regarding this matter.

Thank you,

Thomas G. Knight
Chief of Staff

Appendix F-5:
Response to Inquiry (USCG), 25 June 2012

U.S. Department of Homeland Security
United States Coast Guard

Commander
United States Coast Guard
Pacific Area

Coast Guard Island, Bldg 51-6
Alameda, CA 94501

Staff Symbol: PAC-33
Phone: (510) 437-2942
Fax: (510) 437-3774

16000
25 Jun 2012

MEMORANDUM

From: J. D. DOHERTY, CAPT
CG PACAREA (PAC-33)

Reply to Attn of: Ms. Lori Mazzuca
Marine Protected Species Program Manager
(510) 437-3211

To: Ms. Lynne Barre
NMFS Northwest Region

Subj: RESPONSE TO REQUEST FOR INFORMATION IN SUPPORT OF L112 STRANDING INVESTIGATION

Ref: (a) Your letter of 16 Apr 2012

1. In response to your request for information that might support your investigation into the L112 stranding, we have reviewed Coast Guard operations and training exercises, as well as civilian activities, along the Oregon coast to as far north at Ledbetter Point, Washington, between 01 and 11 February 2012. We did not learn of any whale strikes or other impact to whales by Coast Guard assets or commercial vessels.

2. During that period, Coast Guard activities included search and rescue, law enforcement, aids to navigation work, and training exercises. Small boats and cutters made over 100 voyages in the area and on the Columbia River. No major cutters transited the area. There were no reported whale strikes.

3. There were 116 large vessel (over 300 gross tons) movements that arrived or departed Sector Columbia River's area of responsibility, which encompasses the Oregon Coast north to Gray's Harbor, Washington. The Coast Guard received no reports of whale strikes for this region during the time in question. Additionally, we are not aware of any explosives being used in the area during that period.

4. If we may be of further assistance with this or any other matter, please contact my Marine Protected Species Program Manager, Ms. Lori Mazzuca, at the phone number above or email at Lori.L.Mazzuca@uscg.mil.

#

Appendix F-6:
Response to Inquiry (USN), 21 March 2012

Brent Norberg - NOAA Federal <brent.norberg@noaa.gov>

Fwd: ORCA at Long Beach, WA

1 message

Jaclyn Taylor <jaclyn.taylor@noaa.gov> Wed, Mar 21, 2012 at 8:47 AM
To: Brent Norberg <Brent.Norberg@noaa.gov>, Lynne Barre <Lynne.Barre@noaa.gov>, Kristin Wilkinson <Kristin.Wilkinson@noaa.gov>
Cc: Teri Rowles <Teri.Rowles@noaa.gov>

Hi All,

Please see the email below from Frank, with Navy activities info from January 11.

Thanks,
Jackie

---------- Forwarded message ----------
From: **Stone, Frank V CIV OPNAV, N45** <frank.stone@navy.mil>
Date: Wed, Mar 21, 2012 at 10:38 AM
Subject: ORCA at Long Beach, WA
To: Jaclyn Taylor <jaclyn.taylor@noaa.gov>, Teri Rowles <Teri.Rowles@noaa.gov>

Teri/Jackie,
Have either of you been tracking the necropsy of L112 (Orac) which was found on the shore near Long Beach, WA on 11 Feb? The Navy did no sonar nor any explosives in the area as far back as 11 Jan. Please let me know what you know since there appears to be rumors that Navy sonar or explosives were responsible for the death.

Regards,
Frank

V. Frank Stone, Ph.D.
Chief of Naval Operations
Energy and Environmental Readiness Division (N45)
2000 Navy Pentagon, Room 2E258
Washington, DC 20350-2000
703-695-5271
frank.stone@navy.mil
SIPRNET: frank.stone@navy.smil.mil
http://www.navy.mil/oceans/

