Uncommon Stranding Event of Bottlenose Dolphins (*Tursiops truncatus*) in San Diego, California (October 2015)

Kerri Danil[1], Nicole Beaulieu-McCoy[1], Sophie Dennison[2], David Rotstein[3], Teri Rowles[4], Sarah Wilkin[4]

[1]Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, La Jolla, CA
[2]Marine Mammalogy Radiology, Oakton, Virginia
[3]Marine Mammal Pathology Services, Olney, Maryland
[4]National Marine Fisheries Service, Office of Protected Resources, Silver Spring, Maryland

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Executive Summary

Two freshly dead, adult coastal common bottlenose dolphins (*Tursiops truncatus*), stranded one mile apart from each other on Silver Strand State Beach in Coronado, San Diego County, California on October 21, 2015. On this same day, the U.S. Department of the Navy began a major training exercise (MTE). Ten days later, an additional adult *T. truncatus* stranded dead in advanced decomposition along the same beach. The spatial and temporal similarities of the strandings represented an unusual occurrence for the region.

Post-mortem examinations revealed that all three dolphins were in robust body condition, had no external signs of fishery interaction, and presented with subcutaneous hemorrhage in the head region concentrated along the lower jaws, with subcutaneous emphysema associated with areas of cervical blubber hemorrhage. Hemoabdomen, gas bubbles within the mesenteric and cerebral vasculature, and evidence of recent feeding were observed in both dolphins that were examined in greatest detail. Additionally, cerebral hemorrhage and hemopericardium was observed in one specimen and scant lipids in the lungs of another. The combined findings indicate that the dolphins were exposed to acute, severe trauma, likely anthropogenic in nature.

Based on previous case studies, an underwater detonation, peracute underwater entrapment (PUE) (e.g. net entanglement) or mid-frequency active (MFA) sonar are the most plausible explanations for the observed trauma. However, no underwater detonations were reported as part of the MTE. Three coastal fisheries that target squid, bait, and lobster operate in October that could potentially entrap a coastal *T. truncatus*. However, no squid fishery vessels were operating off San Diego during this time, no bait fishing activity the day preceding the strandings occurred, and lobster pots are unlikely to entrap 3 dolphins at one time. An illegal U.S. fishery or nearshore Mexican fishery could be an alternate source of PUE. MFA sonar was in use on October 19 and 20, with the closest Navy ship within 6 NM of the stranding location. Although MFA sonar seems the most likely source of the observed trauma, PUE from an unidentified fishery or unknown source remain a possibility.

1. Introduction

Coincident with a U.S. Department of the Navy (hereafter referred to as "the Navy") major training exercise (MTE), two common bottlenose dolphins (*Tursiops truncatus*) stranded dead within 1 mile of each other in Coronado, San Diego County, California on October 21, 2015. These strandings met the criteria of an Uncommon Stranding Event (USE): two or more individuals of any cetacean species found alive or dead within a two day period and within 10 miles of one another. The designation of a USE initiated communication between the National Marine Fisheries Service and the Navy. An investigation was launched to consider all plausible causes of the stranding event as outlined in Southern California Stranding Response Plan (Appendix 1), part of the Navy's Letter of Authorization to take marine mammals during the Navy's training activities off southern California (78 FR 78105). The carcasses were refrigerated overnight and necropsies performed the following day at the Southwest Fisheries Science Center in La Jolla, California. Ten days after the initial two *T. truncatus* strandings, an additional *T. truncatus* stranded in the same general area (Figure 1), in a greater state of decomposition. Post-mortem findings of all three dolphins are reported here and possible explanations for their cause of death are discussed.

2. Background

Two *T. truncatus* stocks are recognized along the west coast of the United States: a coastal population of approximately 450-500 individuals that are found within 1 kilometer of shore, and an offshore population of roughly 1000 individuals that are typically found in waters greater than a few kilometers from shore (Carretta et al., 2020; Defran et al., 1999; Lowther-Thieleking et al., 2015). The coastal population is known to move back and forth along the west coast, primarily from Point Conception to San Quintin, Mexico, with little site fidelity (Defran and Weller, 1999; Defran et al., 1999; Feinholz, 1996). In southern California, they are generally within 500 m of shore (Hanson and Defran, 1993) and travel in schools of approximately 20 individuals (Defran and Weller, 1999). Disease outbreaks and anthropogenic threats such as pollution, fisheries, military training activities, and ship strikes put both populations at risk due to their small size. For the coastal and offshore populations, the potential biological removal (PBR) level is 2.7 and 11 dolphins per year, respectively (Carretta et al., 2020). PBR is defined as the maximum number of animals that can be removed from a stock (not including natural mortalities) that allows the population to reach or maintain its optimal sustainable population.

Over the 35 years prior to this stranding event, on average, 3.3 *T. truncatus* stranded per year in San Diego County. Ninety nine percent of San Diego County *T. truncatus* strandings that have been analyzed (n=132) have been genetically identified as the coastal ecotype. No large scale disease outbreaks or impacts from military training exercises have been recorded for the coastal stock. Since 2003, peracute underwater entrapment (PUE) has been documented in four California coastal *T. truncatus*: one entrapment in the net curtain of a dolphin pen enclosure inside a bay, two entangled with rope of unknown origin, and one attributed to a fishery using 3.5 inch mesh gillnet, likely a set or drift gillnet targeting yellowtail, white seabass, or barracuda (Carretta et al., 2020). All of these dolphins had impressions or lacerations associated with the

PUE. Prior to the event described in this document there has only been one other group stranding event for this species in San Diego. In 1963, two adult female *T. truncatus* stranded alive and subsequently died for unknown reasons (Danil et al., 2010). The three *T. truncatus* that stranded with spatial and temporal similarities during the event to be described here truly represent an unusual occurrence for this region.

All three strandings described in this report were found within the Silver Strand Training Complex (SSTC), which is used by the Navy for amphibious, special warfare, and mine countermeasure training activities. The SSTC is located on and adjacent to the Silver Strand, a narrow isthmus separating San Diego Bay from the Pacific Ocean (U.S Department of the Navy 2011). In 2011, a school of long-beaked common dolphins (*Delphinus capensis*) were inadvertently exposed to an underwater detonation in this area during mine countermeasure training activities, which resulted in the death of at least three dolphins (Danil and St. Leger, 2011).

3. Overview of Stranding Event

A dead adult male *T. truncatus* (NEB0075) was reported in Coronado (Figure 1) on October 21, 2015 at 10:20 am by the Navy Region Southwest. The animal appeared to be in fresh condition from the photos that were sent by the reporting party. However, upon arrival at the lab, the genital slit had begun to swell, indicating a transition into an early decomposition code 3 (moderate). The dolphin was placed in a refrigerator at 1300 until the necropsy on the following day.

At 1300 on the same day, Imperial Beach lifeguards reported a dead adult female *T. truncatus* (KXD0280) 1 mile away from the stranding location of NEB0075 (Figure 1). When it was first reported, it was thought that this was a duplicate report of NEB0075 due to spatial and temporal similarities, which delayed initial response by the stranding team. Specimen KXD0280 was picked up at 1800 and placed in a refrigerator at 1900 until necropsy the following day. Although the animal appeared to be in fresh condition in photos taken by the Imperial Beach lifeguards at 1240 (Figure 2), it was a decomposition code 3 (moderate) at the time it was taken off the beach.

Ten days later, on October 31, 2015, a dead adult male *T. truncatus* (KXD0281) in advanced decomposition was reported in Coronado (Figure 1) by Naval Amphibious Base personnel. A partial necropsy was performed at the stranding site.

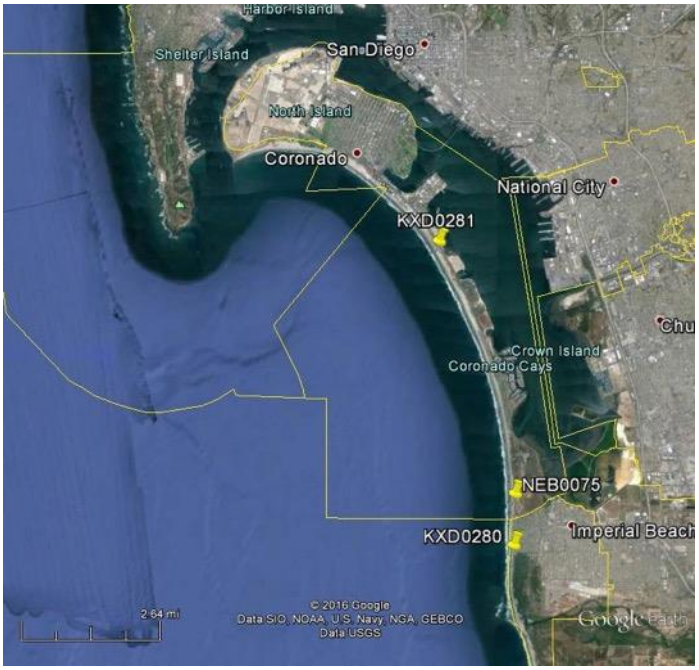

Figure 1. Stranding locations of *T. truncatus* specimens, NEB0075, KXD0280, and KXD0281, along Silver Strand Beach. Yellow lines indicate city boundaries.

Figure 2. Initial photograph of specimen KXD0280 taken by lifeguards.

4. Overview of Known Anthropogenic Activities in the Area

4.1 US Navy Activities

On October 22, 2015 the Navy notified National Marine Fisheries Service (NMFS) that a Major Training Exercise (MTE) began on October 21, 2015. In response to NMFS's Uncommon Stranding Event (USE) notification, the US Navy queried explosive and sonar activities within 72 hours and 80 NM of the strandings. No explosives were reported to have been used in the SSTC area. Two ships used mid-frequency active (MFA) sonar within 80 nm during unit level training for four periods of time: 35 minutes on October 19th and 31 minutes, 22 minutes, and 9 minutes on October 20th, with the closest ship within 6 NM of the stranding location (Department of the Navy, 2017).

4.2 Fishing Activities

Both squid and bait purse seine fisheries are known to operate in nearshore southern California waters and both have had minimal observed marine mammal interactions. The squid purse seine fishery typically operates from October to March, primarily at night, on average using 1.2 inch mesh, 27.5 fathoms deep, with a soak time just over an hour. A pilot observer program from July 2004 to January 2006 documented one unidentified common dolphin (*Delphinus* sp.) death in 135 squid fishery sets (Carretta et al., 2020). No squid fishery vessels were operating offshore San Diego County from October 19-21, 2015 (K. Lynn, California Department of Fish and Wildlife, personal communication, February 26, 2016).

