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THEME SECTION

Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma

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ABSTRACT: Post-mortem examination of dead and live stranded beach-cast pinnipeds and cetaceans for determination of a cause of death provides valuable information for the management, mitigation and prosecution of unintentional and sometimes malicious human impacts, such as vessel collision, fishing gear entanglement and gunshot. Delayed discovery, inaccessibility, logistics, human safety concerns, and weather make these events challenging. Over the past 3 decades, in response to public concern and federal and state or provincial regulations mandating such investigations to inform mitigation efforts, there has been an increasing effort to objectively and systematically investigate these strandings from a diagnostic and forensic perspective. This Theme Section provides basic investigative methods, and case definitions for each of the more commonly recognized case presentations of human interactions in pinnipeds and cetaceans. Wild animals are often adversely affected by factors such as parasitism, anthropogenic contaminants, biotoxins, subclinical microbial infections and competing habitat uses, such as prey depletion and elevated background and episodic noise. Understanding the potential contribution of these subclinical factors in predisposing or contributing to a particular case of trauma of human origin is hampered, especially where putrefaction is significant and resources as well as expertise are limited. These case criteria descriptions attempt to acknowledge those confounding factors to enable an appreciation of the significance of the observed human-derived trauma in that broader context where possible.

KEY WORDS: Seal · Dolphin · Whale · Marine mammal · Entrapment · Entanglement · Vessel strike · Gunshot

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INTRODUCTION AND OVERVIEW

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Background

The Marine Mammal Protection Act (MMPA) was enacted to conserve and manage all marine mammal populations under US jurisdiction. It prohibits the unauthorized 'take' of marine mammals (defined as capture, harassment, or killing of any individual) and requires federal agencies to monitor the status of populations, including the extent to which human activities contribute to marine mammal morbidity and mortality. Section 117 directs the National Marine Fisheries Service (NMFS) and the US Fish and Wildlife Service to prepare stock assessment reports, in which human-caused marine mammal mortalities and serious injuries must be enumerated. In addition, the Marine Mammal Health and Stranding Response Act of the MMPA requires the evaluation of causes of morbidity and mortality in marine mammals, as well as the monitoring of trends in population health.

The nature of serious injury (SI) was reviewed through NMFS workshops in 1997 (Angliss & DeMaster 1998) and more recently in 2008 (Andersen et al. 2008). The goal of the workshops was to enhance consistency in the assessment and reporting of human interaction (HI) injuries within the National Oceanic and Atmospheric Administration (NOAA) by refining criteria used in human interaction determinations. The SI criteria that were developed were with respect to HI injuries sustained by large cetaceans that did not result in an immediate mortality but would likely result in subsequent mortality. In these cases, the animals were seriously injured but still alive. Draft guidelines (Moore & Merrick 2011) and procedures (NMFS 2012a,b) for this were recently published. There has been an excellent attempt to standardize how HI is recognized grossly by personnel responding to marine mammal strandings (Moore & Barco in press). This includes the development of a training manual (Moore & Barco in press), standardized data collection form (see Supplement 1, available at www.int-res.com/articles/suppl/d103p229_supp/), and the provision of training workshops to members of the US Marine Mammal Stranding Network. The protocol presented to stranding response personnel is designed to provide (1) an objective gross evaluation of an animal or carcass that determines whether any signs of HI are present (regardless of whether they may have contributed to the stranding or death of the animal, are pre- or post-mortem, healed or recent); and (2) a subjective finding in which examiners use all available information and their experience to evaluate the likelihood that any observed evidence of HI contributed to the stranding event. Ideally, to reach a conclusion as to whether HI caused the death (or was a contributing cause of the death) requires review of all sample results and veterinary pathological information (Moore & Barco in press). Such information could include history, gross necropsy, histopathology, microbiology, radiology, molecular tests, toxicology, hematology and other diagnostic disciplines. As such the manual falls short of a goal of establishing objective criteria to determine the proximate cause of death (COD), whether it be by HI or not. More rigorous scientific examination of all available information is important for the scientific community and requires the cooperation and collaboration of the veterinary and stranding network communities.

This Theme Section aims to establish case statements for each common class of HI to enable examiners of cetaceans and pinniped cases to generate and interpret gross, histological and other analytical data to conclude if an animal died or was seriously injured as a result of a specific human activity, and to qualify such conclusions in terms of degrees of confidence. It identifies knowledge gaps to complete the needed criteria whereby each casual factor can be recognized as causative, or not, in a particular case. It is critical to recognize that there is a spectrum of the amount and type of data available for the analysis of specific cases that ranges from a single at-sea photograph of a dead or seriously injured animal to a full case history involving necropsy, histology and ancillary investigations. It is important to use the available precedents from all similar documented cases to inform the interpretation of data-poor cases to arrive at the best assessment. On 1 and 2 February 2012, a group of biologists, government scientists and veterinarians convened in a workshop to define evidence necessary to attribute observations of cetacean and pinniped mortality or SI from proximate anthropogenic events that initiated their ultimate demise. It was in no way designed to advise or modify NOAA policy and procedure for the determination of causes of SI and mortality, but the hope remains that this Theme Section will enable such determinations (Fig. 1) to be made on the basis of the best available criteria as summarized herein. While this review works within the relevant legal framework in the United States of America, it should have global relevance in principle.

Methods for investigation of mortality and SI cases

Clinical signs

Investigators should observe and record, preferably with a camera, the behavior of any live animal (see Supplement 2, available at www.int-res.com/articles/suppl/d103p229_supp/). The nature of respiration, response to stimuli, external body condition, and extent, distribution and severity of the trauma, and skin color or discoloration should also be recorded.

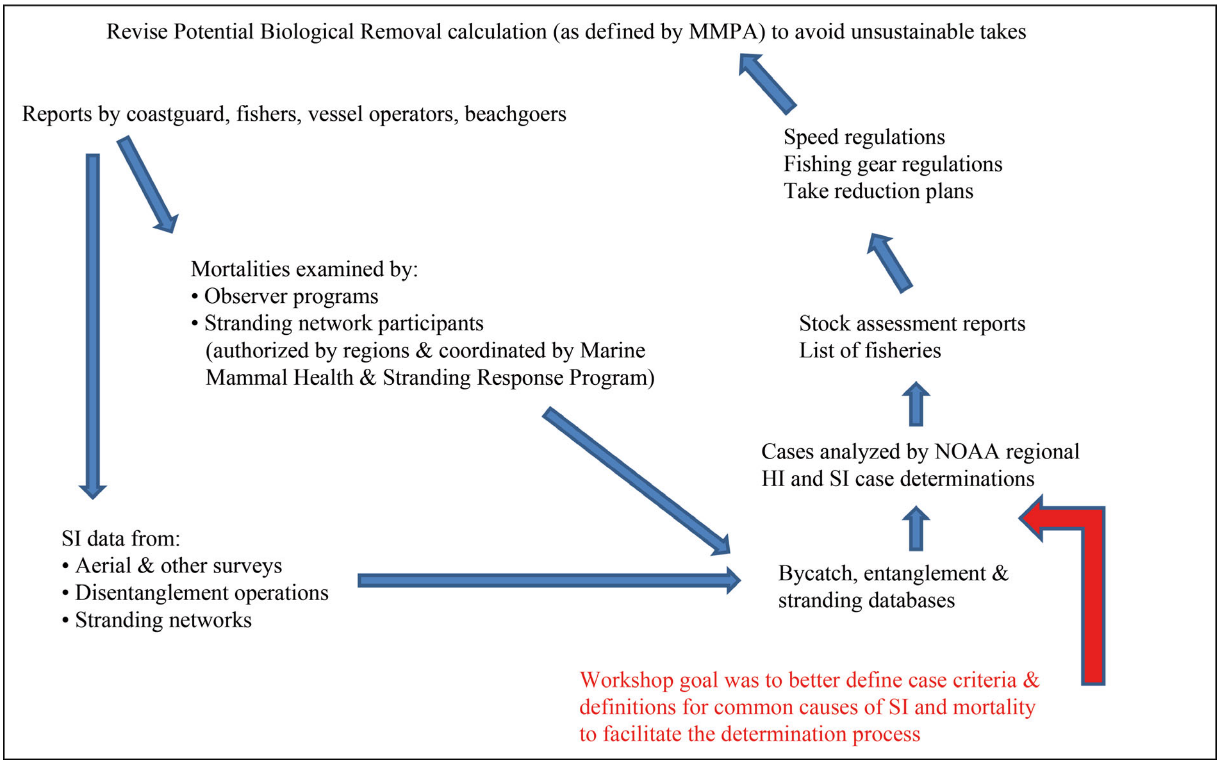

Fig. 1. Potential flow of mortality and serious injury (SI) observations through the generation and review process in the USA in the context of the Marine Mammal Protection Act (MMPA)

Assignment of decomposition condition code

A condition code should be assigned to a deceased individual based on the level of decomposition of a carcass. As sample quality diminishes with decomposition (with higher condition codes), assessing condition can help determine the samples that will be of greatest value. Carcasses can be classified into one of 5 categories, as described by Geraci & Lounsbury (2005). Live animals are Code 1. Code 2 carcasses are those in good (fresh) condition, characterized by having normal appearance, little scavenger damage, fresh odor, lack of bloating, and firm muscle and blubber, among other features (Geraci & Lounsbury 2005). Carcasses with moderate decomposition are Code 3, typically with organs more or less intact, bloating, cracked and sloughing skin, potential scavenger damage, mild odor, blood-tinged oily blubber, soft muscles and intestinal distention. Code 4 carcasses are in poor condition, with advanced decomposition, and are often collapsed and displaying sloughing skin, severe scavenger damage, strong odor, soft blubber, liquefied muscles, and gas-filled intestines. Mummified or skeletal remains are Code 5.

Level A data and first response

Researchers should collect Level A data (species, age, sex, length, location, date stranded) (Supplement 3, available at www.int-res.com/articles/suppl/d103p229_supp/) (Geraci & Lounsbury 2005), and describe and photograph the entire animal and any surface lesions and attached fishing gear if present. Photographs should capture flukes, callosities, or other distinguishing features used for photo-identification of individuals. This can aid in matching an animal with known sighting and history information that can in turn aid in determining the timing of the injury. A skin sample should be collected for genetic analyses. Complete a HI form (Moore & Barco in press).

If a case is floating at sea, towing ashore is a priority. If any attached fishing gear is likely to fall off during towing, it should be removed before moving the animal. Similarly, in the event of a severely impacted carcass, investigators should anticipate the potential separation of body parts. The body part to which any towline is attached should be documented prior to attachment.

Level B data

Detailed data (measurements, patterns, diagrams and photographs from multiple angles) and samples on all injuries and wounds should be collected, and body condition (emaciated, thin or robust) assessed.

Sample collection

Clinical pathology and histopathology

Sample collection from various condition code animals can be guided by Table 1. Blood from live animals and vitreous from fresh dead (Code 2 or 3) animals should be collected for baseline serum chemistry and hematology (from blood) and potassium and creatine kinase (from vitreous); surplus serum should be banked at −70°C. Clinical chemistry tests of important note include creatine phosphokinase, lactate dehydrogenase and aspartate transaminase for muscle damage (capture myopathy/acute rhabdomyolysis).

Thorough tissue sampling should be conducted when practical for all cases according to condition code (Table 1); major organs, lesion, and wound margins and subtending tissue including bone on

Table 1. Valuable sample analysis according to carcass condition code (adapted from Geraci & Lounsbury 2005, Pugliares et al. 2007)

Code	Animal status	Samples of value
Code 1	Live animal	Morphometrics, blood, urine, biopsies, genetics, toxicology
Code 2	Fresh carcass	Morphometrics, blood, urine, histology, cytology, virology (tissue), microbiology (swabs or tissue for culture, tissue for polymer chain reaction (PCR), parasitology, contaminants, biotoxins, life history, genetics
Code 3	Moderate decomposition	Morphometrics, histology (limited), virology (PCR), microbiology (PCR), parasitology, contaminants, biotoxins, life history, genetics
Code 4	Advanced decomposition	Morphometrics, histology (limited), virology (PCR), life history, genetics
Code 5	Mummified or skeletal remains	Morphometrics, life history, genetics

all carcasses should be sampled as practical. Foreign objects should be handled with appropriate instruments (e.g. plastic forceps) to avoid artifactual tool-marks. Histologic sampling of wound margins should be conducted with care to avoid artifactual destruction of physical (e.g. muscle fiber curling or contracture) and cellular (e.g. vital response) indicators of ante-mortem injury.

All lesions should be labeled with a unique identifier, and photographed *in situ*, with a scale and Case ID marker. Lesion identifiers should accompany all subsequent samples derived from each lesion, with the anatomic site of trauma recorded.

In cases with traumatic bone injury, evaluation of the cortical defect or bone fracture margins is recommended. If possible, concurrent collection of urine for urinalysis is recommended for evaluating the presence of muscle and blood breakdown products (myoglobinuria and hemaglobinuria).

Necropsy

Gross necropsies should be performed by experienced prosectors on all carcasses following prescribed pinniped and cetacean protocols (McLellan et al. 2004, Pugliares et al. 2007). Ensure appropriate life history and individual identification characters are collected. Collect detailed documentation (Fig. 2) (e.g. at impact sites, photograph from several angles, measure and diagram, scar/wound patterns, sketch with scale) and histology samples of soft and hard tissue wound margins at various depths to account for differential resolution of peri-mortem and chronic wounds as well as to help rule out exposure artifacts (e.g. salt water or scavenging) (DiMaio & DiMaio 2001). The texture (e.g. curling/fiber bunching, bridging) and histopathology of wound margin and muscle can often provide insights into ante-mortem/peri-mortem vs. post-mortem injury or scavenging (Shkrum & Ramsay 2007). Presence, volume and location of loose and/or adherent well-formed blood clots and frank hemorrhage associated with incised wounds should be noted. Additionally, documentation of wound location may help with ante-mortem vs. post-mortem injury determination; for example, blunt trauma injuries to the dorsum may indicate that a whale was alive when it was struck, as most dead whales float with either the ventral or the lateral midline facing upward (Campbell-Malone et al. 2008).