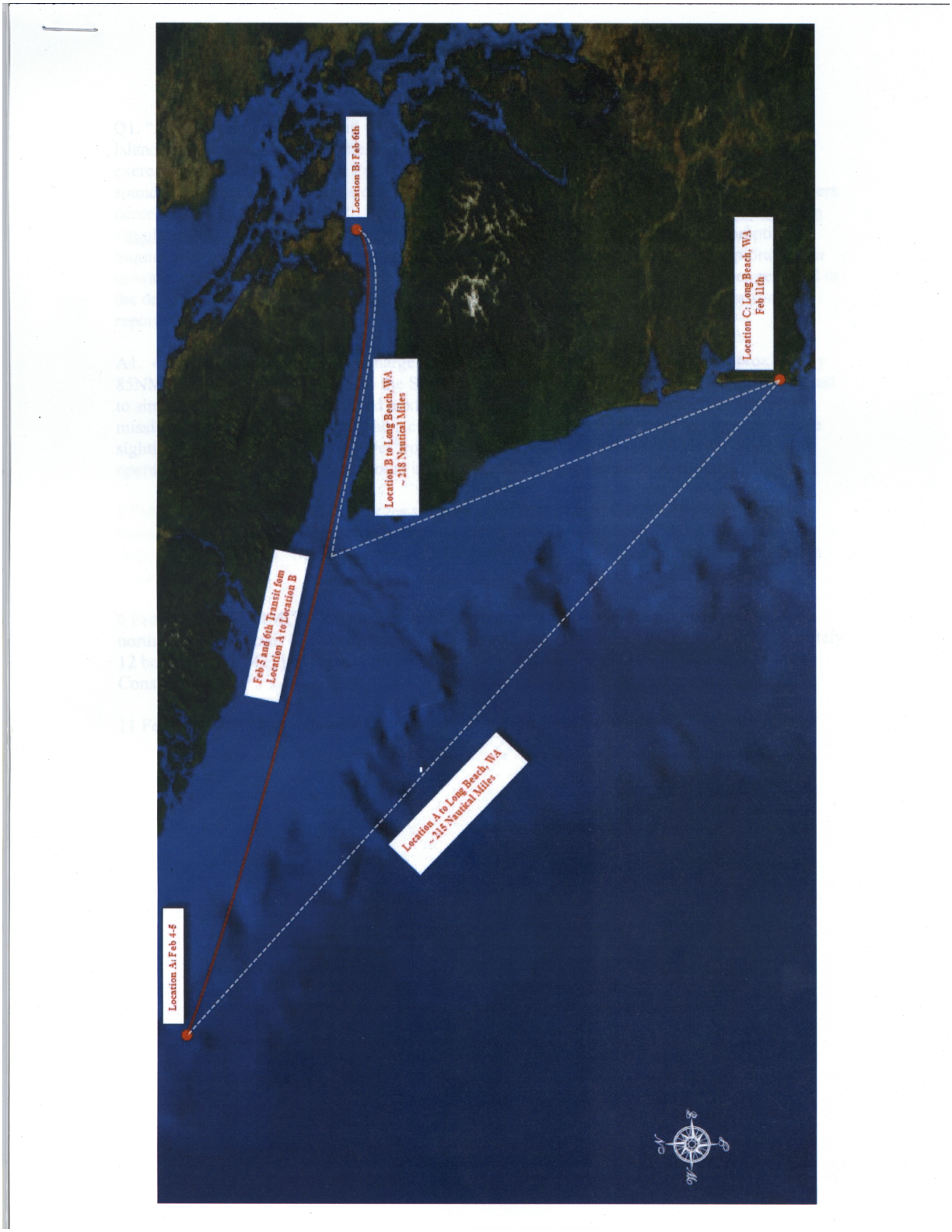
--
Jaclyn Taylor

National Marine Fisheries Service

Office of Protected Resources
1315 East-West Highway
Silver Spring, MD 20910

301-427-8480 (phone)
301-713-2525 (fax)

Appendix F-7:
Response to Inquiry (Royal Canadian Navy), 13 April 2012



Q1. "At the time of the stranding we were advised that local researchers from the San Juan Islands had detected what they believed to be military sonar signals from a Canadian Navy exercise in the Strait of Juan de Fuca on or about February 6, 2012. In addition we received sound recordings made the same day, from a hydrophone array in Haro Strait that the researchers describe as "distant explosions". Recordings were provided by Scott Veirs (sveirs@gmail.com <mailto:sveirs@gmail.com>). The timing of the events prior to the stranding is prompting us inquire whether military exercises or other civilian activities (shipping, resource exploration, or in-water construction) in the Strait or along the west coast may have occurred or been reported in the days prior to the stranding. We would appreciate your assistance in compiling any such reports."

A1. 4 February 2012: A DM211 charge was dropped next to the HMCS Ottawa approximately 85NM northwest of the entrance to the Strait of Juan de Fuca. A DM211 is a 1.4 kg charge used to simulate battle damage - dropped next to a ship and creates a small explosion to simulate missile or torpedo hit. Lookouts had a clear view of the area and there were no reported whale sightings. The kill radius for a diver from the DM211 is approximately 15 yards. Sonar was operated for approximately eight hours in this general location (location A on map).

5 February 2012: Two DM211 charges were dropped next to the HMCS Ottawa, one in the morning and one in the late afternoon approximately 90NM northwest of the entrance to the Strait of Juan de Fuca. Sonar was operated for approximately 11 hours in this general location (location A on map).

6 February 2012: HMCS Ottawa used sonar during the transit from approximately 90NM northwest of the Strait of Juan de Fuca to Constance Bank (location B on map) for approximately 12 hours. Two DM211 charges were dropped next to the HMCS Ottawa in the morning near Constance Bank.