The bait fishery operates year round, using 11/16 inch to ¾ inch mesh size for capturing northern anchovy (*Engraulis mordax*) (Knaggs, 1972). The pilot observer program documented one California sea lion (*Zalophus californianus*) death in 93 observed bait fishery sets. Only one bait company operates fishing vessels along San Diego and they were fishing in the nearshore waters of the Coronado and Imperial Beach areas October 19th and 21st, 2015. However, the company reports that no dolphin interactions occurred during these seining events. A coastal pot fishery for California spiny lobster (*Panulirus interruptus*) operates off San Diego but it would be highly unlikely to ensnare multiple marine mammals at one time in this type of fishery. All other U.S. fisheries operate 3 miles or more offshore, outside of the range of coastal *T. truncatus*. It is possible that coastal Mexican fisheries operate along the U.S/Mexico border.

5. Methods

Necropsies were conducted according to established protocols (Pugliares et al., 2007). A gas score (scale = 0 to 6), used to describe the extent of intravenous gas bubbles (Bernaldo de Quirós et al., 2013), was assigned to areas of the body where gas accumulations were noted. A full suite of tissues, collected for histopathology, were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Additionally, Oil Red O stain was used on formalin-fixed brain, lung, and lymph node for detecting fat emboli. Computed tomography (CT) scans of entire carcasses were not possible due to the weight limit of the available CT scanner. Instead, the heads of the freshest animals, NEB0075 and KXD0280 were submitted for scans. Fecal samples were tested for domoic acid and saxitoxin via ELISA as described by Lefebvre et al. (2016). Morbillivirus PCR tests were performed using the universal

morbillivirus primers directed against the phosphoprotein (P) gene (Barrett et al., 1993) followed by nested primers specific for morbillivirus. Genetic differentiation of coastal vs. offshore ecotypes of *T. truncatus* was determined as described in Lowther-Thieleking (2015).

6. Post-Mortem Examinations

6.1 Gross Observations

6.1.1 NEB0075

The necropsy examination of a 295 cm long, 271.5 kg adult male *T. truncatus* commenced at approximately 0830 on 22 October 2015. The dolphin was robust and the body coloration was dark grey, with no obvious cape (Figure 3). The skin had begun to wrinkle, creating fine linear creases in various areas. Numerous healed and unhealed rake marks were present across the body. Transverse linear creases in the throat region were present, common in many *T. truncatus*. Lingual papillae were present but much reduced. A 2 cm wide bruise was present at the dorsal insertion point of the left pectoral fin and upon incision hemorrhage in the blubber was apparent. The distal portion of the left pectoral fin was absent and completely healed. The genital slit was swollen with the tip of the penis protruding. There were three 1 – 2 cm healed notches in the dorsal peduncle. Healed notch and rake marks were present on the flukes.

Figure 3. Lateral view of NEB0075.

Severe hemorrhage in the blubber extended circumferentially (both dorsal and ventral aspects) from the rostrum to the cervical region, dissipating caudally and ending in the mid-thoracic region (Figure 4). There was extensive hematoma formation in the muscle surrounding the skull, concentrated along the muscles of the lower jaws (Figure 5). Extensive subcutaneous emphysema (0.2 cm thick) was present in the dorsal superficial fascia just posterior to the head (Figure 5), extending laterally to the deep fascia underneath the left scapula (Figure 6). A 6 cm x 0.75 cm stingray barb was embedded in the left dorsal lateral muscle anterior to the dorsal fin, lying longitudinally with the barb apex directed caudally. There was associated hemorrhage in the surrounding muscle (12 cm x 24 cm area) but there was no associated scar in the dermis or perforation of organs found (Figure 6).

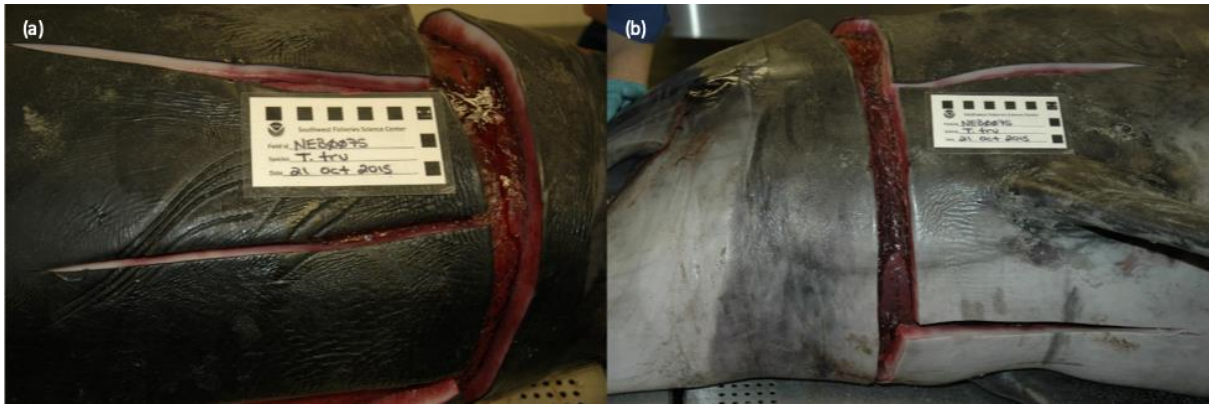

Figure 4. Subcutaneous hemorrhage in the dorsal (a) and ventral (b) aspects of blubber of NEB0075.

Figure 5. Severe diffuse hematoma along the muscles of the lower jaw (a) and subcutaneous emphysema in the dorsal superficial fascia (b) of specimen NEB0075.

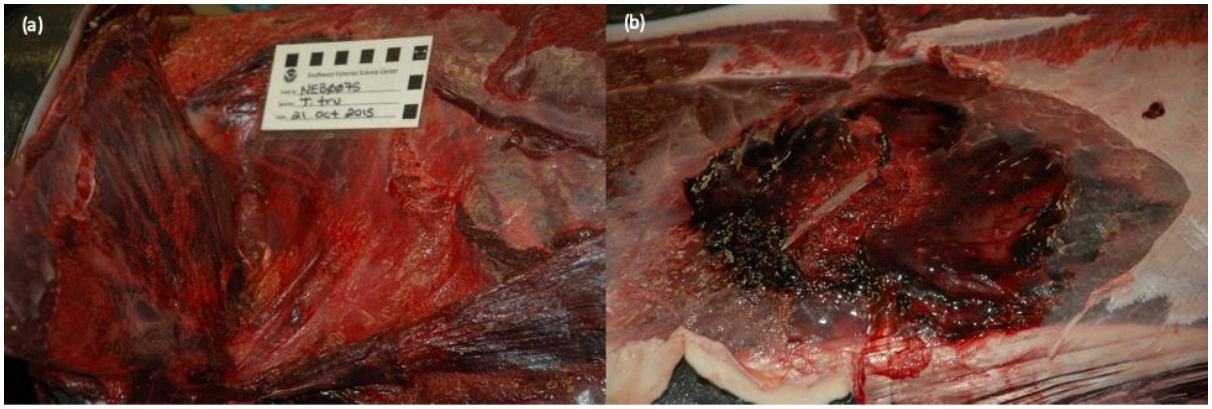

Figure 6. Subcutaneous emphysema in the deep fascia underneath the scapula (a) and an embedded stingray barb (b), in specimen NEB0075.

There were thin hair-like worms present in the trachea. Light raised areas were diffusely spread across the lungs and hard nodules (0.4 cm in diameter) were diffusely scattered across right lung. A 2 cm mass was present on a lymph node associated with the right lung. There was 140 ml of blood present in the pericardial sac. The apex of the heart was dark red and this coloration continued approximately two centimeters into the parenchyma upon incision. The epicardium in

this area was gelatinous in texture (Figure 7). There were two centrally located 0.5 cm focal circular areas of dark red tissue in the spleen (Figure 8).

Figure 7. Dark discoloration at apex of heart with associated gelatinous texture, dorsal (a) and ventral (b) aspects, specimen NEB0075.

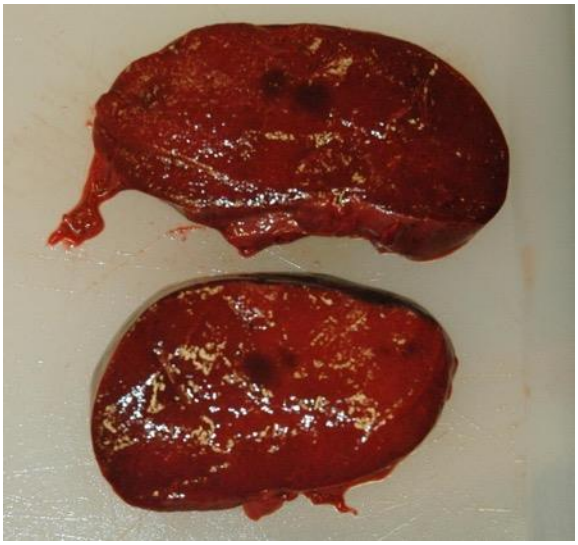

Figure 8. Focal round dark areas in spleen of specimen NEB0075.

Nematodes, likely *Anisakis* spp., were present in the esophagus. One liter of blood was present in the abdominal cavity. The forestomach contained digested prey items and a low nematode load. Three gastric ulcers, approximately 4 cm in diameter, were present. No perforations of the stomach were observed. There were a few bubbles with small discontinuities of blood flow in the mesenteric vasculature (gas score 2). Feces were watery and red-tinged. Brain was dark pink with gas bubbles within the cerebral vasculature that created large discontinuities in blood flow within some veins, filling some sections completely with gas (gas score 4) (Figure 9).

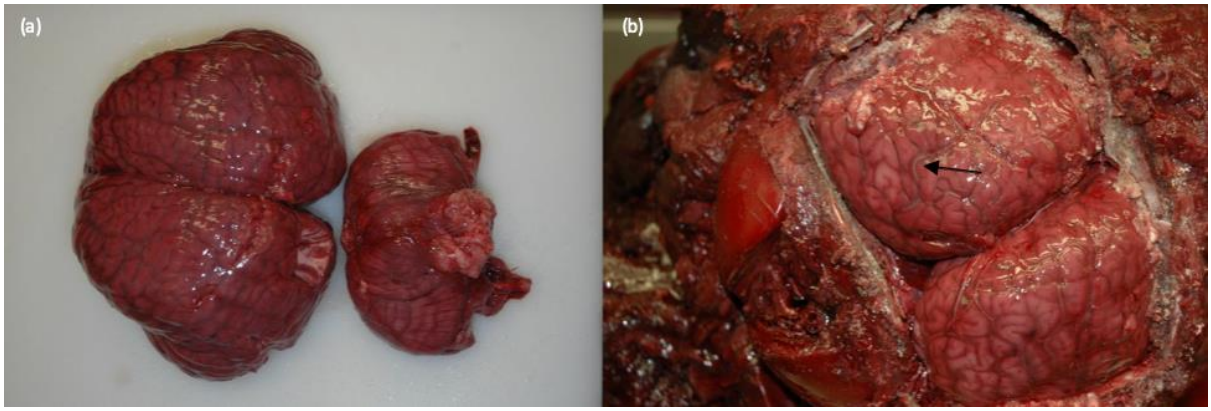

Figure 9. The brain of specimen NEB0075 was dark pink (a) with gas bubbles (arrow) within the vasculature (b).