Abdominal displacements, herniation and visceral ruptures should be carefully documented, as these can be post-mortem changes. The pulmonary system should be examined for evidence of froth, or water and/or blood aspiration. Measuring the specific gravity and chemical constituents of airway fluids may help to discriminate between body fluid and seawater.

Stomach chambers should be examined for foreign ingesta, and for presence of blood, as maxillofascial and upper respiratory tract injuries can result in hemoptysis and subsequent ingestion of blood, which provide strong support for ante-mortem timing of injuries.

All major organs should be examined and sampled to exclude other possible causes of death. Focused sampling of liver, kidneys, and lymph nodes should be conducted for evidence of ante-mortem blood drainage from the site of injury and erythrocyte degradation. Regional and global lymph node examination and histological sampling may help narrow down extent of damage and/or post-injury survival time based on presence and degree of erythrocyte turn-over/degradation.

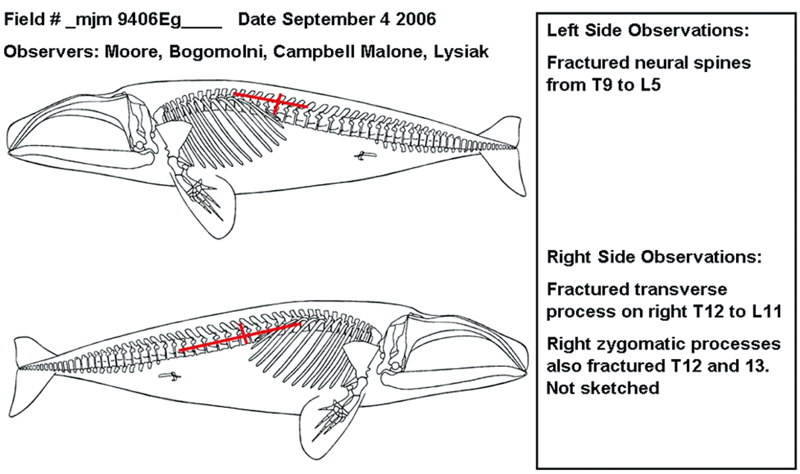

Fig. 2. Image of the fractured vertebral elements discovered during necropsy of MJM 9406 Eg. Fractures (shown in red) were recorded on necropsy data sheets described in McLellan et al. (2004)

Histological staining

Examining all samples, especially relating to gross images and descriptions if practical, is primarily of use in diagnosing intercurrent conditions. Conventional histologic staining, such as hematoxylin and eosin (H&E), is recommended with additional special stains undertaken at the discretion of the pathologist. Oil Red O or osmium tetroxide post-fixation may detect the presence of fat embolization (Fernández et al. 2005), which has been associated with traumatic injury to lipid-rich tissues (e.g. long bone fractures with marrow exposure or adipose tissue injury) (Shkrum & Ramsay 2007). Note that samples must be collected for adipose staining and that these cannot be put through routine processing. In the case of bone fractures, histological evaluation of fracture margins should also be undertaken, although no changes will be detected if death is peracute. Okajima staining for hemaglobin may better define protein casts in renal tubules, if there is a myoglobinuria or hemoglobinuria.

Microscopic evaluation of regional lymph nodes to assess draining hemorrhage and of kidney to evaluate proteinuria associated with myoglobin or hemoglobin is recommended. Electron microscopy, special stains for pathogens, phosphotungstic acid hematoxylin-fibrin, and Trichrome-collagen for fibrosis may also be considered.

Report

Reporting of gross necropsy findings should follow a standardized protocol (McLellan et al. 2004, Pugliares et al. 2007). A gross report should include as detailed a life history (from catalog ID if applicable) and case history as possible, and sketches, measurements, descriptions and samples taken and their disposition. Diagrams and photographs of significant lesions should be supplied with the gross report. Use of HI forms (Supplement 1) and/or injury-specific forms (Supplement 2) is recommended. Once ancillary diagnostic findings (e.g. histology) are available, a final case report and interpretation should be prepared with conclusions as to likely COD. COD from anthropogenic trauma should be rated as confirmed, probable or suspect.

Case criteria

The subsequent sections of this Theme Section include criteria for the following diagnostic trauma categories: peracute underwater entrapment; chronic entanglement; blunt vessel; sharp vessel; and gunshot. For each trauma type described, case definition material is mostly presented as follows: background, signalment, epidemiology, clinical signs, host response, injury-specific gross necropsy, toxicology, and histology findings, and COD assignation.

Degrees of confidence

Scoring 'confirmed, probable and suspect' cases as defined in the Glossary (Appendix 1) remains an inexact science for cetacean and pinniped trauma mortality investigations. The traumatic event is rarely witnessed or documented, the time from death is generally not known and for large whales the investigation is often hampered by the inability for large equipment to reach the site to aid the necropsy. Constraints also include loss of tissue from scavenging, combinations of chronic entanglement and resolved past lesions, additional post-mortem insults from vessel collision, tissue degradation and dissolution from putrefaction, beach abrasion and finally, animal condition prior to the insult. Each category includes separate well-defined forensic findings and provides case reports that best illustrate each of these findings. As more large whale necropsy findings are published world-wide these categories can become more codified. These problems of size are less extreme for smaller pinnipeds and cetaceans, but overall the same limitations apply.

General recommendations

(1) A periodic retrospective peer analysis should be undertaken of the prevalence of each lesion type for each trauma type in past necropsy cases based on COD confidence levels, including information on location and depth of wounds and entanglements; trauma dorsal or ventral to the center of gravity of the animal; organ displacement or herniation; whether there was an acute, subacute or chronic mortality; and the review of pathology cases in order to determine the frequency of observing each type of injury/gross lesion. Such an analysis would confirm assumptions on past cases, strengthen the conclusions from specific observations and increase confidence with enhanced sample sizes for categorizing mortalities.
(2) Cross-correlation of injury data from live and dead animals should be used to evaluate lethality criteria.
(3) All necropsy teams should adopt standardized protocols of examination and reporting (e.g. necropsy team leader [NTL] / logistics coordinator / offsite coordinator) (McLellan et al. 2004) to maximize data comparability.
(4) Increased effort should be made for systematic archiving, reporting, and sharing of data, such as open/limited access online researcher databases for archiving of trauma determinations.

(5) Greater transparency and communication should occur between managers, NTLs, and interest groups in order to enhance future diagnostic quality, and consequent implementation of suitable conservation measures to mitigate impacts.

(6) Communication should occur between east and west coast stranding programs and managers regarding cases, to increase the learning curve based on national caseload rather than regional loads.

PERACUTE UNDERWATER ENTRAPMENT OF PINNIPEDS AND CETACEANS

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Background

By-catch has been identified as a major cause of mortality in individual marine mammals (Kuiken et al. 1994, Kuiken 1996, Kirkwood et al. 1997, Northridge & Hofman 1999). More importantly, there is increasing evidence of the negative ecological impact of by-catch from specific commercial fisheries on many cetacean populations (Northridge & Hofman 1999). For example, several observer-based studies have reported high or unsustainable mortality in harbor porpoises *Phocoena phocoena* due to by-catch in commercial gill-net fisheries (Tregenza et al. 1997, Moore & Barco in press) and in trawl-caught common dolphin *Delphinus delphis* (Peltier et al. 2012).

Case definition

Clinical signs

Clinical signs of peracute underwater entrapment in fishing gear are unlikely to be seen in most cases due to the essentially cryptic nature of immersion in fluid. However, pathological findings in peracute drowning due to underwater entrapment often suggest some degree of physical struggle associated with often marked muscular exertion.

Signalment

For cetaceans, any age and sex can be affected by accidental entanglement in commercial fishing gear (by-catch) although biases have been found for particular species and fisheries. For example, juveniles were over-represented in some studies of by-caught animals (stranded and retrieved directly from fishing vessels) (Kirkwood et al. 1997, Siebert et al. 2001, Jauniaux et al. 2002, Jepson 2006), while in other studies they were under-represented (*Delphinus delphis* and *Stenella longirostris* by-catch in tuna purse-seine fisheries) (Danil & Chivers 2007, Larese & Chivers 2009). Male *D. delphis* have been over-represented in both Pacific and Atlantic fisheries (Ferrero & Walker 1995, Murphy 2004, Westgate & Read 2007, Danil et al. 2010) and there are anecdotal reports that juvenile male bottlenose dolphins *Tursiops truncatus* on the US east coast are also over-represented.

Epidemiology

All types of fishing gear pose significant global threats to welfare and conservation status of exposed pinnipeds and cetaceans (Read et al. 2006, Moore & Barco in press). Pinnipeds, odontocetes and mysticetes can be affected (Jepson 2006, Moore & Barco in press, Cassoff et al. 2011). Gillnet entrapment (Figs. 3 & 4) seems to occur in all regions of the globe where gillnets are employed (Read et al. 2006). Underwater entrapment in mobile gear also occurs widely but may be rather more cryptic (Northridge & Hofman 1999).

Injury-specific gross necropsy findings

These gross necropsy findings are based on a number of sources (Kuiken et al. 1994, Kuiken 1996, Moore & Barco in press). The diagnosis of peracute underwater entrapment (by-catch) when circumstances of death/discovery or external findings are obvious can usually be made on gross examination alone without the need for histopathology. Histopathology and microbiology are useful investigative tools to help rule out other causes of death. In addition, assays for algal toxins (e.g. domoic acid and others), may also be useful investigative tools to rule out underlying or pre-existing conditions that may predispose animals to HI.

Contact with fishing gear

Evidence of contact with fishing gear is not required to confirm an underwater entrapment mortal-

[1]For author affiliations, see Supplement 5 at www.int-res.com/articles/suppl/d103p229_supp/

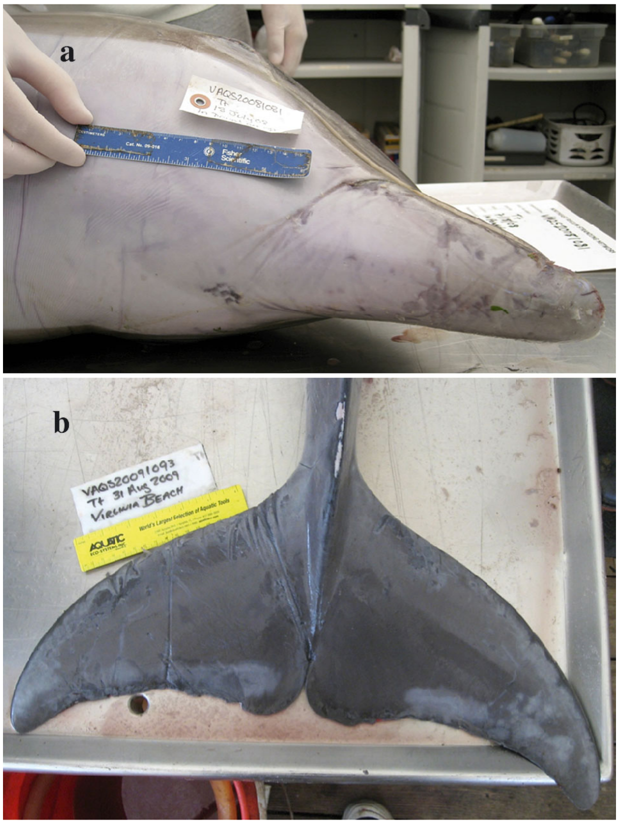

Fig. 3. Linear impressions from net entanglement on the (a) rostrum and (b) fluke of a bottlenose dolphin *Tursiops truncatus*. VAq cases (a) VAQS20081081 Tt and (b) VAQS20091093 Tt

ity. Entrapment can occur without direct contact with fishing gear, such as when surrounded by fish in the end of a mobile trawl. Evidence includes (1) fresh linear skin lesions and furrows (e.g. net marks; Figs. 3 & 4) in or around the mouth, fin or tail (Moore & Barco in press) or encircling one or more extremities (Fig. 5); (2) bruises in body regions consistent with entanglement such as peri-mandibular (Fig. 6), peri-

Fig. 4. Impressions from entanglement of a harbor porpoise *Phocoena phocoena* in gillnet. WHOI case DO8760Pp

scapular and thoracic *rete mirabile*; and (3) fractures (in pinnipeds and small odontocetes) and associated hemorrhage and soft tissue maceration in the mandible, other parts of the cranium, and ribs. Further, whether or not strandings occurred in the vicinity of fishing activity may assist in making a determination.

Evidence of hypoxia

Lack of oxygen (hypoxia) can be evidenced by wet, moist, glistening and heavy lungs (edematous lungs). Persistent froth in the airways (Fig. 7) may be red-tinged, but can also be white and clear; some froth may be stable, likely depending on protein content, surfactants, or mucus. Generalized congestion (dark red lungs) can also suggest lack of oxygen. Fluid in the primary and secondary airways may or may not be evident grossly. Occasional hyperinflation of lungs and emphysema have been observed (Jepson et al. 2000, Jepson 2006).