11 Feb 2012: L112 strands near Long Beach, WA (location C on map).

Appendix F-8:
Response to Inquiry (FVOA), 26 April 2012

FISHING VESSEL OWNERS' ASSOCIATION
INCORPORATED

4005 20TH AVE. W., ROOM 232
SEATTLE, WASHINGTON 98199-1290
PHONE (206) 284-4720 • FAX (206) 283-3341

SINCE 1914

April 26, 2012

Lynne Barre, Chief
Puget Sound Ecosystem
And Marine Mammals Branch
NOAA/NMFS – NW Region
7600 Sand Point Way N.E., Bldg. 1
Seattle, WA 98115

Dear Chief Barre:

I am responding to your letter of April 18, 2012 regarding the death of a killer whale which may have occurred in early February 2012 off the Columbia River. The Fishing Vessel Owners' Association (FVOA) is a trade association of family-owned longline vessels that target sablefish and halibut. Most of our operations are off Alaska; however, we have a number of vessels that target sablefish off of Washington, Oregon and California.

Our vessels are smaller, between 50 to 85 feet in length and crew three to six persons. Our fleet, due to weather does not start their operations off the lower coast until March or April. The speed of our vessels is between seven to twelve knots. We do not have any mothership operations like you might have with the whiting fishery.

Based on your description of the whale, I do not think our fleet members would have been fishing during early February and killer whales are so agile and mobile, I cannot imagine one of our vessels killing one while traveling.

I will offer one concern for our fleet that may play a part in the death of the killer whale. Much of the commercial fishing fleet that operate from California to Washington are constantly at risk to freighter traffic that does not use the proper shipping lanes going north or south between Canada and Los Angeles. There have been occurrences with foreign freighters that have run down or nearly run down vessels in our organization over the years. Recently, the F/V Lady Cecelia, a trawler based out of Warrenton, Oregon, sank off Leadbetter Point near Willapa Bay. The sinking was suspicious to many in our industry as trawl vessels do not typically role over so fast that the crew does not have time to get to a life raft. There is a suspicion in the fishing community that the Lady Cecelia may have been run down.

LATITUDE: 47° 39' 36" NORTH
LONGITUDE: 120° 22' 58" WEST

WEB PAGE
WWW.FVOA.ORG

The killer whale that you are concerned about may have been run down by freighter traffic as well. Freighter traffic that tries to save time and distance from Juan de Fuca to Los Angeles by turning southbound too early have been a safety problem for over 30 years that I have been managing our Association.

Sincerely,

Robert D. Alverson
Manager

RDA:cmb

Recent NOAA Technical Memorandums

published by the

Northwest Fisheries Science Center

NOAA Technical Memorandum NMFS-NWFSC-

132 Anderson, L. E., and M. Plummer. 2016. Puget Sound recreational shellfishing survey: Methodology and results. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-132, 38 p. doi:10.7289/V5/TM-NWFSC-132.

131 Waples, R. S. 2016. Small investments with big payoffs: A decade of the Internal Grants Program at the Northwest Fisheries Science Center. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-131, 80 p. doi:10.7289/V5/TM-NWFSC-131.

130 Puget Sound Recovery Implementation Technical Team. 2015. Puget Sound Chinook salmon recovery: A framework for the development of monitoring and adaptive management plans. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-130, 146 p. NTIS number PB2016-100691. doi:10.7289/V5/TM-NWFSC-130.

129 Hard, J. J., J. M. Myers, E. J. Connor, R. A. Hayman, R. G. Kope, G. Lucchetti, A. R. Marshall, G. R. Pess, and B. E. Thompson. 2015. Viability criteria for steelhead within the Puget Sound distinct population segment. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-129, 332 p. NTIS number PB2015-105188. doi:10.7289/V5/TM-NWFSC-129.

128 Myers, J. M., J. J. Hard, E. J. Connor, R. A. Hayman, R. G. Kope, G. Lucchetti, A. R. Marshall, G. R. Pess, and B. E. Thompson. 2015. Identifying historical populations of steelhead within the Puget Sound distinct population segment. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-128, 155 p. NTIS number PB2015-103741. doi:10.7289/V5/TM-NWFSC-128.

127 Roni, P., G. R. Pess, T. J. Beechie, and K. M. Hanson. 2014. Fish–habitat relationships and the effectiveness of habitat restoration. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-127, 154 p. NTIS number PB2014-108836.

126 Russell, S., and M. S. Ruff. 2014. The U.S. whale watching industry of Greater Puget Sound: A description and baseline analysis. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-126, 171 p. NTIS number PB2014-105939.

125 Sloan, C. A., B. F. Anulacion, K. A. Baugh, J. L. Bolton, D. Boyd, R. H. Boyer, D. G. Burrows, D. P. Herman, R. W. Pearce, and G. M. Ylitalo. 2014. Northwest Fisheries Science Center's analyses of tissue, sediment, and water samples for organic contaminants by gas chromatography/mass spectrometry and analyses of tissue for lipid classes by thin layer chromatography/flame ionization detection. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-125, 61 p. NTIS number PB2014-104055.

124 Anderson, L. E., and S. T. Lee. 2013. Washington and Oregon saltwater sportfishing surveys: Methodology and results. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-124, 61 p. NTIS number PB2014-101405.

NOAA Technical Memorandums NMFS-NWFSC are available at the Northwest Fisheries Science Center website, https://www.nwfsc.noaa.gov/index.cfm.