Significant gross findings from this adult male *T. truncatus* included hemoabdomen, hemopericardium, subcutaneous hemorrhage and emphysema in the head region. The embedded stingray barb was likely incidental.

6.1.2 KXD0280

The post-mortem examination of a robust 269.4 cm, 193 kg female *T. truncatus* commenced at approximately 1200 on October 22, 2015 (Figure 10). Signs of scavenging were apparent on the ventrum and overall the skin was beginning to slough and wrinkle. The distal tip (1 cm) of the pectoral fin was exposed. The urogenital openings were swollen.

Figure 10. Lateral view of specimen KXD0280.

There was 1 – 1.5 milliliters of blood within the globe of the eye (Figure 11). Upon incision of the left pinna, ecchymoses of the blubber was apparent. Petechiae and echymoses in the cervical and melon blubber were present with underlying low to moderate subcutaneous emphysema (Figure 12). Diffusely, an expansive hematoma was present in the subcutaneous space, circumferentially extending from the rostrum to the cervical region, concentrated along the muscles and blubber associated with both lower jaws (Figure 13).

Figure 11. Free blood within the globe of the eye of specimen KXD0280.

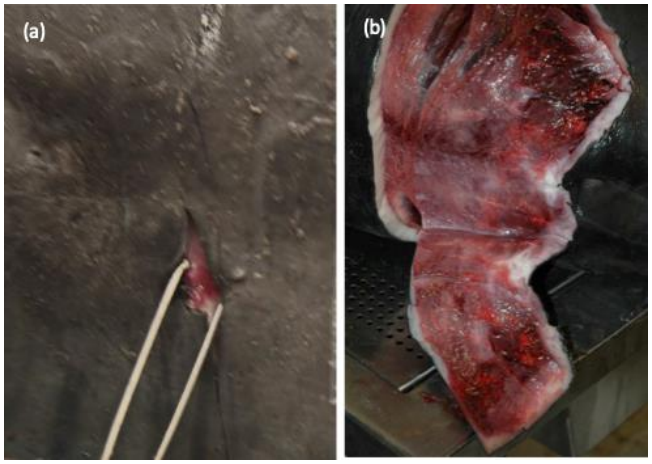

Figure 12. Echymoses of the blubber at the left pinna (a) and hemorrhage in the cervical blubber with associated subcutaneous emphysema (b), in specimen KXD0280.

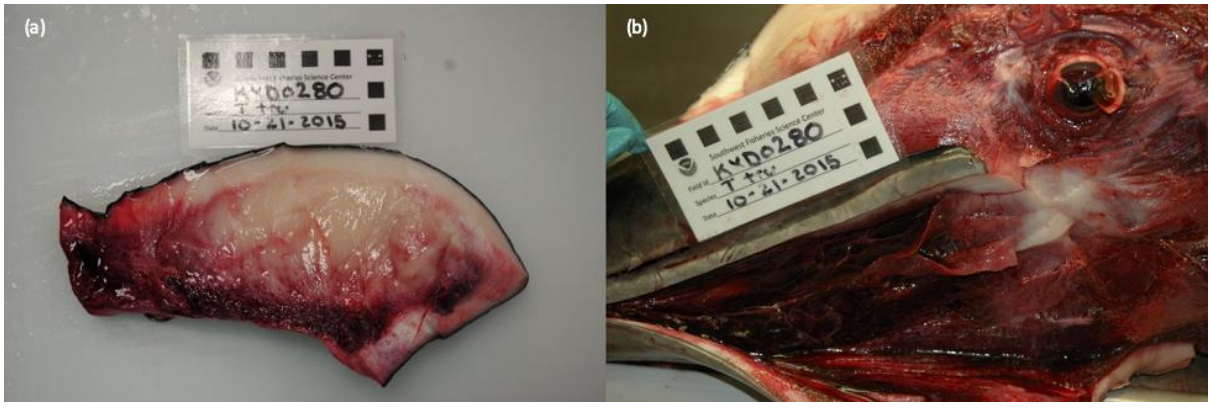

Figure 13. Echymoses and petechiae in the melon fat (a) and hematoma in the lower jaw muscles (b) of specimen KXD0280.

Froth and bloody fluid were present in the trachea. There were calcareous hard nodules throughout both lumpy, dense, dark, and pink lungs. Two otoliths were observed in the anterior

region of the right main bronchus. Congestion or hemorrhage was concentrated on the right side of the heart where the tissue throughout was saturated with blood (Figure 14).

Figure 14. The right ventricle of specimen KXD0280's heart was saturated with blood.

There was 1.5 L of blood in the abdominal cavity. A focal darkened region within the parenchyma of the spleen was apparent. The left adrenal was enlarged compared to the right adrenal (24.1 g vs. 9.3 g) and a focal dark area on the cranial aspect was present. Areas of congestion or hemorrhage were present on both the cranial and caudal aspects of the right adrenal (Figure 15).

Figure 15. Left (a) and right adrenal (b) of specimen KXD0280.

The forestomach contained 305 g of digested stomach contents and 4 ulcers. A red viscous fluid was present in the main stomach. Intestinal fluid was watery and pink in color. Feces were mustard yellow in color. The right liver lobe was almost twice the size of the left side and was very firm. The left liver lobe was lumpy and dimpled. Liver parenchyma appeared normal. Dark discoloration along the edges of the pancreas that extend into the parenchyma was

observed and overall the tissue was friable. There was a 2 cm region of dark black viscous material lining the left kidney, which was slightly friable overall. The right kidney was friable with a dark discoloration on the outer capsule and there was pooled dark viscous material, possibly blood, in the capsule (Figure 16). The bladder was empty. A moderate amount of superficial hemorrhage was present in the caudomedial aspect of both hemispheres of the cerebrum. There were gas bubbles within the cerebral vasculature that created large discontinuities in blood flow within some veins, filling some sections completely with gas (gas score 4) (Figure 17). There was a mucus plug in the cervix and two corpora albicantia were present on the left ovary, indicating that this dolphin was sexually mature.

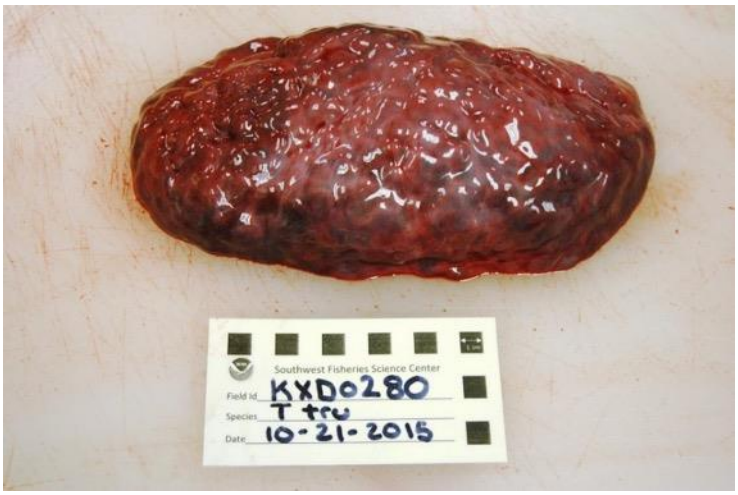

Figure 16. Dark viscous material lining the kidney of specimen KXD0280.

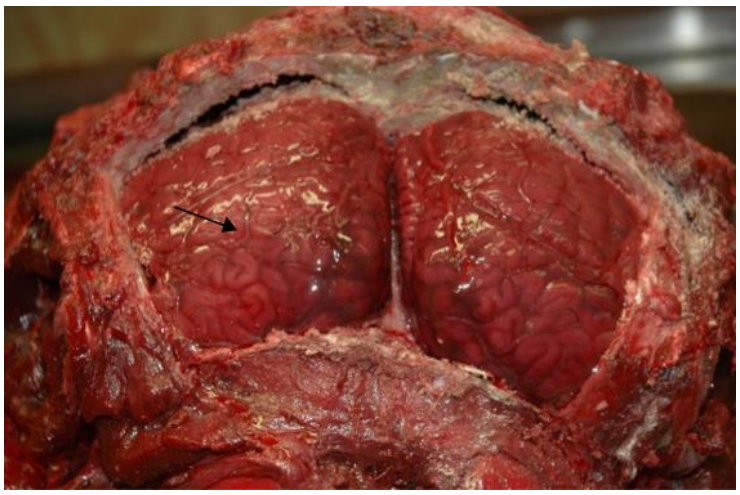
Figure 17. Superficial hemorrhage in the medial aspect of both hemispheres of the cerebrum and gas bubbles within the vasculature (arrow) of specimen KXD0280.

Significant gross findings from this adult female *T. truncatus* included hemoabdomen, subcutaneous hemorrhage and emphysema in the head region, unilateral (left) adrenal enlargement, and petechiae/ecchymoses in the melon blubber.

6.1.3 KXD0281

This robust male *T. truncatus*, 307.2 cm in length, was examined on the beach on 31 October 2015. Approximately 75% of the skin was missing and the carcass was very oily. The body was bloated, with the penis and eyes protruding. The phalanges of the right pectoral fin were exposed and the teeth were loose in the jaw from decomposition (Figure 18).

Figure 18. Lateral view of specimen KXD0281.

Patchy circumferential hemorrhage in the cervical blubber was apparent, focused bilaterally and ventrally, with associated hemorrhage in the underlying muscles (Figure 19). Hemorrhage in the muscles and blubber associated with the lower jaws was also present. All organs were intact but extremely friable. The liver was discolored with black splotches that extended superficially into the parenchyma. A diffuse dark red discoloration was observed on the serosal surface of both emphysematous kidneys (Figure 20). Brown liquid digesta was present inside the forestomach. The right testis was approximately 200 g and 215 mm long. Significant gross findings from this adult male *T. truncatus* included subcutaneous hemorrhage in the head region.

Figure 19. Circumferential hemorrhage in the cervical blubber of specimen KXD0281.

Figure 20. Gross observations of the muscles and blubber associated with the lower jaw (a, b), liver (c), and kidney (d) of specimen KXD0281.

6.2 Computed Tomography (CT)

6.2.1 NEB0075

6.2.1.1 Imaging Findings

Multifocal and extensive gas accumulation was present, consistent with the decapitated status of the head being scanned. Several mandibular teeth were absent with lucent sockets, confirmed to originate from post-mortem extraction. No evidence of skull fractures were identified. The tympanoperiotic complexes were intact.

There was mildly increased soft tissue within the peribullous sinuses bilaterally. Some fluid/soft tissue attenuating material was present bilaterally within the pterygoid sinuses with greater volume on the left than the right. The pterygoid bones were normal in appearance. There was soft tissue swelling ventral to the tongue/mandible observed with altered attenuation of the ventral blubber layer at this level on midline. Both eyes were in situ and are within normal limits. A large dorsal to dorsolateral cutaneous laceration was present over the calvarium without changes to the underlying bone. The laceration was very linear and is most likely post-mortem.