Fig. 5. Large mesh net marks on the fur of a grey seal *Halichoerus grypus*. IFAW case CCSN04-153Hg

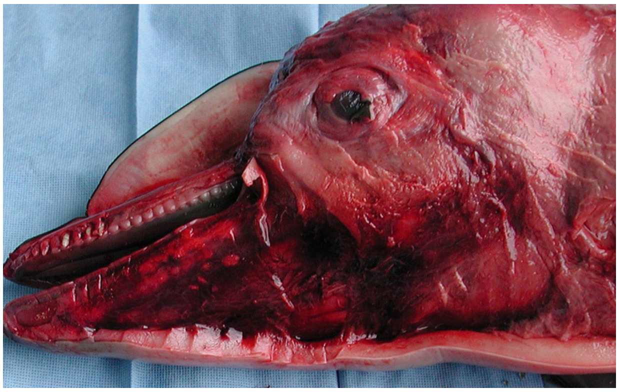

Fig. 6. Skin and blubber removed and pre mortem subcutaneous and fascial hemorrhage apparent around the left mandible of bottlenose dolphin. VAq case VMSM10021035 HEM

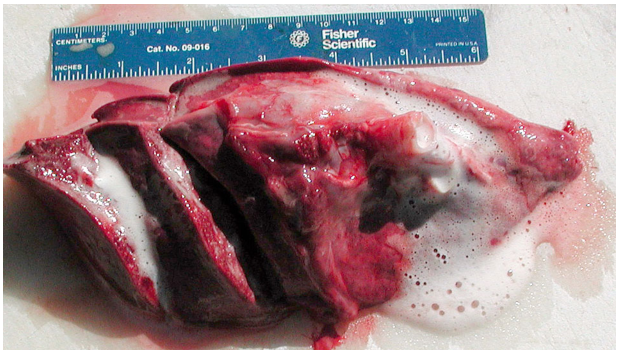

Fig. 7. Incised harbor porpoise lung showing copious froth exuding from cut surfaces and airways. VAq case VMSM20041006

Fig. 8. Amputated flukes in a net-entrapped bottlenose dolphin. VAq case VMSM20011123

Fig. 9. Post-mortem abdominal wall incision in net-entangled bottlenose dolphin. VAq case VAQS 20121008

Fig. 10. Tooth rake marks (oval) and penducle line impression (arrow) in bottlenose dolphin. VAq case VAQS20111014

Although histology would not be required to confirm the diagnosis of pulmonary edema, microscopic assessment of the tissues would be critical to exclude other underlying or pre-existing conditions that may have contributed to entanglement or even death, such as septicemia, parasitemia, cardiomyopathy, or forms of vasculopathy.

Physical trauma

Evidence of physical trauma during the release from a net would include the amputation of fins, flukes, or tail (Fig. 8) (Cox et al. 1998), a penetrating incision into a body cavity (Fig. 9), or rope around the tail stock that was added to enable removal from a net (Fig. 10).

There are no pathognomonic clinical signs or lesions for an animal that died from peracute underwater entrapment. However, there are a number of findings on necropsy that are consistent with this cause of mortality. By-caught harbor porpoises (usually caught in bottom set gill-nets or tangle nets using wide-meshed monofilament nylon nets) typically exhibit characteristic cuts on the edge of the mouth, fin or tail and sometimes have encircling lesion(s) around the head (e.g. Fig. 4). In contrast, common dolphins (mostly suspected to be caught in mid-water pelagic trawl fisheries) typically present with few external cutaneous lesions, although amputated fins/flukes, fractured beaks and broken teeth are sometimes identified (Kuiken et al. 1994, Jepson 2006, Deaville & Jepson 2011).

Pulmonary lesions are similar to those seen in death due to asphyxia in terrestrial mammals and include pulmonary congestion, edematous lungs, and presence of a fine persistent whitish (or blood-tinged) froth within the airways. Pleural or pericardial petechial hemorrhages and bullous emphysema have also been proposed as criteria for diagnosis of cetacean by-catch in the UK (Kuiken et al. 1994, Kuiken 1996) but are very rarely seen (Jepson et al. 2000). Edematous lungs and the presence of persistent blood-tinged or white stable foam in the airways are thought to result from asphyxia associated with hypoxic damage to the integrity of alveolar membranes leading to leakage of erythrocytes and proteinaceous fluid into the alveoli (Modell 1981, Davis & Bowerman 1990, Lunetta & Modell 2005). However lung edema can also occur post mortem, following intrinsic contractions of the atrio-ventricular and sino-atrial nodes in the heart; contractions may persist for up to 5–10 min. As blood is ejected, it increases the hydrostatic pressure in the pulmonary microvasculature with seepage or suffusion of fibrin and edema into the alveolar spaces. Subsequent mixing with residual air may then result in stable froth formation. The role of possible traumatic asphyxia is not understood, but should be considered given the compression and physical restraint involved in net-entanglement events (Shkrum & Ramsay 2007).

Intravascular gas bubbles have been found in fresh (Code 2) odontocetes that were by-caught at considerable depth (>100 m) and probably relate to nitrogen off-gassing post mortem from supersaturated tissues as the by-caught carcass returns to the surface (Moore et al. 2009). Bubbles may not be seen in Code 2 carcasses which have been by-caught at shallower depths.

Health status must be considered in confirming an underwater entrapment mortality. Nutritional condition of by-caught small cetaceans is often good to moderate, or not emaciated in the case of pinnipeds. Evidence of recent feeding is commonly presented either by whole prey within the stomach, partially digested ingesta mixed with hard parts (skeleton, squid pen, squid beaks, or otoliths) or chyle within intestinal lymphatics.

Histological findings

Histopathology of underwater entrapment is non-specific and may include severe bilateral pulmonary congestion, pulmonary edema (Fig. 7) and foci of intra-alveolar hemorrhage (Fig. 11). Common findings include alveolar hyperinflation and emphysema and constriction of smooth muscle sphincters within terminal bronchioles. Other organs e.g. liver, kidneys, and thoracic *rete mirabile* are also typically congested (grossly and histologically). Contusions are found in many locations including peri-cranial and peri-scapular tissues, skeletal muscles (such as epaxial and hypaxial muscles) and often within the thoracic *rete mirabile*. In these particular anatomic regions, the pericranial and prescapular contusions are subcutaneous, muscular or track along fascial planes. This is likely related to the greater potential space in these anatomical sites, relative to other areas where skin is tightly interdigitated with the subcutaneous tissue (e.g. peduncle and dorsal fin). The thoracic *rete* contusions may be related to shearing or laceration of vessels associated with entanglement and trauma, congestion and diapedesis or some other process.

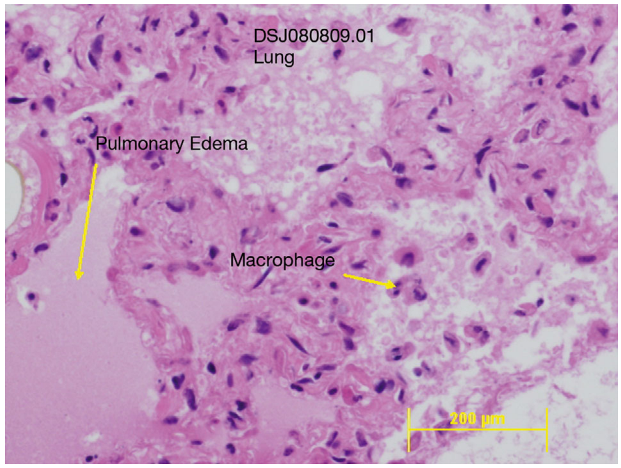

Fig. 11. Flooding of alveolar spaces with edema fluid (long arrow) and occasional aggregates of macrophages (short arrow) in an underwater-entrapped Pacific white sided dolphin *Lagenorhynchus obliquidens*. SWFSC case DSJ080809.01. Photo: D. Rotstein

Confounding circumstances

Certain circumstances may complicate the determination of COD. Evidence of non-peracute death (see 'Chronic entanglement' section, p. 240) include wound repair suggestive of a longer time course. Lesions, either pre-mortem (e.g. rake marks from conspecifics or contraspecifics; Fig. 10), or pre- or post-mortem (e.g. from scavengers or predators) may lead to surface lesions that need to be discriminated from fishing gear trauma. Further, post-mortem marks, such as from towing around peduncle/tail, beach casting, bird scavenging, impressions from transport bag (some body bags have a weave pattern that may be artifactually imprinted on the surface of the carcass during freezing and transport), and impressions from lying on other uneven surfaces must be recognized. Alternative causes of fatality, such as traumatic injury (e.g. propeller injury; see 'Sharp trauma' section, p. 251) or due to poor nutritional status, must also be considered. Finally, decomposition state can lead to loss of critical information and can preclude COD determination.

Moderate to low levels of some persistent organic pollutants such as polychlorinated biphenyls (PCBs) and pesticides such as DDTs and dieldrin have been associated with cases of entrapment (by-catches) in pinnipeds and cetaceans, including cases of UK-stranded harbor porpoises (Jepson et al. 2005, Hall et al. 2006) in contrast to higher levels in diseased animals. Levels of exposure to biotoxins are also likely to be low or absent in tissues or stomach contents.

Examination of frozen and then thawed carcasses is often useful; although net marks should ideally be examined prior to freezing, most by-catch related injuries and lesions will survive the freezing process (e.g. subcutaneous bruising, foam in the trachea, fin/fluke amputations). Some additional decomposition may occur and tissue will be less suitable for histopathology following freeze or thaw.

Data gaps and research needs

Gross and histological examination of mysticetes drowned in fishing gear should be undertaken. Further, the gross and histological appearance of drowned cetacean lungs should be compared between different depths of drowning to establish if phenomena such as hyperinflation are characteristic of a particular depth. Finally, we suggest that the specific gravity and chemical constituents of airway fluids should be more frequently measured to discriminate between body fluid and seawater.

COD assignation

Table 2 lists conditions necessary and sufficient to assign a case to a category of confirmed, probable or suspect. To assign a case to a particular level of confidence, a case must conform to one of the sets of conditions given in the various columns for each level. For instance, entanglement would be 'confirmed' as COD if a dead cetacean was (1) reported by a fisheries observer; or (2) showed net marks; or (3) was entangled in gear and Code 3 or less; or (4) was entangled in gear, had food in its stomach and was in good nutritional condition.

Table 2. Criteria sets for diagnosis of underwater entrapment in pinnipeds and cetaceans. For explanation of Codes and scorings 'Confirmed', 'Probable' and 'Suspect' see 'Introduction and overview' and Appendix 1

Criterion	Confirmed				Probable[a]			Suspect
Cetaceans								
Reported by fisheries observer	✓							Most parsimonious conclusion based on observer experience
Entangled in gear			✓	✓				
Code 2 or 3			✓					
Froth in lungs					✓	✓	✓	
Whole or partially digested prey in stomach				✓	✓	✓	✓	
Bruising around appendages/neck					✓	✓	✓	
No other significant gross pathology					✓	✓	✓	
Good nutritional status				✓	✓	✓	✓	
Net marks		✓						
Rope/line marks					✓			
Amputation/body slit						✓		
Rostral/mandibular fractures							✓	
Pinnipeds								
Reported by fisheries observer	✓				Most parsimonious conclusion based on observer experience			Most parsimonious conclusion based on observer experience
Entangled in gear			✓	✓				
Code 2 or 3			✓	✓				
Code 2		✓						
Bruising around appendages/neck				✓				
Redness in eyes (Code 2)				✓				
Net marks		✓		✓				
Gas bubbles in blood vessels/heart (Code 2)		✓						

[a]For cetaceans, fewer criteria than those shown may be sufficient for a 'Probable' diagnosis, according to the most parsimonious conclusion based on observer experience

CHRONIC ENTANGLEMENT TRAUMA OF PINNIPEDS AND CETACEANS

Michael J. Moore[1], Mendy Garron, Lanni Hall, Allison Henry, Scott Landry, Heather Pettis, Jooke Robbins, David Rotstein, Julie van der Hoop, David Mattila

Background

Wherever fishing gear/marine debris and marine animals overlap there is potential for entanglement. Although entanglement frequency is not well-understood in most areas of the world, it is known to be prevalent in some species and populations (Henderson 2001, Hofmeyr et al. 2006, Robbins et al. 2007, Moore et al. 2009, Neilson et al. 2009, Raum-Suryan et al. 2009, Robbins 2011, Henry et al. 2012, Knowlton et al. 2012). Many whales are repeatedly entangled (Knowlton et al. 2012). Therefore it is critical to discern potential causal relationships in any case of mortality or SI. When an entanglement event occurs that is not immediately lethal through underwater entrapment, the incident can be acute or chronic. This usually occurs when the strength of the animal exceeds that of the gear, enabling a return to the surface to breathe. Chronic cases may involve severe wounds from the entanglement trauma, persistent entanglement or both, whenever the trauma exceeds the capacity for first intention healing.

Case definition

Signalment

Juveniles show a higher risk of becoming entangled (Pemberton et al. 1992, Lien 1994, Arnould & Croxall 1995, Moore et al. 2009, Knowlton et al. 2012, Robbins 2011), but all age classes and genders can be affected.

Epidemiology

Entanglement can persist if fishing gear or marine debris remain attached to the animal without causing immediate death through peracute underwater entrapment (see previous section, p. 235). However, even if gear/debris detaches, severe wounds can remain (Fowler 1985, Heyning & Lewis 1990, Alzueta et al. 2001, Jepson 2006).

[1]For author affiliations, see Supplement 5 at www.int-res.com/articles/suppl/d103p229_supp/

Injury-specific response protocol

In addition to the data and sample collection methods described in the 'Introduction and overview' section, the following steps should be considered to fully document a chronic entanglement case. Describe and photograph gear and any associated wounds (see Supplement 2 for a standardized datasheet). Sketch the best understanding of entanglement. If a case is floating at sea, towing ashore is a priority. If the gear is in any way likely to fall off during towing, then document it in place and remove it before moving animal. Document the body part to which any towline is attached prior to towing. Collect any entangled body parts if practical and examine in laboratory if possible, imaging with CT or MRI if available, and undertake further gross dissection. Tag, collect and submit all gear removed to gear specialists for identification.

Clinical signs

A cetacean or pinniped that comes ashore, or is found floating at sea with signs of entanglement may have had a prior (likely healed) entanglement unrelated to the stranding event, a recent (unhealed) entanglement event that led directly to its death, or a prior entanglement that has contributed to death due to a recurring impairment or trauma. Acute deaths from entanglement may result from drowning or severe trauma (i.e. bleeding out from a traumatic incision, laceration or amputation) during the anchoring phase of the event, when the animal is tethered to a fixed point by the netting or line. Death from chronic entanglement may result from physical injuries of entanglement (described in 'Host response', p. 244) and/or the impairment and energetic burden of the entanglement on the animal.