6.2.1.2 Diagnostic Interpretation

Cause of death has not been determined. Ventral oral cavity/rostrum swelling was present. Differential diagnoses include: bruising, edema, cellulitis. Mild peribullous and pterygoid sinusitis, likely within normal limits for the free-ranging status of the dolphin.

6.2.1.3 Imaging Conclusions and Comments

A specific cause of death has not been determined from the imaging study. The significance of the swelling observed ventral to the tongue and mandible is of uncertain significance and determination of its occurrence pre- or peri-stranding was challenging from imaging alone.

6.2.2 KXD0280

6.2.2.1 Imaging Findings

The head has been removed from the body and multifocal and extensive gas accumulations consistent with this were present. Several teeth were absent from the mandible with lucent sockets, confirmed to be from post-mortem extraction. There was no evidence of skull fracture observed. There was increased soft tissue/fluid accumulation within the pterygoid sinuses bilaterally and within the peribullous sinuses that was mild. The pterygoid bones were normal in appearance. The tympanoperiotic complexes were normal.

6.2.2.2 Diagnostic Interpretation

No cause of death was identified from the imaging study. Mild bilateral pterygoid and peribullous sinusitis, likely within normal limits for the free-ranging status.

6.2.2.3 Imaging Conclusions and Comments

Mild bilateral pterygoid and peribullous sinusitis was found but was considered very mild and well within normal limits for free-ranging dolphins, based on personal experience.

6.3 Histopathology

6.3.1 NEB0075

In the lung there was multifocal alveolar flooding with flocculent eosinophilic proteinaceous substances (Figure 21). There was multifocal mild to occasionally moderate interstitial fibrosis and occasional alveolar hyperinflation. In the lung, there were small deposits of Oil Red O-positive staining that correspond to the flocculent fluid within the alveoli and within macrophages within alveolar lumina. The lipid was attributed to cellular product (cholesterols) from pneumocytes that were freely within alveoli and within macrophages.

There was moderate congestion in the spleen. Splenic periarteriolar lymphoid sheaths were moderately cellular. Lymphocytes in the lymph node were present in moderate numbers. Lymphoid follicles were well-formed.

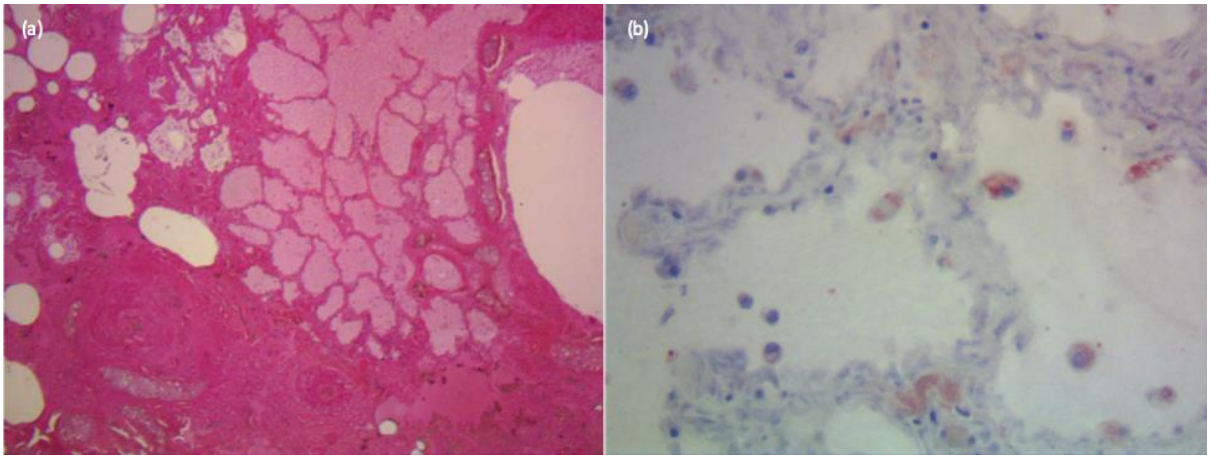

Figure 21. Occasional clusters of alveoli were flooded with foamy proteinaceous substance (a) and Oil Red O-positive stain (lipid) was found in a few alveoli and within alveolar lumina (b).

There was regionally extensive moderate mucosal epithelial hyperplasia of the forestomach. There was focal umbilication of the mucosal epithelium. The submucosa was multifocally infiltrated by lymphocytes, plasma cells, and macrophages forming nodular aggregates (Figure 22).

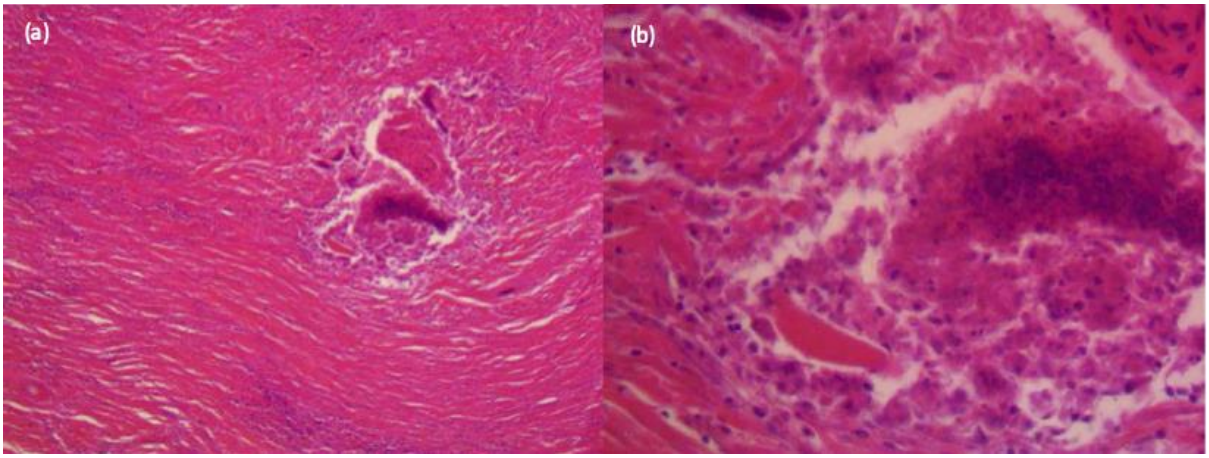

Figure 22. Hyperplasia in the gastric mucosa (a) and lymphoplasmacytic and histiocytic nodular aggregates in the submucosa (b).

Epididymal ducts were filled with abundant spermatazoa within a lightly eosinophilic proteinaceous substance. Testicular seminiferous tubules were lined by plump germ cells. There were few to occasionally moderate spermatagonia and few spermatazoa. The interstitium was prominent.

Fourteen sections of spinal cord and brain were examined. There was distention of myelin sheaths primarily within the ventral tracts (artifact, presumptive). In the brain, there was multifocal perivascular clearing with no deposition of protein or inflammatory cells.

Occasionally, there was infrequent disruption of the neuropil by clear spaces without associated hemorrhage or inflammation (Figure 23). Scant lacey proteinaceous substance was present within occasional spaces.

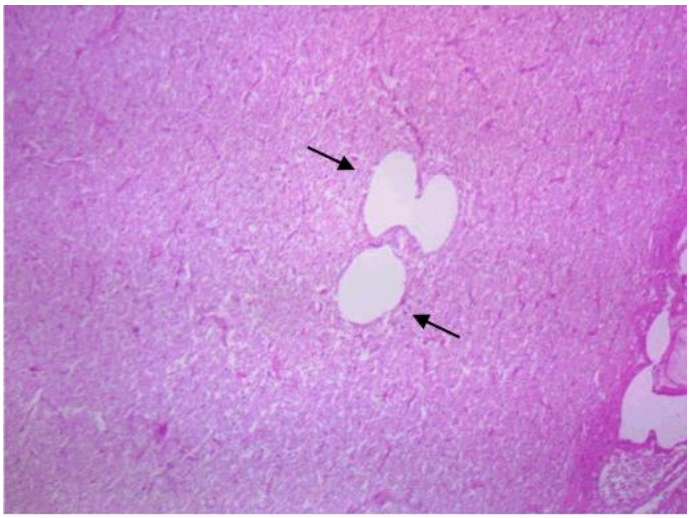

Figure 23. Although rare, clear spaces (arrows) that expanded the neuropil in the brain were observed.

There was accumulation of serum within the chamber of the eye. In the masseter muscle, there was separation of myofibers by eosinophilic granular substance (edema) (Figure 24). Eosinophilic proteinaceous fluid was present within the blubber. Oil Red O stains were applied to brain and lymph node; there was no evidence of lipid within blood vessels or within the parenchyma of these tissues.

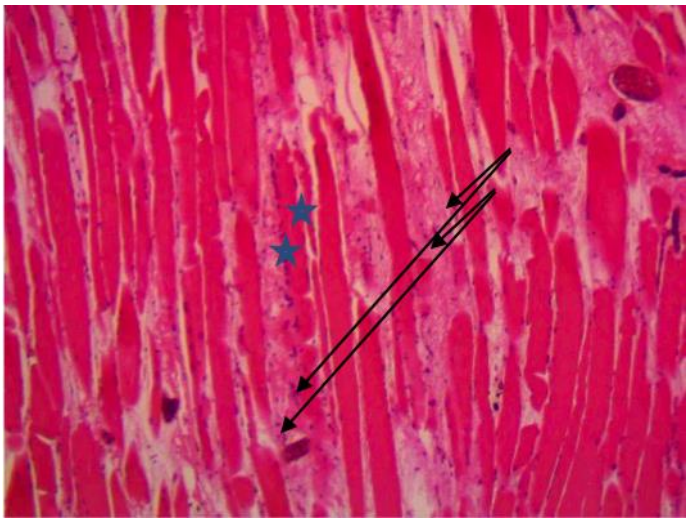

Figure 24. In the masseter muscle (star), the space between myofibers (interstitium) is expanded by lightly eosinophilic granular fluid (edema), indicated by arrows.

No significant histological findings were found in the following tissues: skin, intestine, penis, trachea, tongue, urinary bladder, skeletal muscle, adrenal gland, pancreas, heart, pituitary gland, main stomach, kidney, diaphragm, and liver.

6.3.1.1 NEB0075 Histopathology Conclusions

The cause of stranding of this cetacean was not determined with histopathological evaluation. Lesions in the respiratory, digestive, and musculoskeletal system were incidental to mild. Pulmonary fibrosis was mild and non-specific as this is a chronic change. Prior endoparasitism,

bacterial, fungal, or viral infection could have previously occurred resulting in the fibrosis (scarring). No infectious agents were observed. Gastritis was parasite associated and was not beyond levels observed in other cetaceans finding.

6.3.2 KXD0280 Histopathology

There was multifocal flooding of alveoli with lightly eosinophilic proteinaceous fluid. There was multifocal interstitial fibrosis. There were occasional foci of bronchiolar collapse and fibrosis leaving small islands of cartilage (Figure 25). The bone marrow was 70% cellular. Megakaryocytes were present at 2 to 4 per 100X field. Myeloid and erythroid cells exhibited eosinophilic pallor with diminished morphologic features. Myeloid cells were present in all stages of storage and maturation. Erythroid cells were present in all stages. The M:E ratio was 3:1. Tonsillar lymphoid tissue was moderately cellular. There was a single gland that is moderately dilated and contained a concretion of clumped to lamellar basophilic mineral. Lymph node was moderately cellular with loosely formed follicles. Splenic periarteriolar lymphoid sheaths were moderately cellular. Within the capsule, there were multifocal accumulations of golden-brown amorphous pigment (hematodin) surrounded by fibrous connective tissue, lymphocytes, plasma cells, macrophages, and few multinucleated giant cells (Figure 26). Periarteriolar lymphoid sheaths were moderately cellular.

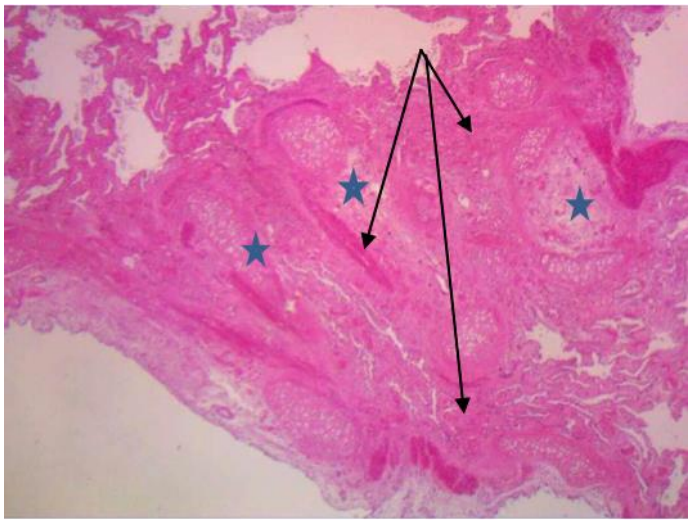

Figure 25. In the lung, the normal structure of the bronchiole with a central space ringed by cartilage is disrupted with the lumen absent and cartilage (star) displaced and surrounded by fibrous connective tissue (collagen) (arrows).

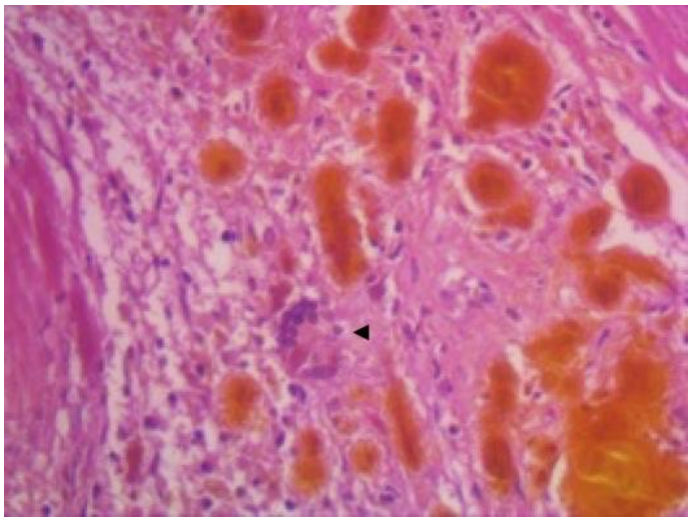

Figure 26. Within the capsule, there was deposition of golden pigment (hematoidin). The pigment was surrounded by mixed inflammatory cells including multinucleated giant cells (arrow). Multinucleated giant cells are macrophages which coalesce and phagocytize foreign material. Hematoidin is an erythrocyte breakdown pigment at a site of hemorrhage (e.g., yellowing of a bruise).

Hepatocytes exhibited multifocal, mild cytoplasmic vacuolation. In the ventricle there were small accumulations of eosinophilic proteinaceous fluid. The capsule of the kidney had deposits of basophilic granular substance. Twelve sections of brain and spinal cord were examined. In the brain, there was random, mild extravasation of erythrocytes in the meninges. Homogenous and granular eosinophilic fluid was present within the anterior and posterior chamber of the eye.

There was a focally extensive periadrenal accumulation of lymphocytes and plasma cells. In the adrenal gland, there was expansion of the periadrenal soft tissue by fibrin, necrotic cellular debris, neutrophils, and macrophages. There were scattered colonies of bacterial cocci (Figure 27). Vessels exhibited hypereosinophilia with a loss of nuclei. Occasional vessels contained clumped eosinophilic substance (fibrin, suspect). Within the adrenal gland, there were abundant colonies of bacterial cocci. Occasional foci had surrounding neutrophils and macrophages. Large bacilli were present within scattered vessels without an associated inflammatory response (Figure 28). Masseter myofibers were separated by an eosinophilic proteinaceous substance.

No significant histological findings were found in the following tissues: skin, fore stomach, trachea, cervix, aorta, tongue, uterine horn, pituitary gland, main stomach, pancreas, mammary gland, and kidney.

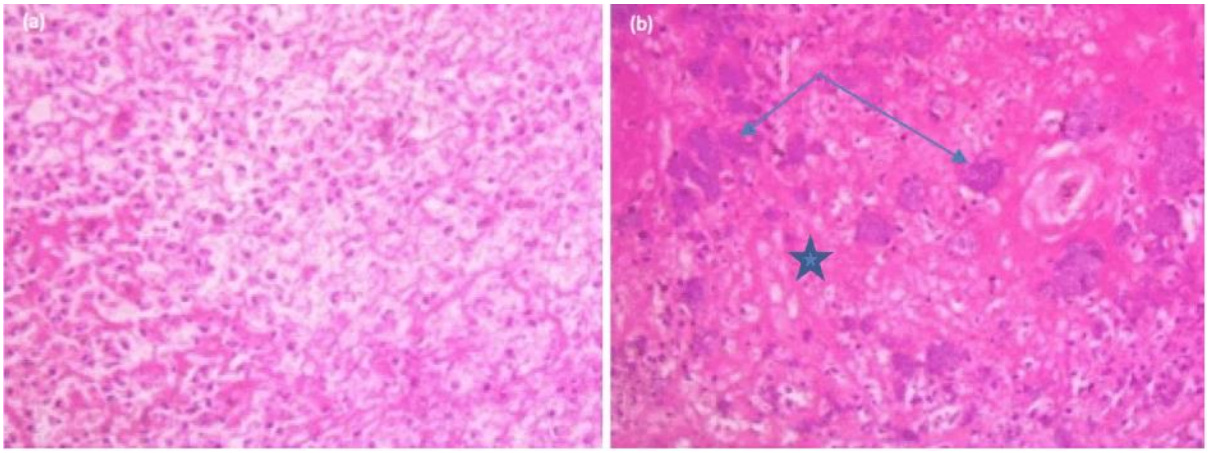

Figure 27. In the peri-adrenal stroma, there were mixed inflammatory cells (a); bacterial colonies (arrows) were present within a fibrinous exudate (star) and inflammatory cells within the peri-adrenal soft tissue (b). Fibrin is an acute phase protein which occurs in inflammatory process.

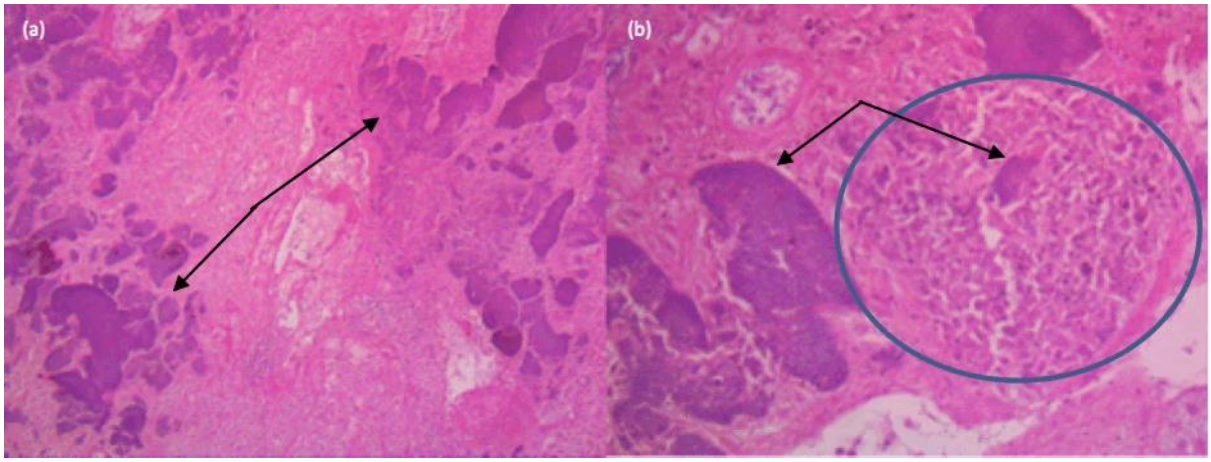

Figure 28. In the adrenal gland (a), there were numerous bacterial colonies (arrows). Some colonies were surrounded by mixed inflammatory cells. Higher magnification (b) highlights the bacterial colonies (arrows) and inflammatory cells, primary neutrophils (circle).

6.3.2.1 KXD0280 Histopathology Conclusions

Histopathological findings were observed in the respiratory, urogenital, musculoskeletal, and endocrine system. Of these, the bacterial adrenalitis was a significant finding. Bacteria were observed in large numbers with no response to a histiocytic and neutrophilic response. Bacterial invasion would have been embolic, but the primary source was not determined. No vegetations (bacterial vegetative endocarditis) were observed and there were no external skin wounds. Special stains for bacteria as well as immunohistochemistry for *Toxoplasma gondii* (though no cysts were observed) could be done to better define and rule out pathogens. It is possible that this cetacean was septicemic which could have led to stranding. Petechiations and ecchymoses could be associated with the vasculitis, and while this would be the differential for the hemorrhage observed in the cranial region, disseminated hemorrhage would be expected with septicemia.

Fibrosis in the lung may have occurred from prior parasitic or other infection in the lung. No infectious agents were observed and the impact on this animal was minimal. Disruption of the bronchioles with consolidation of cartilage within a fibrous stroma likely accounted for the grossly palpable nodules. Edema and serum exudation of the masseter muscle coincides with the grossly observed hemorrhage in the head region.

Splenic siderotic plaques were an incidental finding. This indicates a site of prior hemorrhage with degradation of senescent erythrocytes resulting in deposited hematoidin, an erythrocyte breakdown pigment.

6.4 Ancillary Tests

Morbillivirus: PCR results indicate that the lung and brain of specimens NEB0075 and KXD0280 were negative.

Biotoxins: Fecal samples from both NEB0075 and KXD0280 were below the detection limit for both domoic acid and saxitoxin.