Evidence of a recent entanglement event is determined by one or more of the following: the presence of gear; gear impressions and/or unhealed injuries such as abrasions, lacerations and contusions at multiple sites on the body; and damaged baleen or teeth. No single mark is likely to be conclusive of an entanglement but cumulatively, these injuries will show evidence of wrapping of at least one anatomic site, or connections of gear between multiple parts of the body. The most common attachment sites are the mouth/head, the flippers and the tail insertion. Injuries associated with an entanglement should be most prevalent at and behind the primary entanglement attachment site(s), as linear marks tend to lead aft due to the forces of drag in free-swimming cases (Cassoff et al. 2011). Cases in which there are linear (or other) marks on the body should be considered consistent with entanglement, but without a clear pattern of wrapping around at least one appendage, protuberance or snout, are not necessarily diagnostic. Single linear marks can also be suggestive of blunt trauma.

Signs of an acute event include the signs above, but the outward conditions of the injuries are fresh/uninfected and do not yet show any healing response. Body condition, skin condition, and cyamid loads are not necessarily altered. There may also be evidence of peracute underwater entrapment (see previous section, p. 235).

Subacutely, the animal may be weak, listing or motionless on the water surface, vocalizing, unresponsive to human approach, and with poor exhalations, appendages or segments of torso submerged due to weight of gear or line, restricted range of motion of flippers, or frank hemorrhage. The animal may show suboptimal body condition, cyamid spread and discoloration, dull skin, or grey or white cutaneous mottles (Pettis et al. 2004).

Signs of a chronic entanglement event include the recent entanglement evidence as defined above, but the lesions appear infected and or damaged with significant host response. There may be expansion of the defect through abrasion or excoriation or incision, with a host response characterized by remodeling, epidermal proliferation, or depigmentation (Fig. 12). If the wound margins are apposed, repair may occur with both first and second intention healing, or if the defect is too large or the area too mobile, the wound may persist with exposed granulation tissue and possible contraction and stricture formation. In some animals, there may be a loss of range of mobility and function, as well as possible atrophy secondary to ischemia. The entangling gear may persist and remain embedded in lacerations (Fig. 13; Moore et al. 2013). Alternatively an acute entanglement may have resolved via disentanglement or shedding of gear, but residual tissue damage, such as structural damage to developing bones, may lead to chronic lesions (Fig. 14). The whale may exhibit emaciation (Figs. 15 & 16a), abnormal skin condition (overall or just at constricted appendages; Fig. 16b), and higher than average cyamid loads often at wound sites and body depressions (Figs. 17 & 18). Large whales commonly carry rope and nets (Figs. 16b & 19). Ingested debris such as nets and other products can be found, especially in sperm whales (Jacobsen et al. 2010). Pinnipeds can have neck-encircling debris such as gillnet, toys (e.g. frisbees), packing strips, and salmon flashers (Figs. 20 & 21; Raum-Suryan et al. 2009). Small odontocetes and pinnipeds can carry and/or consume recreational fishing gear, monofilament and braid, longline, fishing lures and debris such as clothing. Factors contributing to SI and mortality from entanglement include drag-induced negative energy balance and consequent emaciation (Fig. 15), infection, amputation of fluke or flipper, hemorrhage, baleen malocclusion and loss of ram filtration, tissue (Fig. 22), vessel (Fig. 23), and bone damage and constriction (Figs. 12 & 14), ischemia (Fig. 23), atrophy (Fig. 24), and reduction in range of motion (Fig. 14).

It is important to recognize that some large whale species such as the North Atlantic right whale (*Eubalaena glacialis*; NARW) usually float after

Fig. 12. Dissection of left flipper recovered from an entanglement resulting in fibrocartilageneous proliferations of a North Atlantic right whale *Eubalena glacialis*. 1: scapula; 2: shoulder joint; 3: mass of fibro-cartilaginous and partially ossified repair tissue. Catalog: NEAq Eg #2301; VAq case VAQS2005-1008Eg

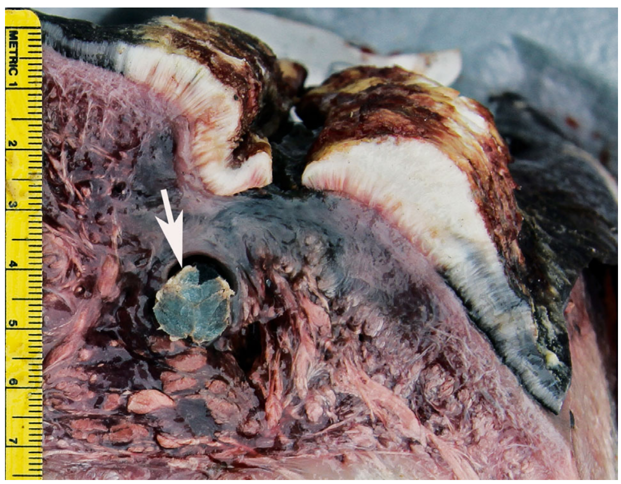

Fig. 13. Section through lip of a North Atlantic right whale, with rope embedded in the left lip (arrow) with severe scar development. This animal was sighted gear-free in February 2010 and then with chronic entanglements in December 2010. It died in January 2011. Catalog: NEAq Eg #3911; FFWC case EgNEFL1103 (Fig. 6 from Moore et al. 2013)

Fig. 14. North Atlantic right whale stranded off Cape Lookout, NC, Jan 2009. The 2007 calf was sighted Aug 2007 in the Bay of Fundy with recent peduncle entanglement lacerations. This trauma was assumed to have deformed developing vertebrae, such that the animal developed a severe scoliosis that eventually debilitated the animal and precipitated a live stranding. (a) Intact animal on beach. (b) Dissection showing deformed spinal column. Catalog: NEAq Eg #3710; UNCW case CALO 0901

Fig. 15. Severe emaciation in a Bryde's whale *Balaenoptera brydei* following a chronic oral entanglement (not visible in this image). Note cargo strap round peduncle was added post mortem for moving animal on beach. UNCW case WAM587

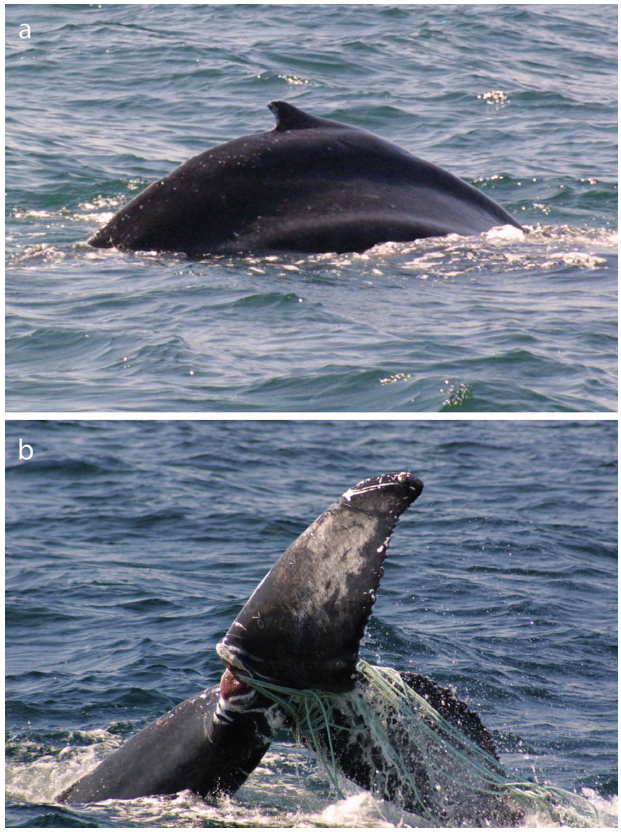

Fig. 16. (a) Flank of an emaciated humpback whale *Megaptera novaeangliae* with (b) severe caudal entanglement with gillnet gear incising the fluke. PCCS case WR-2002-07

Fig. 17. Cyamid spread and nuchal fat loss in North Atlantic right whale. Catalog: NEAq Eg #1102; photo: PCCS

Fig. 18. Head of North Atlantic right whale showing cyamid spread and granulation tissue around rope embedded in the rostrum. Catalog: NEAq EG #1102; photo PCCS

Fig. 19. North Atlantic right whale May 25, 2008. First sighted with entangled right flipper (upper left of image) March 17 2004, gear still attached March 2010, last seen Jan 16, 2012 in moderate condition, but unclear if still entangled. Catalog: NEAq Eg #3346; photo: NEFSC

Fig. 20. California sea lion *Zalophus californianus* chronically entangled in gillnet. TMMC case CSL 4909 (Chelsea)

Fig. 21. Northern elephant seal *Mirounga angustirostris* with a chronic entanglement in a toilet seat. Ano Nuevo, CA. Photo: TMMC

Fig. 22. Damage to North Atlantic right whale blowhole. Rope was entwined around the left baleen plates, passed across the mouth, exiting the right side and then taughtly embedded over the left nares before tightly constricting around the left flipper. Catalog: NEAq Eg #2301 VAq; VAQS2005-1008Eg

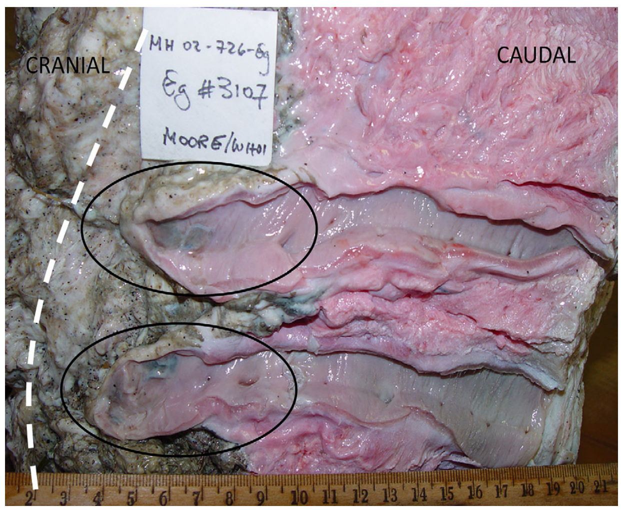

Fig. 23. Dissection of North Atlantic right whale peduncle. Chronic stricture around the ventral fluke insertion severed the 2 lateral superficial veins draining the fluke. These then healed, as seen in this image. The walls of the veins are dissected to show their blind ending at left (black ovals). The course of the lacerating rope is shown with a dashed white line. Catalog: NEAq Eg #3107; photo: WHOI

Fig. 24. Chronic entanglement resulting in severe necrosis and tissue defects in a live entangled humpback whale. PCCS case WR-2006-15

death, but are more likely to sink if they have become emaciated and thus less buoyant due to depletion of lipid stores (Allison et al. 1991, Reisdorf et al. 2012). Also, not all persistent entanglements are fatal (Fig. 19), although the long-term sub-lethal effects of these persistent entanglements are not clear.

Host responses

Host responses include emaciation and, in right whales, parallel white lines radiating from the blow hole (also called 'rake' marks; Fig. 25) (Pettis et al. 2004). The timing of these changes appears to be variable. Skin discoloration (mottling from extreme sloughing), swath lesions (Fig. 25), and flipper discoloration are also observed. Note the ability to detect skin color change depends on normal skin color: white humpback whale flippers and right whale belly patches show bruises, whereas black skin does not. In contrast, skin edema and sloughing is better seen in black skin. Persistent spinal deviation, altered respiration and lethargy have also been observed. There are numerous other causes of these host responses, but in the context of persistent entanglement, these changes are commonly related to trauma (Fig. 14).

Injury-specific gross necropsy findings

These can include hematoma, edema, and shock (multi-systemic congestion and disseminated intravascular coagulation). Ingested material and debris may be found. Incompletely healed scars with or without associated abscessation, fractures, and serous atrophy of fat are also present at times. Disuse osteopenia; muscular and skeletal damage such as jaw, flipper, spine fracture; disuse atrophy; and formation of pseudoarthroses in the jaw have been described (Moore et al. 2004, Cassoff et al. 2011).

Fig. 25. Rake marks (linear white marks below blowhole) and swath lesions anterior to the rake marks (Hamilton & Marx 2005) in North Atlantic right whale after severe entanglement injury. Catalog: NEAq Eg #1608; photo: PCCS

Lesions can also include granulation, abrasion, laceration, impression, incision, and constriction (Moore et al. 2004, Cassoff et al. 2011). Fishing gear may be embedded to various depths (Figs. 13, 16b, 18 & 20) and may be accompanied by massive fibro-osseous periostitis (Fig. 12) (Moore et al. 2004, Cassoff et al. 2011).

Toxicology

Biotoxins may impair mental awareness, change behavior and precipitate anthropogenic trauma, although this has not been documented in large whales.

Histological findings

Proximate effects can include fibrosis (early, late), acute to chronic inflammation (osteomyelitis, dermatitis, or cheilitis), hemorrhage (acute/chronic-macrophages, hemoglobin pigments such as hematoidin), fibrin, vascular thrombosis, localized infection (open wound) (acute and chronic), myofiber degeneration/fragmentation/necrosis/mineralization, bony response, and cardiac and skeletal muscle contraction bands.

Host response effects can include serous atrophy of fat/edema, muscle atrophy, osteopenia (also requires gross information such as flipper weight), metabolic/stress-adrenal cortical hypertrophy, medullary hyperplasia, and adrenal cortical lipoidal degeneration.

COD assignation

Confirmed

Sufficient evidence to conclude that entanglement was the proximate COD requires a combination of the above factors that logically lead to a major decline in health, resulting in death from consequent factors, such as inanition from emaciation, metabolic exhaustion from increased drag, exertional myopathy, overwhelming infection or starvation, or amputation, secondary to the chronic effects of ischemic necrosis and loss. The entangling material need not still be present at the time of death. The post-mortem condition of cases examined to date has rarely allowed diagnosis of ultimate COD, thus inference from the above at-sea observations, gross necropsy data, and histological information as available, need to be assimilated into an assessment of the most parsimonious interpretation of the reason(s) for the demise of the animal.