Genetics: Based on the best information available, NEB0075, KXD0280, and KXD0281 were genetically assigned to the California Coastal Stock. This is further corroborated by the dorsal fin match of KXD0280 to the coastal Southern California Bight (SCB) catalog (SCB dolphin no. 12054).

7. Discussion

The gross exam, radiology, histopathology and ancillary testing did not indicate a common infectious disease or biotoxin among these animals. The two fresh specimens were found to be negative for morbillivirus, domoic acid, and saxitoxin. The common finding for all three *T. truncatus* included: coastal stock designation, robust body condition, no external signs of fishery interaction, subcutaneous hemorrhage in the head region concentrated along the lower jaws, and subcutaneous emphysema associated with areas of cervical blubber hemorrhage. In addition, both dolphins that were examined in more detail both had additional consistent findings such as partially digested food in the stomach, hemoabdomen, and gas bubbles within the mesenteric and cerebral vasculature. The combined findings indicate that the dolphins died from severe acute trauma. Vessel strike and inter- or intraspecific aggression were ruled out as differentials for this type of trauma due to a lack of evidence typical of these events. Vessel strikes typically result in unilateral blunt or sharp trauma and inter- or intra-specific aggression results in bilateral focal hemorrhage and/or rib or skull fractures, accompanied by rake marks (Cotter et al., 2011; Moore et al., 2013; Sarah M. Wilkin et al., 2012). These contrast the diffuse circumferential hemorrhage and lack of fractures observed in the dolphins described in this study.

Possible explanations for the observed findings in common with all three specimens are: exposure to an underwater detonation, peracute underwater entrapment (PUE), or exposure to mid-frequency active (MFA) sonar. Perimandibular hemorrhage has been reported in cetacean mortalities associated with PUE (Jepson et al., 2013) while mandibular hemorrhage in the "acoustic" fats has been associated with underwater detonations and MFA sonar (Bernaldo de Quirós et al., 2019; England et al., 2001; Fernandez et al., 2005; Ketten, 2005; St. Leger et al.,

2011). In addition, the robust body condition of all three dolphins and recent feeding observed in the 2 stomachs examined, indicate death was acute, which also supports all three hypotheses. Gas emboli have been reported for underwater detonation, MFA sonar and PUE cases (Bernaldo de Quirós et al., 2019; Fernandez et al., 2005; Moore et al., 2009; St. Leger et al., 2011), but it is possible that the observed mesenteric and cerebral gas was present due to putrefaction or in the case of the cerebral gas, from decapitation (Harms et al., 2012). Hemorrhage in the brain of one specimen (KXD0280) was found and scant lipids in the lungs of another (NEB0075); these conditions have been observed in beaked whales associated with MFA sonar, albeit more severe and extensive (Bernaldo de Quirós et al., 2019; England et al., 2001; Fernandez et al., 2005). Fat emboli have also been observed in underwater detonation cases (St. Leger et al., 2011). All hypotheses are plausible explanations considering nearshore fishing activity occurs in this area, underwater detonations have occurred in the area previously (Danil and St. Leger, 2011), and MFA sonar occurred in the area in the days preceding the strandings of the two carcasses that were in fresh to moderate decomposition (NEB0075 and KXD0280). However, no underwater detonation was reported as part of the MTE. A lack of squid fishing effort and no bait fishing activity the day preceding the strandings combined with no dolphin interactions reported gives less support for the PUE hypothesis. A U.S. fishery operating illegally in nearshore waters or a Mexican nearshore fishery would be a more likely source of PUE but there is no data to support or refute these as possible sources.

There are other observations or lack thereof that are not congruent with any of the hypotheses. Hemorrhage in the lungs, bronchi, trachea, and esophagus have been noted in underwater detonation cases (St. Leger et al., 2011) but were not observed in this study. If one is to consider PUE as the cause of death, the lack of external signs of PUE such as net/line/rope marks, severed appendages, or sliced abdomen are perplexing. One would think that the force of a struggle required to cause some of the observed traumatic lesions would leave external signs of that struggle in the form of impressions, abrasions, or lacerations. If MFA sonar is considered as the cause of death, hemorrhage around the ears might be expected as this has been noted in mass strandings of beaked whales associated with MFA sonar (Fernandez et al., 2005; Ketten, 2005), and this was not observed. However, it is important to note that pathologies observed in relation to MFA sonar are from beaked whales and could present differently in small delphinids such as *T. truncatus* whose behavior and habitat differ.

Other conditions such as hemoabdomen that were observed in both fresh carcasses and hemopericardium that was noted in one specimen, have not been reported in relation to PUE or MFA sonar. Hemoabdomen is normally associated with either bleeding masses from major organs or significant trauma to the abdomen. No masses or trauma to any of the organs was observed. Hemopericardium can be caused by thoracic trauma or myocardial infarction. Subcutaneous emphysema was also observed in both fresh dolphins, which can be caused by infection, malignancy, medical procedures, and blunt, penetrating, or blast trauma (Aghajanzadeh et al., 2015; Horrocks, 2001). It is likely that the hemoabdomen, hemopericardium, and subcutaneous emphysema are a result of trauma, although it is not clear whether that trauma was from an underwater detonation, PUE, MFA sonar, or some other unidentified source. Although the bacterial adrenalitis noted in specimen KXD0280 was

significant, it was likely an underlying condition, considering the trauma that was common among all three strandings.

The tight correlations in time, space, and gross necropsy observations for all three *T. truncatus* discussed here, suggest that they were all impacted by the same acute event. Although KXD0281 stranded 10 days after the first two dolphins, the advanced decomposition state of this dolphin suggests that it died long before it was first observed on the beach and likely around the time the first two were observed. All three dolphins clearly suffered severe acute trauma, likely anthropogenic in nature, although the exact cause cannot be confirmed. An underwater detonation, PUE, or MFA sonar are suspected differentials.

8. Acknowledgements

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10. Appendix 1.
Southern California Stranding Response Plan

Southern California Stranding Response Plan for Hawaii-Southern California Training and Testing (HSTT) Study Area
November 2013

Strandings

Marine mammal strandings, as defined by the Marine Mammal Protection Act (MMPA), have occurred throughout recorded history, although U.S. stranding programs have only been keeping consistent records in some cases as long as the last the last three decades but more commonly the last decade. Strandings may result from many different causes, including, for example, infectious agents, biotoxicosis, starvation, fishery interaction, ship strike, unusual oceanographic or weather events, sound exposure, or combinations of these stressors sustained concurrently or in series. In many cases, a cause of stranding or death cannot be unequivocally determined for a number of reasons. Approximately five marine mammal stranding events in the Mediterranean Sea, Caribbean Sea, and Eastern Atlantic Ocean and involving beaked whale species have been associated with mid-frequency active sonar (MFAS), however, scientific uncertainty remains regarding the exact combination of behavioral and physiological responses that link MFAS exposure to strandings (though several mechanisms have been theorized). Available evidence suggests that in some cases it may be the presence of additional specific environmental or physical conditions working in confluence with the exposure of marine mammals to MFAS that can potentially result in a stranding. The National Marine Mammal Stranding Network (created under the Marine Mammal Health and Stranding Response Program Act (MMHSRPA)) consists of over 100 organizations partnered with the National Marine Fisheries Service (NMFS) to investigate marine mammal strandings in U.S. waters. Among other things, the plan discussed below is intended to contribute to the better understanding of why strandings occur.

Introduction to the Stranding Response Plan

Pursuant to 50 CFR Section 218.74, the plan outlined below will be included by reference and summarized in the Hawaii-Southern California Training and Testing (HSTT) final rule and included fully as part of (attached to) the Navy's MMPA Letter of Authorization (LOA), which indicates the conditions under which the Navy is authorized to take marine mammals pursuant to Navy training activities, involving Navy sonar or explosive detonations conducted off the coast of Southern California. This Stranding Response Plan is specifically intended to outline the applicable requirements the authorization is conditioned upon in the event that a marine mammal stranding is reported off the Southern California Coast during a *major training exercise* (MTE) (see glossary below). As mentioned above, NMFS considers all plausible causes within the course of a stranding investigation and this plan in no way presumes that any strandings are related to, or caused by, Navy training activities, absent a determination made in a Phase 2 Investigation as outlined in Paragraph 7 of this plan, indicating that active Navy sonar or explosive detonation in the HSTT Study Area were a cause of and/or contributed to the stranding. This plan is designed to address the following three issues:

- **Mitigation** – When marine mammals are in a situation that can be defined as a *stranding* (see glossary below), they are experiencing physiological stress. When animals are stranded alive, NMFS believes that exposing these compromised animals to additional known

stressors would likely exacerbate the animal's distress and could potentially cause its death. Regardless of the factor(s) that may have initially contributed to the stranding, it is NMFS' goal to avoid exposing these animals to further stressors. Therefore, when live stranded cetaceans are in the water and engaged in what is classified as an *Uncommon Stranding Event* (USE) (see glossary below), or when live stranded cetaceans are being returned to the water as part of the stranding response, the shutdown component of this plan is intended to minimize the exposure of those animals to Navy sonar and explosive detonations, regardless of whether or not these activities may have initially played a role in the event.

- **Monitoring** – This plan will enhance the understanding of how Navy sonar and other active acoustic sources, and explosives (as well as other environmental conditions) may, or may not, be associated with marine mammal injury or strandings. Additionally, information gained from the investigations associated with this plan may be used in the adaptive management of mitigation or monitoring measures in subsequent LOAs, if appropriate. We note that detections of stranded marine mammals off the Southern California Coast are typically accomplished using passive surveillance (i.e. individuals conducting their normal activities happen to see an animal and report it to the stranding network). If surveys or expanded active detection efforts are specifically used during Navy training exercises, we expect that the number of strandings detected during training may be higher relative to other times because of the increased targeted effort, and, again, does not presume that the detected strandings are related to or caused by Naval activities.

- **Compliance** – The information gathered pursuant to this protocol will inform NMFS' decisions regarding compliance with Sections 101(a) (5) (B and C) of the MMPA.

In addition to outlining the necessary procedural steps for the Navy to undertake in the event of a USE during an MTE (as required by the LOA), this document describes NMFS' planned participation in stranding responses off the Southern California Coast, as NMFS' response relates specifically to the Navy requirements described here. The NMFS Marine Mammal Health and Stranding Response Program (MMHSRP) and the participating California Stranding Networks have specific responsibilities regarding marine mammal strandings and marine mammal unusual mortality events (UMEs) pursuant to Title IV of the MMPA and, in some cases, their Stranding Agreements (under Section 112c of the MMPA). This document does not serve to replace or preclude any of the procedures currently in place for NMFS' response to UMEs or to any normal operations of the stranding network. NMFS will pursue any activities to fulfill obligations relative to UMEs any time that a trigger is reached as determined by the Working Group on Marine Mammal Unusual Mortality Events. This document highlights (or adds to) applicable existing (and in development) protocols and procedures to be used with the specific circumstances and specific subset of strandings addressed here, namely a USE off the Southern California Coast during an MTE. This document has been reviewed and approved by the NMFS staff responsible for conducting and overseeing the referenced activities and this plan will be implemented by NMFS to the degree that resources are available and logistics are feasible.