Probable

A finding of probable would arise if some or all of the above factors were present, but carcass quality could not allow confident linkage of entanglement evidence with observed condition of the mortality.

Suspect

Suspect cases would have evidence of current or past entanglement, without sufficient findings to link the entanglement to major consequent changes in the animal, but that still had a suggestion of linkage.

BLUNT FORCE TRAUMA INDUCED BY VESSEL COLLISIONS WITH LARGE WHALES

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Background

Collisions between watercraft and pinnipeds and cetaceans can have adverse effects on the health of individual animals as well as the population status of some endangered species (e.g. NARW) (Kraus et al. 2005). The severity and type of trauma resulting from a collision depends on a number of factors, including vessel speed and size (Fig. 26) (Laist et al. 2001, Vanderlaan & Taggart 2007), the angle of impact, the anatomic site of contact with the body, and whether

Fig. 26. Humpback whale and cruise ship in Glacier Bay, Alaska. Photo taken under NMFS Scientific Research Permit No. 945-1776-00

Fig. 27. Manipulating North Atlantic right whale up to a high position on the beach for necropsy. The left fluke was missing when initially discovered, thus rolling the carcass is more practical than the usual attachment of a hawser to the peduncle, despite the fact that rolling with machinery increases the risk of post-mortem bone damage. Catalog: NEAq Eg #1909; photo: VAq

the strike occurred ante- or post-mortem. In contrast to sharp traumatic injuries to large whales typically associated with propeller strike, blunt force trauma may be attributed to a number of physical insults. As most necropsy data on large whales are collected from carcasses hauled up onto beaches, care should be taken interpreting pre- and post- mortem trauma that could be caused by the necropsy itself (Fig. 27). Here, we will focus on blunt trauma injuries associated with ante-mortem vessel collisions with large whales.

Broadly, blunt trauma injuries fall into 4 categories, defined by DiMaio & DiMaio (2001) as (1) abrasions, (2) contusions, (3) lacerations, and (4) fractures of the

[1]For author affiliations, see Supplement 5 at www.int-res.com/articles/suppl/d103p229_supp/

skeletal system. Campbell-Malone et al. (2008, p. 51) characterized blunt force trauma as follows: 'mechanical stress applied to a body causes blunt force trauma. To cause a blunt force injury, stress applied to the tissue must be great enough to deform the elastic or viscoelastic tissue beyond its ability to recover or maintain integrity. This can occur in situations where (1) the magnitude of the applied stress is greater than the ultimate strength of the tissue; (2) the stress is imparted in an unnatural direction, loading the tissue in a direction with weaker material properties; or (3) the stress is applied to mechanically inferior pathologic tissue.' As a result, blunt force trauma disrupts the integrity of tissues and can result in impact abrasions, contusions (hematoma), tears, shears, and crush injuries.

Blunt force trauma injuries in large whales may be attributed to a number of physical insults, including impact of the body against a surface (e.g. beach, piers, or moorings) or contact of a moving object with the body. In a vessel collision, blunt force trauma injuries result from contact with a non-rotating feature of the vessel (e.g. bow, hull, rudder, or skeg), while sharp force trauma injuries involving incising wounds typically result from contact with the sharp, rotating propeller of the vessel. Vessel collisions may involve blunt trauma, sharp trauma, or both. Blunt force trauma injuries from collisions can range from non-lethal superficial abrasions and contusions to severe lethal impact wounds (Campbell-Malone et al. 2008). Ante-mortem blunt force trauma collision injuries vary in their presentations; however, common features include the presence of a well-defined focal area of subcutaneous hemorrhage and edema (Fig. 28), torn/physically disrupted muscle (Figs. 29 & 30), fractured bones, and disruption of organ systems (Rommel et al. 2007, Campbell-Malone et al. 2008).

Blunt force trauma may be attributed to a number of human and nonhuman interactions; due to the lack of clinical history, surveillance and necropsy data, it is often difficult to resolve the specific nature of the injury and relative proportions of these 2 forms of physical trauma to injury or death in populations of animals. Inadvertent and malicious boat or propeller strikes are recognized in pinnipeds, ottarids and mustelids, and ship strikes of large cetaceans are likely related to a combination of increased vessel speed and size and aggregations of animals in areas of high vessel traffic (e.g. shipping lanes). Attempted predation and aggression between conspecifics to establish territory and breeding hierarchy may also be considerations.

Fig. 28. Blue whale *Balaenoptera musculus* with focally extensive subcutaneous and possibly muscular hemorrhage. SBMNH case SBMNH-2007-19

Fig. 29. Fin whale *Balaenoptera physalus* beached after removal from the bow of a vessel. Note major thoracic depression coinciding with impact site. VAq case VAQS20071011_Bp

Fig. 30. Sei whale *Balaenoptera bopealis* showing areas of chafe and contact with ship's bow. VAq case VMSM2003_1006Bb

Although entanglement injuries may also be considered sharp or blunt force trauma, they will not be considered in this section.

Blunt force trauma of natural origins

Non-anthropogenic blunt force injuries, such as those associated with intra- and inter-specific aggression can lead to mortality, are well-described elsewhere for small cetaceans (Ross & Wilson 1996, Patterson et al. 1998, Dunn et al. 2002) but not for large whales as yet.

An age-related injury is specifically recognized in neonatal bottlenose dolphins with infanticide (Dunn et al. 2002) and harbor porpoises, killed by bottlenose dolphins (Ross & Wilson 1996). No external lesions may be apparent on necropsy and reflection of the skin may reveal focally extensive subcutaneous hemorrhage within the throat, thoracic and abdominal regions. Massive thoracic and abdominal hemorrhage with occasional lung and liver ruptures are recognized. Physical trauma has also increasingly been documented in walruses; due to receding ice levels, increasing numbers of adult, subadult and juvenile animals haul out on shore and when startled, stampede and trample primarily young animals (Fay & Kelly 1980). Attempted predation and aggression with conspecifics may also be considerations. Impacted animals may present with protruding and occasional hemorrhagic eyes, swollen heads, appendages and random regions throughout the trunk. Involvement of appendages or the facial region may impede normal locomotion (loss of function) and interfere with foraging and predation. In cetaceans, animals may present with no apparent external lesions, superficial poorly circumscribed transverse to oblique linear white depressions, focally extensive pallor, subcutaneous swelling or asymmetry of the torso (vertebral dislocation).

Case definition

Signalment

In large whales all age classes and both sexes are susceptible to blunt force trauma induced by vessel collisions. Stranding data indicate that in some species calves and juveniles may be at a higher risk of vessel collisions than adults (Wiley et al. 1995, Knowlton & Kraus 2001, Laist et al. 2001, Panigada et al. 2006, Douglas et al. 2008, Carrillo & Ritter 2010, Neilson et al. 2012), although in blue whales vessel strikes have primarily involved adults. However, it is unknown if young animals are more likely than adults to be struck by vessels (based on differences in their behavior, sightability, or other factors) and/or if young animals are more likely to suffer lethal injuries from blunt force trauma because of their smaller body size.

Epidemiology

Most commonly, anthropogenic blunt force injuries are associated with vessel strikes, although impact with piers, moorings and other submerged structures may also be considerations. Blunt force trauma is possible whenever vessels and animals are in close spatial proximity. Blunt force trauma wounds have been observed in an array of small and large cetaceans, ranging from coastal delphinids to mysticetes (Wells & Scott 1997, Moore et al. 2004, Van Waerebeek et al. 2007, Wells et al. 2008). Vessel collisions, including those involving fatal vessel strikes, have been documented during all months of the year (Laist et al. 2001, Panigada et al. 2006). Mapping of documented blunt force injury trauma cases may provide valuable insights into additional contributing factors (e.g. NARW calving grounds) (Neilson et al. 2012, van der Hoop et al. 2013).

Fourteen of the 30 NARW for which necropsy reports were examined by Moore et al. (2004) were determined to have been killed by vessel collisions. This represents at least 3% of the population, and is probably an underestimate given the uncertainties regarding carcass recovery rates. Similarly, Campbell-Malone et al. (2008) found that 21 of 40 NARW necropsies identified vessel collision as the most probable COD, with 9 (22.5%) resulting specifically from blunt trauma. Van der Hoop et al. (2013) summarized data from 1762 mortalities (all known) and serious injuries (likely fatal) involving 8 species of large whales in the Northwest Atlantic (23.5°N to 48.0°N), from 1970 through 2009. Vessel strike was the third leading determined COD (171/750) for all species combined, and the leading determined COD for fin (59/116) and right (38/87) whales.

Injury-specific response protocol

In addition to the data and sample collection methods described in the 'Introduction and overview' section (e.g. Fig. 31), the following steps should be considered to fully document a blunt-trauma case.

Fig. 31. Field response team collecting external morphometrics on the fetus of North Atlantic right whale on 14 January, 2005. Catalog: NEAq Eg #2143 'Lucky'; photo: UNCW

Fig. 32. Live humpback whale (southeastern Alaska ID #954 in 2008) The origin of the injury, first seen in 1989, is unknown, however the lesion closely resembles confirmed bow strike lesions. Photo: Steve Lewis (taken under NMFS Scientific Research Permit No. 14122)

Fig. 33. Focal pallor (arrow) on the left flank of a live humpback whale approximately 1 h after the whale was struck at that point by the bow of a 72 ft (~22.2 m) catamaran in Glacier Bay on Aug 31, 2011. NMFS AK Region accession #2011142

The carcass should be fully flensed to examine skeletal elements for evidence of blunt trauma and either fracture, subluxation or luxation with hemorrhage (Fig. 2; see 'Introduction and overview').

Ancillary diagnostic samples (e.g. for biotoxin testing, bacteriology, virology, parasitology, hormonal testing) should be collected when practical to exclude or confirm the possible contribution of other pathologic factors to ante-mortem morbidity and possible predisposition to vessel collision. Such diagnostics can include diagnostic evaluation of fluids or swabs from pleural and abdominal cavities, as well as tissue cultures and bacteriology of major internal organs. If there is evidence of material transfer (e.g. hull paint), sample the tissue or object and preserve.

If blunt trauma is found, diagrams and photographs of the lesions should be supplied with the gross report (Figs. 2, 28 to 30).

Clinical signs

Typical signs include impaired locomotion, abnormal body posture/positioning, lethargy, subcutaneous swelling, external discoloration of the skin at the site of impact, impaired or forced respirations, disarticulation or malocclusion of the mandible, prolapsed eyes, periorbital swelling and conjunctival hemorrhage, hemorrhage from the nares or blowhole, shock, unresponsiveness, inappetence or anorexia. Localized to regional asymmetric subcutaneous swelling with elevation of the affected area above the plane of adjoining, normal skin may occur. Conversely, impacted areas can show marked depression at the site of the injury related to destruction of underlying tissue (Figs. 29 & 32). The external aspect of the impact site may be de-pigmented with white transverse to oblique poorly circumscribed bands or depressed furrows (Fig. 30) or focally extensive pallor (Fig. 33) related to more superficial abrasions, erosions, and lacerations. Visual health assessment (cyamid load, color) can provide information on general health status (Pettis et al. 2004). Circulatory assessment via infrared thermography can also be very instructive in relation to hemorrhage and edema in the acute phase of injury to inflammation, wound healing, and possible resolution with more chronic non-lethal progression of the injury. Varying degrees of wound resolution with primary and/or secondary intention healing may be observed.

Clinical diagnostic data are often unavailable; however, depending on the time between impact and subsequent clinical evaluation or post-mortem examination, an inflammatory leukogram, electrolyte imbalances, possible anemia, and elevated serum amyloid A (SAA) and creatinine kinase may be observed.

Injury-specific gross necropsy findings

Field observations of floating or beach-cast animals may include localized to regional asymmetric subcutaneous swelling with elevation of the affected area above the plane of adjoining, normal skin. The external aspect of the impact site may be depigmented with white transverse to oblique poorly circumscribed bands or depressed furrows or focally extensive pallor related to more superficial abrasions, erosions, and lacerations (Fig. 29).

However, in cases of impact with smooth objects, there may be no externally visible signs of blunt trauma, or signs may only be observed following internal examination and retrospective correlation of external lesions. On reflection of the skin, variably extensive subcutaneous hemorrhage (Fig. 34), which may track dependently as well as extend deep into the adjoining skeletal musculature (Fig. 35), is typically observed. Evaluation of the impact site for associated subcutaneous edema and hemorrhage is imperative. Should the animal survive impact/previous injury, resolution of the hemorrhage should be apparent on gross exam and histopathology. In more severely affected animals, physical impact may result in muscle shredding, especially surrounding bones, and rupture of the liver or spleen, or lung with massive hemoperitoneum or hemothorax, respectively.

Histological samples should be collected and the location and extent of the defect described.

Large volumes of dark red serous fluid are commonly observed in the abdominal cavity, and to a much lesser extent, thoracic cavities; it is important to differentiate this fluid from acute hemorrhage by detection in the latter of fibrin strands on serosal surfaces, spontaneous clot formation on exposure to air, and other criteria. Herniation, transposition and prolapse of internal viscera may also be apparent. If the injury is localized to the head, careful dissection of the cranium and assessment of the superficial brain for coup contre coup lesions is recommended. Brain swelling and occasional herniation of the brain stem and posterior cerebellum through the foramen magnum may also occur. Cranial, mandibular, scapular and rib fractures and luxation and subluxation of vertebrae at the point of impact with occasional comminuted fractures of lateral or dorsal processes may also be apparent (Figs. 36 to 38).