General Notification Provisions

If, at any time or place (i.e., not just in southern California and not just during the activities covered under NMFS' regulations), Navy personnel find a *stranded* marine mammal (see glossary below) either on the shore, near shore, or floating at sea, NMFS requests the Navy contact NMFS immediately (or as soon as clearance procedures allow) as described in the CA HSTT Stranding Communication Protocol. NMFS requests the Navy provide NMFS with species or a description of the animal (s), the condition of the animal(s) (including carcass condition if the animal(s) is/are dead – see glossary for condition codes), location, time of first discovery, observed behaviors (if alive), and photo or video (if available).

In addition, NMFS requests that in the event of a ship strike by any Navy vessel, at any time or place, the Navy do the following:

- Navy immediately report to NMFS the species identification (if known) or a description of the animal, location (lat/long) of the animal (or the strike if the animal has disappeared), and whether the animal is alive or dead (or unknown)
- As soon as feasible, report to NMFS the size and length of the animal, an estimate of the injury status (e.g. dead, injured but alive, injured and moving, unknown, etc.), vessel class/type, length, speed, heading, and operational status.
- Provide NMFS a photo or video, if possible

If any marine mammal is observed either injured or exhibiting signs of distress after an explosive detonation, NMFS requests the Navy report to NMFS the date and time of the sighting, location (lat/long) of the animal(s), species identification (if known) or a description of the animal(s), and description of the animal's status (e.g. dead, injured but alive, injured and moving, unknown, etc.). NMFS further requests that the Navy take any feasible actions to recover any carcasses.

Operational Response Plan

This section describes the specific actions the Navy must take in order to comply with the Hawaii-Southern California Training and Testing (HSTT) LOA if a USE is reported to the Navy off the Southern California Coast coincident to, or within 72 hours of an MTE. This Stranding Response Plan will include an associated CA HSTT Stranding Communication Protocol, which will indicate, among other things, the specific individuals (NMFS Office of Protected Resources - HQ senior administrators) authorized to advise the Navy that certain actions are prescribed by the Stranding Response Plan. A glossary is included at the end of this document. Words included in the glossary are italicized in this section the first time they are used.

1. <u>Initial Stranding Response</u> - The NMFS regional stranding network will respond to reports of stranded marine mammals in areas where there is geographic coverage by the stranding network, when feasible. All cetaceans that are responded to will receive examination appropriate to the condition code of the animal and the feasibility of the logistics. If a *qualified* individual determines that the stranding is a *USE*, NMFS staff (or other qualified individual) will initiate a *Phase 1 Investigation*. NMFS will immediately contact appropriate NMFS and Navy personnel (pursuant to the CA HSTT Stranding Communication Protocol). NMFS and Navy will maintain a dialogue, as needed, regarding the identification of the USE and the potential need to implement shutdown procedures.

2. *Shutdown* Procedures – Shutdown procedures are not related to the investigation of the cause of the stranding and their implementation is in no way intended to imply that Navy sonar is the cause of the stranding. Rather, as noted above, shutdown procedures are intended to protect cetaceans *exhibiting indicators of distress* and involved in a USE by minimizing their exposure to possible additional stressors (Navy sonar or explosive detonations), regardless of the factors that initially contributed to the USE. Only individuals specifically identified in the CA HSTT Stranding Communication Protocol (NMFS Protected Resources – HQ senior administrators) will be authorized to advise the Navy of the need to implement shutdown procedures (pursuant to the Stranding Response Plan/LOA).

a) If live (*Condition Code 1*) or freshly dead (*Condition Code 2*) cetaceans are involved in the USE, the Navy will implement the following procedures:

- If live cetaceans involved in the USE are in the water (i.e., could be exposed to sonar), NMFS will advise the Navy of the need to implement shutdown procedures as defined in the glossary (pursuant to the Stranding Response Plan/LOA).

- NMFS will coordinate internally, with the Navy, and with other agencies and entities with the intent of obtaining aerial survey arrangements. If an aircraft is available, a survey will be conducted within 14 nm (on the shore and in the water near the coast) of the stranding to look for additional animals that meet the USE criteria. NMFS will request that the Navy assist with aerial surveys, as resources are available.

    - If no additional animals that meet the USE criteria are found (including if no aircraft were available to conduct a survey), and the originally detected animals are not in the water, and will not be put back in the water for rehabilitation or release purposes, or are dead, NMFS will advise the Navy that shutdown procedures need not be implemented at any additional locations.

    - If additional cetacean(s) meeting the USE criteria are detected by surveys, the shutdown procedures will be followed for the newly detected animal(s) beginning at 2(a) above.

- If a qualified individual determines that it is appropriate to put live animals that were initially on the beach back in the water for rehabilitation or immediate release purposes, NMFS will advise the Navy of the need to implement shutdown procedures pursuant to the Stranding Response Plan/LOA.

b) If the Navy finds an injured, entangled or dead cetacean floating at sea during an MTE, the Navy shall notify NMFS (pursuant to CA HSTT Stranding Communication Protocol) immediately or as soon as operational security considerations allow. The Navy should provide NMFS with the information outlined in the general notification provision above, as available. Based on the information provided, NMFS will determine if a

modified shutdown (i.e. a shutdown other than those described here, based on specific information available at the time) is appropriate on a case-by-case basis.

c) In the event, following a USE, that: a) qualified individuals are attempting to herd animals back out to the open ocean and animals are not willing to leave, or b) animals are seen repeatedly heading for the open ocean but turning back to shore, NMFS and the Navy will coordinate (including an investigation of other potential anthropogenic stressors in the area) to determine if the proximity of Navy sonar operations or explosive detonations, though farther than 14 nm from the distressed animal(s), is likely decreasing the likelihood that the animals will return to open water. If so, NMFS and the Navy will further coordinate to determine what measures are necessary to further minimize that likelihood and implement those measures as appropriate. Navy and NMFS will maintain a dialogue regarding the plan to return the animal(s) to the water.

d) If no live (Condition Code 1) or freshly dead (Condition Code 2) cetaceans are involved in the USE, shutdown procedures need not be implemented.

3. Restart Procedures

- If at any time, the subject(s) of the USE die or are euthanized, NMFS will immediately advise the Navy that the shutdown around that animal(s)'location is no longer needed.

- Shutdown procedures will remain in effect until NMFS determines that, and advises the Navy that, all live animals involved in the USE have left the area (either of their own volition or following herding by responders). Leading up to restart, NMFS will coordinate internally, with the Navy, and with other federal and state agencies with the intent of securing arrangements to track the movement of the herded animals (via aircraft, vessel, tags, etc.) following the dispersal of the animals involved in the USE. If the Navy has restarted operations in the vicinity of the animals, NMFS and the Navy will further coordinate to determine (based on location and behavior of tracked animals and location/nature of Navy activities) if the proximity of Navy sonar operations is likely increasing the likelihood that the animals re-strand. If so, NMFS and the Navy will further coordinate to determine what measures are necessary to minimize that likelihood and implement those measures as appropriate.

4. Information - Within 72 hours of the notification of the USE the Navy will inform NMFS where and when they were operating Navy sonar or conducting explosive detonations (within 80 nm and 72 hours prior to the event). Within 7 days of the completion of any exercises that were being conducted within 80 nm or 72 hours prior to the event, the Navy will further provide available information to NMFS (per the CA HSTT Stranding Communication Protocol) regarding the number and types of acoustic/explosive sources, direction and speed of units using Navy sonar, and marine mammal sightings information associated with those training activities. Information not initially available regarding the 80 nm, 72 hours, period prior to the event will be provided as soon as it becomes available. The Navy will provide NMFS investigative teams with additional relevant unclassified information as requested (or classified information to designated NMFS staff), if available.

5. <u>Phase 1 Investigation</u> – Because of the variability of available resources across California stranding network agencies, NMFS cannot currently commit, in advance, to the specific degree of investigation that will be conducted for any given stranding. Given the current fiscal environment and the reduction in federal funding, response resources are limited in some geographic regions. The ideal responses (Phase 1 and 2 Investigations) are described below (here and in # 7), and NMFS will respond in the indicated manner when resources are available and it is logistically feasible:

Within 4 weeks of a USE (when feasible), NMFS will conduct and complete the Phase 1 Investigation (list of procedures typically included in Phase 1 investigation are included in the Glossary of this document, description of actual procedures are contained in the Standard Operating Procedures) for all USEs that occur along the Southern California Coast coincident with MTEs. Results from the Phase 1 Investigation will be categorized in one of the two ways discussed below and trigger the indicated action:

- If the results of the Phase 1 Investigation indicate that the USE was likely caused by something (such as entanglement or ship strike) other than sonar or explosive detonations authorized by the Navy's LOA, then the USE investigation will be considered complete as related to the MMPA authorization.

- If NMFS cannot conclude that the stranding was likely caused by something other than MFAS or explosive detonations authorized by the Navy LOA, rather, the results of the Phase 1 Investigation range from completely inconclusive to including potential early indicators that acoustic exposure could have played a role, then a Phase 2 Investigation will be conducted by qualified individuals, under the direction of NMFS staff, and an individual case report will be prepared for each animal (list of procedures typically included in Phase 2 investigation are included in the Glossary of this document, description of actual procedures are contained in the Standard Operating Procedures).

6. <u>Memorandum of Understanding (MOU)</u> - The Navy and NMFS developed a National MOU (signed November 2011) that allows the Navy to assist NMFS with the Phase 1 and 2 Investigations of USEs. Pursuant to this MOU, Navy and NMFS developed a Regional Stranding Investigation Assistance Plan (RSIAP) that identifies regional assets, equipment, and locations or services that Navy may be able to provide NMFS in support of USE response and investigations (signed February 2013). The Navy may assist NMFS with the investigations by providing one or more of the in-kind services outlined in the RSIAP, when available and logistically feasible and when they do not negatively affect Navy operational or installation commitments.

7. <u>Phase 2 Investigation</u> – Please see # 5, above. Results from the Phase 2 Investigation (procedures outlined in the Standard Operating Procedures) will be categorized in one of the three ways discussed below and trigger the indicated action:

- If the results indicate that the USE was likely caused by something (such as entanglement, disease, or blunt force trauma) other than Navy sonar or explosive

detonations authorized by the Navy's LOA, then the USE investigation will be considered complete as related to the MMPA authorization.

- If the results are inconclusive which, historically, is the most likely result (i.e. NMFS can neither conclude that the USE was likely caused by something other than acoustic trauma nor conclude that there is a high likelihood that exposure to Navy sonar or explosive detonations were a cause of the USE), then the USE investigation will be considered complete as related to the MMPA authorization.

- If the results of a comprehensive and detailed scientific investigation into all possible causes of the stranding event indicate that there is a high likelihood that Navy sonar was a cause of the USE, one of the following will occur:

    - If the total mortalities determined to be caused by Navy sonar or explosive detonation do not exceed the number analyzed for the 5-yr period in the regulations, they will be recorded (to add on to if there is another stranding) and NMFS will take no further action beyond that indicated in 8, below.

    - If the total mortalities determined to be caused by Navy sonar or explosive detonation exceed the number analyzed for the 5-yr period in the regulations, NMFS will begin the process of determining whether or not suspension or withdrawal of the authorization is appropriate.

The Navy will be provided at least ten working days to review and provide comments on NMFS' summary and characterization of the factors involved in the USE. NMFS will consider the Navy's comments prior to finalizing any conclusions and/or deciding to take any action involving any take authorization.

8. USE Response Debrief and Evaluation – Within 2 months after a USE, NMFS and Navy staff will meet to discuss the implementation of the USE response and recommend modifications or clarifications to improve the Stranding Response Plan. These recommendations will feed into the adaptive management strategy discussed below.

9. Adaptive Management - The regulations under which the Navy's LOA (and this Stranding Response Plan) are issued contain an adaptive management component. This gives NMFS the ability to consider the results of the previous years' monitoring, research, and/or the results of stranding investigations when prescribing mitigation or monitoring requirements in subsequent years. In the event that NMFS concludes that there is a high likelihood that Navy sonar or explosive detonations were a cause of a USE, NMFS will review the analysis of the environmental and operational circumstances surrounding the USE. In subsequent LOAs, based on this review and through the adaptive management component of the regulations, NMFS may require the mitigation measures or Stranding Response Plan be modified or supplemented if the new data suggest that modifications would either have a reasonable likelihood of reducing the chance of future USEs resulting from a similar confluence of events or would increase the effectiveness of the stranding investigations. Further based on this review and the adaptive management component of the regulations, NMFS may modify or add to the existing monitoring

requirements if the data suggest that the addition of a particular measure would likely fill a specifically important data or management gap. Additionally, the USE Debrief and Evaluation discussed above (in combination with adaptive management) will allow NMFS and the Navy to further refine the Stranding Response Plan for maximum effectiveness.

Communication

Effective communication is critical to the successful implementation of this Stranding Response Plan. Very specific protocols for communication, including identification of the Navy personnel authorized to implement a shutdown and the NMFS personnel authorized to advise the Navy of the need to implement shutdown procedures (NMFS Protected Resources HQ – senior administrators) and the associated phone trees, etc. (to be included in the document entitled "CA HSTT Stranding Communication Protocols") are currently in usable draft form and will be finalized for the HSTT by December 2013 and updated yearly (or more frequently, as appropriate).

The Stranding Response Plan is dependent upon advance notice to NMFS (HQ and West Coast Region Long Beach Office) of the planned upcoming MTE. The CA HSTT Stranding Response Communication Plan outlines the exact procedure for the Navy to notify the West Coast Region Long Beach Office 72 hours prior to the start of an MTE. NMFS will keep information about planned MTEs in a confidential manner and will transmit information to NMFS and stranding network personnel responding to USEs to the minimum necessary to accomplish the NMFS mission under this plan.

Glossary:

Condition Code – a method for evaluating the stage of decomposition of a stranded animal or carcass. Codes range from live animals (Code 1) to skeletal remains (Code 5) (modified from Marine Mammals Ashore: A Field Guide for Strandings by J.R. Geraci and V.J. Lounsbury).

- Code 1: Live animals
- Code 2: Freshly dead. The carcass is in good condition (fresh/edible), as if it has just died.
- Code 3a: The carcass is in fair condition, with only slight decomposition or scavenger damage. There may be slight bloating and a minimal smell.
- Code 3b: The carcass is moderately decomposed with obvious bloating, some sunburn (blackening and cracking of the skin), sloughing or missing skin, and scavenger damage.
- Code 4: The carcass is in an advanced state of decomposition with a strong odor, skin may be entirely missing, and there is likely extensive scavenger damage.
- Code 5: Mummified or skeletal remains. Skin may be draped over skeletal remains and any remaining tissues are desiccated.

Major training exercise (MTE) – An MTE, within the context of this document, means

- Joint Task Force Exercise (JTFEX.)/Sustainment Exercise (SUSTEX)– 6 events annually (typically around 10 days per event although length can vary)
- Composite Training Unit Exercise (COMPTUEX) – 4 events annually (typically around 21 days per event per event although length can vary)

- Integrated ASW Course (IAC) Phase II – 4 events per year (typically around 2-days per event although length can vary)
- Ship ASW Readiness and Evaluation Measuring (SHAREM)- 2 events annually

Note: Sonar is typically not in use throughout an entire event.

Exhibiting Indicators of Distress – Animals exhibiting an uncommon combination of behavioral and physiological indicators typically associated with distressed, out of habitat, or stranded animals. This situation would be identified by a qualified individual and typically includes, but is not limited to, some combination of the following characteristics:

- Marine mammals continually circling or moving haphazardly in a tightly packed group – with or without a member occasionally breaking away and swimming towards the beach.
- Abnormal respirations including increased or decreased rate or volume of breathing, abnormal content or odor
- Presence of an individual or group of a species that has not historically been seen in a particular habitat, for example a pelagic species in a shallow bay when historic records indicate that it is a rare event.
- Abnormal behavior for that species, such as abnormal surfacing or swimming pattern, listing, and abnormal appearance

Phase 1 Investigation – A Phase 1 Investigation, for the purposes of this document, will typically include the following tests and procedures (which are described in NMFS' Standard Operating Procedures):

- Demographics of the stranding
- Environmental parameters
- Behavioral assessment of group
- Live animal
    - physical examination
    - blood work
    - other diagnostics such as AEP or ultrasound
    - assessment or treatment
- Dead animal
    - External examination and external human interaction evaluation
    - Morphometrics
    - Photographs
    - Diagnostic imaging including CT/MRI scans or ultrasound as appropriate and feasible
    - Necropsy with internal examination, descriptions, photographs and sample collection

Note that several factors will dictate whether all or a subset of these procedures are conducted, including:

- The condition of a carcass
- For live cetaceans - the time it would take necessary personnel and equipment to arrive at the site
- Availability (both in time and space) of resources and feasibility of implementation

Phase 2 Investigation – A Phase 2 Investigation, for the purposes of this document, will typically include the following tests and procedures (which are described in NMFS' Standard Operating Procedures):

- Analyses and review of diagnostic imaging obtained in Phase I
- Histopathology
- Special stains
- Ancillary diagnostics (e.g., PCR for infections, gas emboli)
- CT of ears
- Additional diagnostic imaging as needed
- Histology of ears
- Case summaries
- Review

Note that several factors will dictate whether all or a subset of these procedures are conducted, including:

- The condition of a carcass
- Logistics for transport
- Available resources
- Validated diagnostic techniques

Qualified – NMFS has a rigorous set of standards and training in place to qualify stranding responders, however, the stranding network is a largely volunteer network, there is significant variability from one area to another. In the Standard Operating Procedures, NMFS will identify the minimum qualifications necessary for individuals to make the determinations necessary to carry out this plan. Not all qualified individuals (veterinarians, technicians, etc.) will be NMFS employees. However, only specific individuals (NMFS Protected Resources, HQ – senior administrators) indicated in the CA HSTT Stranding Communication Protocol will be empowered to advise the Navy of the need to implement shutdown procedures.

Stranding – an event in the wild in which:

(a) a marine mammal is dead and is –

(i) on the beach or shore of the United States; or

(ii) in waters under the jurisdiction of the United States (including any navigable waters); or

(b) a marine mammal is alive and is –

(i) on a beach or shore of the United States and unable to return to the water;

(ii) on a beach or shore of the United States and, although able to return to the water, is in apparent need of medical attention; or

(iii) in the waters under the jurisdiction of the United States (including navigable waters), but is unable to return to its natural habitat under its own power or without assistance.

Shutdown Procedures – The act of the Navy ceasing operation of sonar or explosive detonations within a designated area for a designated time. The time is designated by the Restart Procedures (# 3, above). The designated area, for the purposes of this document, is an area within 14 nm of any live, in the water animal involved in the USE. This distance (14 nm) is the distance at which sound from the sonar source is anticipated to attenuate to approximately 140-

145 dB (SPL). The risk function predicts that less than 1% of the animals exposed to sonar at this level (mysticete or odontocete) would respond in a manner that NMFS considers Level B Harassment. As indicated above in 2(d), if this distance appears too short (i.e., the proximity of sonar use may likely be deterring the animals from returning to the open water), NMFS and the Navy will further coordinate to determine what measures are necessary to further minimize that likelihood and implement those measures as appropriate.

Uncommon Stranding Event (USE) – A stranding event that takes place during an MTE and involves any one of the following:

- Two or more individuals of any cetacean species (i.e., could be two different species, but not including mother/calf pairs, unless of species of concern listed in next bullet) found live on shore or dead on shore or dead floating in the water within a two day period and within 10 miles of one another.
- A single individual or mother/calf pair of any of the following marine mammals of concern: Guadalupe fur seals, beaked whales of any species, *Kogia* sp., short-finned pilot whales, humpback whales, sperm whales, blue whales, fin whales, or sei whales
- A group of 2 or more cetaceans of any species exhibiting indicators of distress.

Supplemental Documents

CA HSTT Stranding Communication Protocol – This document includes all of the communication protocols (phone trees, etc.) and associated contact information required for NMFS and the Navy to carry out the actions outlined in this Stranding Response Plan. This document is currently in usable draft form and will be finalized by December 2013 and updated yearly (or more frequently, as appropriate).

Standard Operating Procedures (SOPs) for HSTT – This document (which is currently in a usable draft form) contains protocols for the procedures that are necessary for NMFS staff to implement this Stranding Plan including:

- A protocol for the stranding responders that outlines the actions to take in the event of a USE during MTEs
- Protocols for the investigators that describe in detail the procedures implemented for conducting the Phase 1 and Phase 2 Investigations

Memorandum of Understanding –The National MOU (finalized in November 2011) established a framework whereby the Navy may assist with response and investigations of USEs. Regional Stranding Investigation Assistance Plans (RSIAPs) were developed and are the implementing plans of the National MOU. The RSIAPs identify regional assets, equipment, and locations or services that Navy may be able to provide NMFS in support of USE response and investigations. The RSIAP between the Navy Region Southwest and the NMFS West Coast Region Long Beach Office was finalized and signed in February 2013.

LOA Stranding Plans in Other Geographic Regions

The frequency and nature of strandings (naturally occurring or otherwise), the nature of military operations, and the NMFS resources and qualified staff available for stranding response, can be

highly variable in different geographic regions, and sub-regions within those regions. Measures and procedures developed for and implemented in this Stranding Response Plan may not be appropriate, or even possible, in other geographic regions. As the need arises, NMFS and the Navy will work together to develop appropriate Stranding Response Plans for other geographic regions based on available information and resources. This Stranding Response Plan is not intended to serve as a template for other geographic regions, and, in fact, Stranding Plans for other areas may be significantly different.