Fig. 34. North Atlantic right whale focal hemorrhage in lateral blubber with sample location identified by 2 incisions. Note epidermis has been shed post mortem in this region of the animal. WHOI case MJM0906Eg

Fig. 35. Deep hemorrhage on the lateral surface of the left ribs of a North Atlantic right whale. UNCW case KLC 022 Eg

Histological findings

Histologic findings in whales with blunt trauma vary considerably based on anatomic site of impact,

Fig. 36. North Atlantic right whale axial skeleton laid out in sequence to locate multiple fracture of the left transverse processes, and a shattering of the lumbar vertebra in the green gloved hand. WHOI case MJM 9406Eg

Fig. 37. Humpback whale with (a) the right mandible protruding laterally beyond the contour of the skull and (b) the compound fracture in the right mandible. VAq case VMSM 961010

deep visceral versus more superficial subcutaneous involvement, carcass condition code, and temporal association between wound injury and death (e.g. peracute, acute, chronic) (Campbell-Malone et al. 2008). In peracute lethal situations, no microscopic cellular responses may be apparent. In the early stages of injury, hemorrhage (Fig. 39) admixed with variable amounts of edema and fibrin deposition may be apparent in the subcutis and subjacent musculature. If the injury is non-lethal, the hemorrhage may resolve in 14 to 21 d with a progression from red or blue (2 to 4 d) to green (4 to 7 d) to yellow (7 to 14 d) discoloration of the area associated with metabolism of hemoglobin, hematoidin and hemosiderin. These color changes may not be easily identifiable on whales with very dark epidermis or carcasses only photographed floating at sea. Myocellular degeneration and necrosis may be apparent.

Over time, inflammatory cells are recruited to the affected area with fibroplasia, neovascularisation and eventual granulation tissue formation. Secondary microbial involvement is unlikely in acute cases; however, if a pre-existing bacterial infection was present, localization to the hematoma and hemorrhage with abscessation may occur. Clostridial myositis has been documented (Greenwood & Taylor 1978); however, this is uncommon. Detailed microscopic review of select live beach-cast animals with prolonged recumbency has not demonstrated any indication of compartmental type syndrome (Mabee & Bostwick 1993) or myoglobinuria. Abdominal or thoracic visceral fractures with hemorrhage and fibrin deposition, as well as draining hemorrhage in regional lymph nodes may be evident.

Toxicology

At present, anecdotal evidence suggests a possible correlation of elevated domoic acid and vessel interactions with select pinniped and cetacean species. Biotoxins (domoic acid, brevetoxin, saxitoxin) have been documented in some instances of large whale

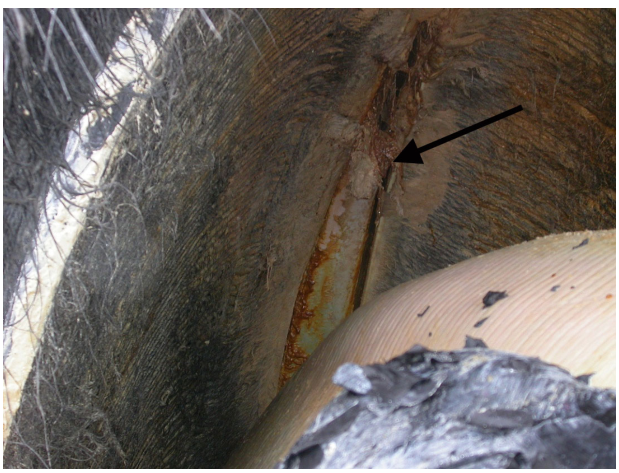

Fig. 38. Oral rete in North Atlantic right whale, with evidence of soft tissue tearing and coagulated blood in the baleen racks (arrow). Deep to the tears was a complete fracture of the premaxillary and maxillary rostral elements. Catalog: NEAq Eg #1004 'Stumpy'; VMSM 2004 1004 Eg

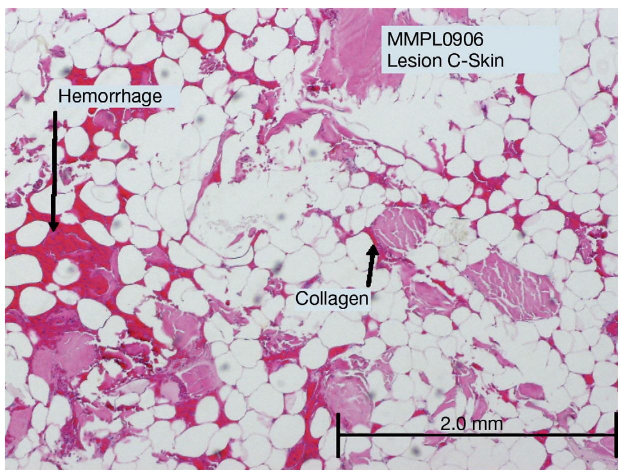

Fig. 39. Extravasation of erythrocytes in the fibroadipose of a Bryde's whale. Considered to be most suggestive of an antemortem occurrence, though hypostatic congestion could also occur if the abrasions were located in a region that would be considered 'dependent'. MMPL0906. Photo: D. Rotstein

strandings (Geraci et al. 1989). More comprehensive post-mortem examinations of suspect animals and screening for harmful algal blooms may provide valuable insights into the potential contributing roles of these compounds (see case report V10-137 *Mn*, 7/30/2010 from Douglas Island, Juneau, Alaska; NMFS AK Region accession #2010089).

Confirmed versus suspect cases

Confirmed

Witnessed and documented strikes or mortality with corroborating lesions are the most confident factors to confirm a case (e.g. Whale 68, NMFS AK Region accession #2011038; SBMNH case SBMNH2007-20 *Mn*). The sum of the observations should lead to death by blunt trauma being the most parsimonious explanation for the case to be confirmed. Such cases should include a number of the following: frank hemorrhage with edematous fluid in the subcutaneous tissue; hematoma formation; laceration or rupture with hemorrhage within the skeletal musculature; hemothorax; hemoperitoneum; visceral displacement, herniation or rupture; skeletal fractures, luxations or subluxations with associated hemorrhage; microscopic fat emboli; acute hemorrhage; edema; rhabdomyocytolysis; subcapsular and medullary draining hemorrhage in regional lymph nodes; history of animal on bow of vessel. Evidence of chronic lesions (pyothorax, abscessation) may accompany findings.

Probable

This conclusion requires a necropsy (as practical), blunt-trauma sequelae, and histopathology supportive of gross findings of trauma if available to be collected (e.g. MMPL0906 Bb, VAQS 20051017 Bp, KMS 374 NEAq Eg #1308 UNCW). A 'probable' case will have similar gross necropsy and histopathology findings to a 'confirmed' case but insufficient information to conclude that other interpretations of COD are not as likely.

Suspect

Cases where necropsy was limited or no necropsy was conducted, or cases with advanced decomposition and blunt-trauma sequelae, but limited or no histopathology findings of trauma, cannot be rated more confidently than 'suspect'. Unilateral and localized abrasions with epidermal pallor, depressed bands or furrows of depigmented epidermis and subcutaneous swelling may be considerations. Abnormal body posture/positioning, vertebral fractures and distension of the abdominal cavity may also support blunt trauma. Prolapse of the colon, reproductive tract or stomach in fresh dead animals may also suggest deep blunt force trauma. Blunt-trauma sequelae and/or bony lesions consistent with blunt trauma may be present; the carcass may or may not have other signs of pathology from entanglement or disease (e.g. UNCW case NCARI 006 Eg).

SHARP TRAUMA INDUCED BY VESSEL COLLISIONS WITH PINNIPEDS AND CETACEANS

Alex M. Costidis[1], Michelle Berman, Tim Cole, Amy Knowlton, William A. McLellan, Janet Neilson, D. Ann Pabst, Stephen Raverty

Background

Watercraft collisions can take the form of blunt-force impacts involving contact between the animal and some non-rotating feature of the vessel (e.g. hull, rudder, skeg), sharp-force injuries such as incising and/or chopping wounds typically generated by the sharp, rotating propeller of the vessel, or a combination of the 2 types of injuries (Lightsey et al. 2006, Rommel et al. 2007, Byard et al. 2012). Additionally, a less distinct category is occasionally observed in which a relatively sharp but non-rotating feature of a vessel (e.g. skeg or rudder) has enough speed and sharpness to penetrate the skin, causing a combination of blunt and sharp-force injuries likely best defined as a chop wound. Sharp-trauma injuries to cetaceans and pinnipeds range from mild nonfatal superficial nicks to severe amputations and other lethal wounds (Kreuder et al. 2003, Campbell-Malone et al. 2008).

Injuries resulting from sharp force trauma in pinnipeds and cetaceans vary in their presentation; however, a common feature is the presence of more than one approximately parallel and roughly equidistant laminar incising wound ranging from simple linear to abruptly curvilinear or sigmoid (S- or anti-S

[1]For author affiliations, see Supplement 5 at www.int-res.com/articles/suppl/d103p229_supp/

shaped) defects (Rommel et al. 2007). This presentation is typically associated with propeller-induced trauma. Vessel-related sharp-force trauma can also present as a single wound composed of a linear or curved incision or chop wound. This is more common in cases involving contact with a large propeller, relative to the animal's size, along appendages and external protuberances, or along regions of significant convexity of the animal's surface where only one propeller blade makes contact. The depth of penetration of the propeller is related to the proximity of the propeller to the animal as well as the propeller radius and the type of propeller (e.g. exhaust-through hub vs. narrower shaft-driven hub without exhaust-through hub). The type of propeller (e.g. performance propeller with high rake vs. working propeller with low rake) can dictate the degree of curvature of the incising wounds. The distance between successive propeller wounds (i.e. cut span) is sometimes representative of the propeller pitch but can vary substantially due to operational characteristics of the vessel. Although not definitively diagnostic of propeller or vessel type, cut span can sometimes provide valuable information when coupled with other wound features such as wound shape, chord length and chord depth (Rommel et al. 2007). It should be noted that the distance between successive propeller wounds is also affected by the gape of the wound. Since incising wounds have a tendency to gape due to intrinsic (e.g. Langer's lines due to skin/blubber fiber orientation) and extrinsic forces (e.g. shearing and stretching forces generated by locomotory movements) it can hinder interpretation of cut span (DiMaio & DiMaio 2001, Rommel et al. 2007). Stab and puncture wounds are also considered to be sharp trauma wounds but are not typically associated with watercraft-induced wounds.

Non-anthropogenic sharp-force injuries can lead to mortality but are often sporadic (e.g. infanticide, attempted predation) or not associated with significant trauma (e.g. conspecific rake marks) and are therefore not discussed.

Case definition

Signalment

There is no gender bias known at the present time, though there could be bias due to ship strikes in calving grounds where females are more common (e.g. NARW) (van der Hoop et al. 2013). Wounds have been documented to heal, only to break down years later during pregnancy (Fig. 40). Age biases appear to exist with respect to likelihood of ship strike, with juveniles showing a greater tendency to be struck (Wiley et al. 1995, Hamilton et al. 1998, Knowlton & Kraus 2001, Laist et al. 2001, McLellan et al. 2004, Panigada et al. 2006, Lammers et al. 2007, Douglas et al. 2008, Berman-Kowalewski et al. 2010, Carrillo & Ritter 2010, Neilson et al. 2012). Biases related to recovery rates of perinates/juveniles are not known. Nonetheless, there could be reduced recovery of propeller-struck neonates/juveniles due to smaller size, lesser positive buoyancy due to modest fat reserves, and greater percentage of tissue loss due to predation. It is unknown whether injured perinates/juveniles are more likely to be predated upon or scavenged, and hence less likely to be recovered.

Epidemiology

Most commonly, sharp-force injuries in pinnipeds and cetaceans are associated with watercraft vessel interactions. Sharp-force trauma is possible when-

Fig. 40. North Atlantic right whale presenting large propeller wounds and resolving scars along the dorsolateral aspect of the torso. Injuries were incurred 14 yr previously as a calf. Note the variation in pathologic presentation in this case. In the more cranial region, there is second intention healing, whereas the most caudal incision features partial retraction of the wound margin and exposure of the underlying dermis. There is patchy loss of epidermis due to postmortem exfoliation. Histopathology of the wound margins confirmed healing skin, and the cause of death was attributed to long-past propeller-induced sharp trauma likely opened up by her first full-term pregnancy, with presumptive secondary sepsis/bacteremia. Expansion of the body cavity by the uterus containing the fetus, fetal membranes, and fluids was thought to have resulted in increased abdominal pressure and stretching of the abdomen resulting in structural tension greater than the tensile strength of the scarred propeller wounds. Catalog: NEAq Eg #2143 'Lucky'; photo: UNCW

ever vessels and animals are in close spatial and temporal proximity, as is seen in areas of high vessel traffic, particularly along the continental shelf (Fonnesbeck et al. 2008, Berman-Kowalewski et al. 2010). Propeller wounds have been observed in an array of small and large cetaceans, ranging from coastal delphinids to mysticetes (Wells & Scott 1997, Moore et al. 2004, Van Waerebeek et al. 2007, Wells et al. 2008), as well as sea otters and a number of pinnipeds (Goldstein et al. 1999, Kreuder et al. 2003). Campbell-Malone et al. (2008) found that 21 of 40 NARW necropsies identified vessel collision as the most probable COD, with 11 (27.5%) resulting specifically from sharp trauma. Propeller injuries have been observed during all months of the year; however, specific temporal and spatial exceptions may occur in some species, particularly when feeding or breeding ranges intersect with shipping routes or boating channels (e.g. blue whales and NARW) (Fonnesbeck et al. 2008, Berman-Kowalewski et al. 2010). In some cases (M. J. Moore unpubl. data), drift analyses have been useful for assessing the possible origin of mortality and transiting of shipping lanes. Retrospective studies to identify more frequented shipping lanes may be indicated.

Clinical signs of sharp trauma

Clinical signs

Depending on the location, distribution and severity of the trauma, typical signs may include impaired locomotion, with logging or listing at the water surface; single or multiple approximately parallel, linear to curvilinear, roughly equidistant, shallow to deeply-penetrating incising or chopping wounds with associated hemorrhage or exposed red and/or pink muscle (if acute), or gray to tan/white muscle (if more chronic). In those cases where large diameter blood vessels have been incised, extensive hemorrhage and lethargy may be apparent. In more chronic lesions, wound repair around the margins of the lesions with focal or multifocal, irregular and/or excessive cyamid distribution along body may occur. In those cases of traumatic lethal amputations, portions or entire appendages, trunk or head may be lost.

Diagnosis of sharp trauma is based upon the following: incisions of skin (Figs. 41 to 44) and possibly deeper structures that may vary in size, depth, extent and location; presence or absence of hemorrhage, amputation, pneumothorax, extrusion of thoracic or abdominal viscera; buoyancy abnormalities; secondary infections; or epidermal color changes (e.g. gen-

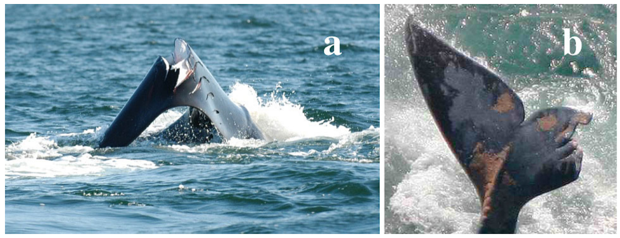

Fig. 41. Flukes of a North Atlantic right whale incised by a propeller: (a) March 10, 2005, (b) September 3, 2005. Note the substantial cyamid spread and tissue failure. The animal has not been sighted since. Catalog: NEAq Eg #2425; photos: (a) NEAq, (b) Tim Voorheis

Fig. 42. Sublethal propeller incisions in the dorsal aspect of a humpback whale calf. Photo: PCCS

Fig. 43. North Atlantic right whale. Extending from the rostrum along the left lateral aspect of the torso, there are 20 curvilinear propeller incisions of varying depth and length with more pronounced 'gaping' along the thoracic wall. Note the absence of visible hemorrhage associated with wound margins (presumptive washout), but blood pouring out of the ventral aspect of cut 8. The white areas of shark scavenging can be seen along many of the propeller wounds; this is a common finding that can affect evaluation and interpretation of sharp-trauma wounds. FWC case GA2006025

Fig. 44. North Atlantic right whale exhibiting fluke amputation at the mid-peduncle and a large gaping propeller incision through the abdominal musculature, partially exposing loops of intestines and providing a portal for salt water to enter the abdomen. UNCW case EgNEFL0602

Fig. 45. North Atlantic right whale showing unilateral amputation of the left fluke with degloving of the skin to the midlevel of the peduncle. Note the angulated and abrupt margin of intact skin, cranial to this defect. The cause of death was attributed to acute propeller-sharp trauma, with hypovolemic shock. The macerated subcutaneous tissue is due to prolonged immersion in salt water and putrefaction. Catalog: NEAq #1909 KMS374; UNCW case

eralized pallor) associated with wound sites. Visual health assessment (cyamid load, skin or fur color, body condition) can provide information on general health status of impacted animals (Pettis et al. 2004). Similar to blunt-trauma cases, circulatory assessment via infrared thermography can also be instructive in relation to wound healing or inflammation. Varying degrees of healing may be present, with primary and/or secondary intention healing visible.

Even shallow open wounds represent an entry point for opportunistic or secondary microbial colonization, proliferation and deeper tissue invasion, which may interfere with wound resolution. Additionally, scar tissue can affect the resilience and function of affected tissue, potentially contributing to reduced tensile strength, as evidenced by right whale 'Lucky' (NEAq #2143) (Fig. 40) or possible restriction in range of motion, with involvement of an appendage.

Clinical observations and diagnostic data are often unavailable; however, inflammatory leukograms, electrolyte imbalances, inflammatory response, anemia, elevated serum amyloid A and creatinine kinase have been observed in live stranded animals and may provide further support regarding prognosis for recovery or immediate COD (Dierauf & Gulland 2001).

Gross necropsy findings

Gross findings of sharp trauma range from small incising wounds to large transecting and/or bisecting chop wounds (Figs. 41 to 43) or amputations (Figs. 44 & 45) (DiMaio & DiMaio 2001, Lightsey et al. 2006, Rommel et al. 2007). Soft tissue damage that penetrates the blubber and deeper muscle layers is often associated with shearing and/or shredding of muscle fibers, severing of large caliber blood vessels and potential hypovolemic shock (Lightsey et al. 2006, Campbell-Malone et al. 2008). Deeper penetrating incisions may extend to and involve internal organs (Fig. 43). Depending on anatomical location, the musculoskeletal and integumentary systems can be differentially affected by shallow and/or deeply penetrating wounds.

The time between initial insult and death may be so abrupt that death may ensue with few microscopic lesions (peracute death). In animals surviving the initial trauma, gross and microscopic changes may range from a defect in the skin with varying degrees of hemorrhage and edema (e.g. Fig. 42), to resolving wounds with primary and second intention healing (Fig. 40). The absence of blood in an incised or chop wound does not discount an antemortem event, as prolonged wound exposure to water results in hemolysis and leaching of blood from wounds. Depending on the anatomic location, such as more mobile regions of skin in the axillae or oral cavity, complete resolution of the injury and scar formation may be compromised due to repetitive motion or lack of apposition of the wound margins. When a large caliber blood vessel is incised or transected massive hemorrhage and exsanguination occurs (e.g. Fig. 45). In many cases, prolonged time between death and necropsy of pinnipeds and cetaceans may result in tissue decomposition and hamper pathologic assessment of wounds.

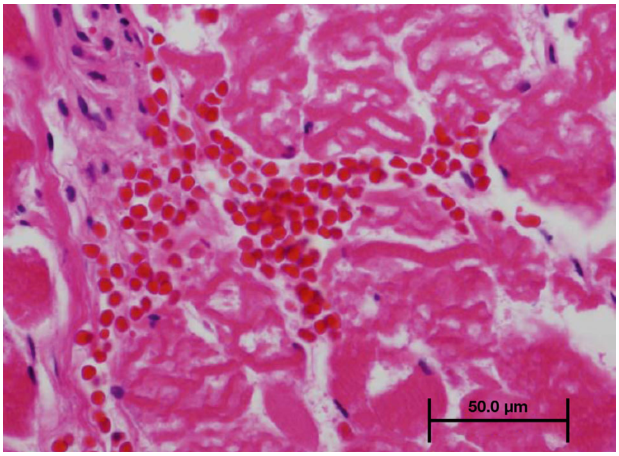

Fig. 46. Photomicrograph of muscle beneath propeller laceration in North Atlantic right whale (see Fig. 44) showing endomysial hemorrhage and muscle degeneration characterized by loss of sarcoplasmic striations, cytoplasmic vacuolation and contraction bands. UNCW case EgNEFL0602

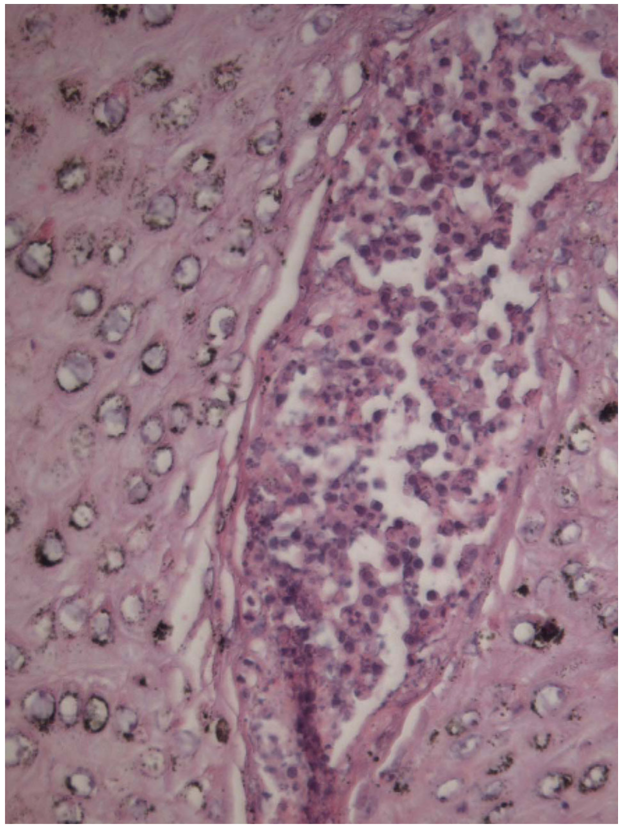

Fig. 47. Dorsal tip of caudal scar, with neutrophils packing vascular channels and infiltrating the supporting stroma of North Atlantic right whale Catalog: NEAq Eg #2143 EgNEFL0501 'Lucky'. See Fig. 40 for case details

Histological findings

The microscopic features of sharp trauma vary considerably and depend on carcass condition code, extent of environmental wound exposure, secondary microbial invasion or cyamid infestation, and stage of pathogenesis of the wound (e.g. peracute, acute, chronic resolving). The histopathological presentation of wound exposure (to salt water, heat, or air drying) in pinnipeds and cetaceans is not well characterized. Typical findings of acute sharp trauma may include subcutaneous edema and hemorrhage with myofiber degeneration, necrosis, and contracture. Wound-associated hemorrhage and myocellular degeneration and necrosis (Fig. 46) can be important determinants of ante- versus peri- or post-mortem injury; however, artifactual loss (e.g. imbibed water due to disruption of the epidermis or autolysis) and/or peracute death before manifestation of microscopic lesions may confound interpretation. Sequelae to sharp injury trauma are typically primary or secondary intention injury-repair (e.g. healing propeller wounds), although secondary microbial involvement, pre-existing systemic disease, and nutritional status of the affected animal may impede or disrupt this process (Fig. 47). Gas introduction via embolization from the wound, as well as pneumothorax may also be present.

Toxicology

Biotoxins (domoic acid, brevetoxin, saxitoxin) may interfere with normal mentation and possibly predispose an animal to traumatic injury. Toxicants and contaminants (e.g. PAHs) may disrupt normal immune function and impede inflammation and normal healing. Gas embolization may also result from sharp wound injury, and if air can be aspirated, chemical analysis to determine ante versus post-mortem gas introduction may be considered (Bernaldo de Quirós Miranda 2011).

Injury-specific response protocol

In addition to the data and sample collection methods described in the 'Introduction and overview' section, the following steps should be considered to fully document a sharp trauma case. Collect wound measurements (e.g. chord length & chord depth) on all propeller wounds to aid in propeller diameter and subsequent vessel size estimation. Additional wound measurements as described in Rommel et al. (2007) can also inform the forensic interpretation.

Although sharp trauma is easily recognizable, assessment of wound injury and differentiation between ante- and post-mortem trauma can be more complex determinations. Examination of sharp trauma lesions should include detailed documentation (e.g. photo, scar/wound sketch/measurements), gross inspection and histologic sampling of soft and hard tissue wound margins at various depths, to differentiate between peri-mortem and chronic wounds as well as assess exposure artifacts, such as salt water, bacterial overgrowth or scavenging (DiMaio & DiMaio 2001). Gross texture of wound margins (e.g. wound margin curling/fiber bunching), and histopathology of wound margin and adjoining or exposed skeletal muscle can often provide insights into ante-mortem, peri-mortem or post-mortem injury (Shkrum & Ramsay 2007). Presence of foreign bodies within the defect (e.g. propeller piece, hull paint transfer) should be well-documented, sampled and maintained through a chain of custody (Supplement 4, available at www.int-res.com/articles/suppl/d103p229_supp/).

In cases of sharp injury to cetaceans, the skeleton should be flensed and bones examined for evidence of sharp- (e.g. bone nicks or shearing) and blunt-force trauma (e.g. fractures, suture separations, displacements). The airways should be examined for water and/or blood aspiration and stomach contents evaluated for blood, as cranial and pulmonary trauma can result in regurgitation and ingestion of blood, respectively, which provide strong support for ante- versus post-mortem injury. If the incised wounds communicate with the thoracic or abdominal cavity, pneumothorax and pulmonary collapse (atelectasis) should be assessed and if indicated, peritoneal/pleural lining sampled for histology and bacteriology.

COD assignation

Due to the gross appearance of lesions associated with sharp trauma, suspect cases are generally not assigned. Post-mortem condition or position of the animal in the water column or on the beach may hamper or preclude diagnostic evaluation. Clinical signs or gross pathology with associated hemorrhage in Code 2 and early Code 3 carcasses are confirmative. In those individuals that present with more advanced autolysis, interpretation of gross findings may be hampered and microscopic assessment of tissues at the margins and beneath the injuries may provide some indication of whether trauma occurred ante- or post mortem.

Confirmed

Cases can be confirmed when they include (1) necropsy findings supporting ante- or peri-mortem ship strike as COD (e.g. Fig. 43, GA2006025); (2) thorough necropsy, open wounds with sharp (incising) or sharp- and blunt-trauma (chop wounds) sequelae, histopathology supportive of gross findings of antemortem sharp trauma (e.g. Fig. 46, EqNEFL0602) or (3) a reported, well documented vessel collision and resultant mortality with carcass present, where a necropsy may not be practical.

Probable

If a carcass is available for study, and necropsy has been carried out as far as practical despite advanced decomposition, open incised wounds with sharp or sharp- and blunt-trauma sequelae are present, but histopathology findings of trauma are limited or not present, then a confidence level of 'probable' may be assigned.

Suspect

Cases which consist of a report/documentation of carcass, no carcass in hand or minimal examination, should be deemed 'suspect'. If necropsy is limited or not done, but open wounds and/or bony lesions consistent with sharp trauma are present, with or without other signs of pathology (e.g. entanglement or disease), a confidence level of 'suspect' could be assigned.

GUNSHOT INJURIES OF PINNIPEDS AND CETACEANS

Katie Moore[1], Frances Gulland, Michelle Berman, Elsa Cabrera, Kerri Danil, Lanni Hall

Background

Gunshot wounds are types of ballistic trauma produced by projectiles or other missiles launched from a firearm. These wounds may be glancing or penetrating, and vary from incidental to lethal. Gunshot wounds will have different characteristics based on the type of firearm, type of ammunition (projectile,

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shotgun pellet), angle of the shot and the distance between the muzzle of the firearm and the animal's body (Fisher & Petty 1977, Goldstein et al. 1999). Gunshot wounds inflicted on pinnipeds and cetaceans are most likely to be inflicted from a distance at which muzzle imprints and gunshot residue will not be present (>1 ft, 30 cm).

Detailed clinical and histological examinations of live and freshly dead small pinnipeds and cetaceans, especially California sea lions *Zalophus californianus*, have demonstrated that animals with gunshot injuries often have multiple active disease processes, such as leptospirosis or domoic acid toxicosis. Thus, determination of the final COD and relative importance of a gunshot injury in causing death can be difficult. The designation of a case as a gunshot case is relatively clear, whereas assigning COD often requires interpretation of all clinical and pathology data by an experienced pathologist.

Case definition

Signalment and epidemiology

There is no consistent presentation to gunshot cases. Although more common in pinnipeds (Goldstein et al. 1999), gunshot trauma can be found in any species, age, or sex, with no specific geographic area or season. Gunshot trauma may occur in clusters associated with overlap of inshore migration and fishery activity specific human activities (Goldstein et al. 1999).

Clinical signs

Clinical presentation varies with the extent and duration of injury, weapon used, body part affected, and presence or absence of infection. Injuries may be associated with external lesions (holes, fistulae, sinus tracts, Figs. 48 to 50), but the skin may be healed over at the site of projectile entry and/or exit (Figs. 51 & 52). Exit wounds may be more obvious than entrance wounds and typically have extruded margins. Bird peck holes are commonly identified as gun shot injuries by the public. Holes do not usually have distinguishing features to conclusively identify them as caused by guns.

Projectiles (such as lead, copper jacket and alloy shots, among others) may be in a different body part than the obvious site of injury. For example a projectile in the thoracic spine can result in paresis of the hind

Fig. 48. Two different holes in a common dolphin *Delphinus delphis*, one with hemorrhage (left), one without (right), associated with projectile entry. Zip-ties were used to probe tracts. SWFSC case KXD0124

Fig. 49. Skin hole in a harbor seal *Phoca vitulina* associated with projectile entry. Photo: MLML

Fig. 50. Skull of long beaked common dolphin *Delphinus capensis* with hole associated with 0.22 caliber projectile entry. SWFSC case KXD0123

Fig. 51. Sinus tract in nasal chambers of California sea lion associated with projectile wound. TMMC case 'Chippy'

Fig. 52 (a) Soft tissue swelling in California sea lion with no skin hole associated with projectile. (b) Soft tissue swelling in (a) lanced to expose projectile. TMMC case 'Chippy'

flippers and perineal edema. Thus, attempts at detecting a projectile should consider the entire body.

Diagnosis

Detection of projectiles associated with tissue damage is the ideal diagnosis of gunshot injury. To determine if the gunshot was the cause of death or SI requires examination of organ and tissues and establishing an association between the projectile and the compromised tissue.

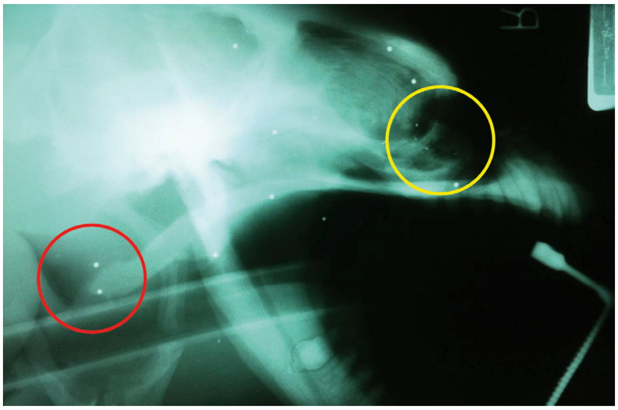

Fig. 53. Radiograph of sea lion head showing lead pellets (red) and trace of gunshot tract (yellow). Photo: TMMC

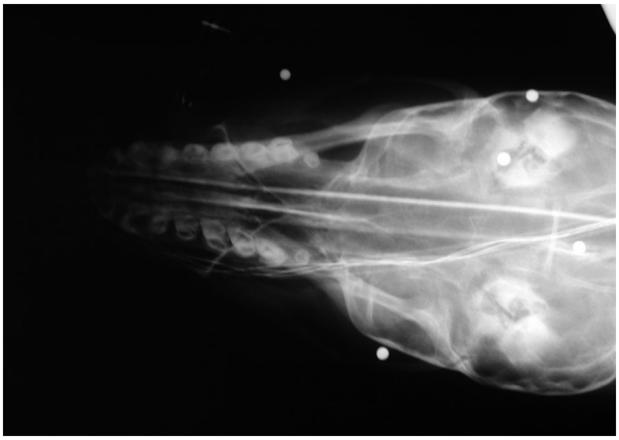

Fig. 54. Radiograph of typical fatal gunshot to a sea lion head. Photo: TMMC

Detection of a projectile may be by radiography (Figs. 53 & 54) or direct observation. Finding a projectile may be problematic as many X-ray machines cannot penetrate thick tissues over about 60 cm; however, it is possible to take radiographs of separated body parts to detect projectiles. Probing tracts with blunt probes can facilitate detection of a projectile and extent of injuries, but samples should be taken first to avoid artifactual damage.

Metal detectors can be useful in some cases to detect some types of projectile, but results are inconsistent and require confirmation by radiography or direct observation.

Perforating holes may also be caused by bird beaks, mammalian canine teeth, gaffs, spears, and crossbow arrows.

Gross necropsy and histological findings

Trauma characterized by hemorrhage, fracture, or secondary infection of any body part may occur in association with a gunshot injury. However, postmortem gunshot wounds can ooze bloody liquid. Different projectiles fragment or pass through to varying degrees.

Response protocol

Live animals

If clinical signs are present, observe potential cases for neurological deficits, general listlessness, and fistulas, which may have associated discharge and swelling. Routine blood chemistry and hematology may show signs of inflammation and or infection.

Level B data

Level B data should include X-ray or CT scan results if available. The animal should be examined for fragments of projectiles to inform gross necropsy.

Necropsy

Investigators should undertake a very careful external examination, labelling, photographing, measuring and describing each lesion. Representative sections should be sampled for histology. Explore all penetrating tracts for the presence of ballistic fragments (Fig. 55). The entire carcass should be skinned in suspected gunshot cases, as projectiles or fragments often lodge under the skin opposite the entry wound. A thorough necropsy is required, to ensure no pre-existing lesions or disease processes which may have adversely affected the animal and might have resulted in a decision to euthanize. The gastrointestinal tract should be examined for any foreign ingesta, and all necropsy findings should be recorded on standardized sampling worksheets.

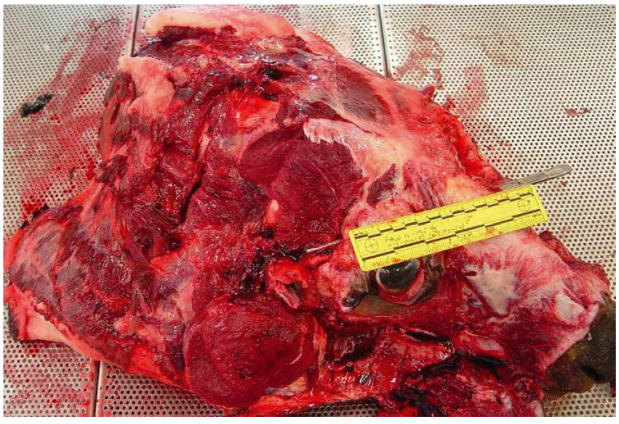

Fig. 55. Use of probes to assess gunshot injury tract in a grey seal. Photo: IFAW/WHOI

COD assignation

Confirmed

This level of confidence is warranted if projectile(s) or projectile fragment(s) are present and associated with massive trauma or organ damage: specifically, skull, vertebral or long bone fracture; hemorrhage; soft tissue or pulmonary abscess; pleuritis; peritonitis; liver, spleen, kidney or intestinal rupture.

Probable

For an injury where projectile or projectile fragments are recovered, but severity cannot be confidently evaluated due to postmortem condition, COD should be assigned as ‘probable gunshot wound’. A defined wound tract associated with severe trauma but no projectile/fragment(s) detected/recovered (especially if no X-ray is available), should also receive this level of COD confidence.

Suspect

This confidence level should be assigned to cases where projectile(s) or projectile fragments are present, but tissue damage or compromised organ function is not confirmed.

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Appendix 1. Glossary

Acute: hemorrhage and edema but no significant longer term host response (hours). The outcome of acute injury may be resolution of the wounds, healing by second intention or progression to a chronic state when the acute response cannot be resolved.

Ante-mortem: before death.

By-catch: the incidental catching of pinnipeds and cetaceans and other non-target species in fishing gear.

Chop wound: an incised wound of the skin, with an underlying comminuted fracture or deep groove in the bone.

Chronic: significant host response such as repair and remodelling, scar formation, secondary bacterial involvement or loss of body condition (weeks to years). Cellular infiltrate may be associated with acute, subacute and chronic stages. It is the proportion or composition of cellular infiltrate that may provide some insights into the time line: neutrophils can be apparent within 15–30 min, but typically are more abundant at 15 h; macrophages generally require 2–3 h, but representative numbers are usually detected in 3 d. The relative proportion of macrophages to neutrophils will be equal or greater by 11 d and range out over several months.

Confirmed: evidence that the most *parsimonious* explanation of the observations is that anthropogenic trauma was the cause and mechanism of SI or death.

Consistent with: evidence or findings that are suggestive but not definitive (see pathognomonic).

Cyamid: a parasitic crustacean whale louse that normally hides in crevices but spreads out over the body surface of a chronically sick animal and post mortem.

Drowning: death from suffocation by submersion in a liquid, usually water (Modell 1981, DiMaio & DiMaio 2001, van Beeck et al. 2005).

Entanglement: the presence of anthropogenic material on the body, or a pattern of incisions, abrasions, lacerations, or impressions reflective of such material. Described and illustrated by Barco & Moore (2010) for instance.

Host response: defense mechanism of the host against an exogenous stressor.

Human interaction (*HI*): interactions and consequences of human activities or materials.

Impression: an impression occurs when a line, net, or other form of debris leaves an indentation, but does not lacerate or abrade the skin/pelt (Moore & Barco in press).

Incidental take: non-intentional interaction with debris or other anthropogenic material that may be attributable to by-catch, but also to non-fishery interaction.

Incision: a cut or incision with clean edges, showing no rounding or tearing.

Laceration: tearing of skin or pelt.

Logistics Coordinator: manages plans for landing, securing, dissecting, recording, sample dissemination and analysis and disposal of a dead whale for necropsy.

Manner of death: explains how the cause of death came about. Manners of death can generally be categorized as natural, accidental, anthropogenic or undetermined.

Mechanism of death: physiological derangement produced by the initiating event that results in death. Examples of mechanism of death would be hemorrhage, septicemia, and cardiac arrhythmia. A particular mechanism of death can be produced by multiple causes of death and vice versa.

Necropsy Team Leader (*NTL*)*:* leads necropsy, directs dissecting, sampling, photographing of necropsy, writes gross report, collates analysis report and completes case report with histopathologist.

Offsite Coordinator: manages interested groups, services and media related to a necropsy.

Pathognomonic: a clinical sign or lesion that is characteristic of a certain condition, hence whose presence means that a particular disease or condition is present beyond any doubt.

Peracute: immediate, or almost immediate (minutes). Hemorrhage and edema may not be evident.

Peri-mortem: at or near the time of death.

Persistent entanglement: presence of entanglement wounds with or without associated gear, for an extended period sufficient to elicit a chronic host response.

Probable: evidence of anthropogenic trauma as the cause of death, or likely progression to death in the case of a SI, but the mechanism is *somewhat* unclear through lack of some critical evidence.

Proximate cause of death: initiating event that immediately or ultimately leads to death, injury or disease that produces a physiological derangement in the body that results in the death of the individual. Thus, although differing widely, the following are proximate causes of death: a gunshot wound to the head, a stab wound to the chest, adenocarcinoma of the lung, and coronary atherosclerosis.

Serious injury (*SI*): an injury that is more likely than not to result in mortality.

Signalment: that part of the history reflecting age and sex.

Skin: the full thickness of epidermis, dermis and lipid-rich hypodermis, the pelt of pinnipeds, and the blubber of cetaceans

Stab wound: a wound that is much deeper than wide.

Subacute: longer-term host response (days).

Suspect: evidence of anthropogenic trauma as the cause of death, or likely progression to death in the case of a SI, but the mechanism is *substantially* unclear through lack of much critical evidence.

Ultimate cause of death: consequent sequel(s) to the proximate event that become the final cause of demise. Examples could include loss of cerebral function, exsanguination, or respiratory failure.

Underwater entrapment: the interaction of a pinniped or cetacean with anthropogenic material that prevents it from returning to the water surface in order to take another breath. The most common type of underwater entrapment is incidental take in fishing gear. Entrapment is most common with smaller animals that lack the power to break out of fixed gear.

Appendix 2. Abbreviations used in the Theme Section, including institutional abbreviations used in table and figure legends

CCSN	Cape Cod Stranding Network (now IFAW Marine Mammal Rescue Research)
COD	Cause of death
FFWC	Florida Fish & Wildlife Commission
HI	Human interaction
IFAW	International Fund for Animal Welfare
NARW	North Atlantic right whale *Eubalaena glacialis*
MLML	Moss Landing Marine Lab
MMPA	Marine Mammal Protection Act
NEAq	New England Aquarium
NEFSC	NOAA Northeast Fisheries Science Center
NMFS	NOAA National Marine Fisheries Service
NOAA	National Oceanographic Atmospheric Adminsitration
PCCS	Provincetown Center for Coastal Studies
SAR	Stock Assessment Report
SBMNH	Santa Barbara Museum of Natural History
SI	Serious injury
SWFSC	NOAA Southwest Fisheries Science Center
TMMC	The Marine Mammal Center
UNCW	University North Carolina Wilmington
VAq	Virginia Aquarium
WHOI	Woods Hole Oceanographic Institution

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