Forensic Science in Marine Mammalogy: Applications and Limitations

Megan Stolen

Abstract

Marine mammals are unique in many ways including their aquatic adaptations, vulnerability to threats, legislation and laws protecting them, and their popularity with humans. Despite their reputation as charismatic megafauna, marine mammals still suffer injuries and death due to anthropogenic causes. Some are on the brink of extinction due to negligence and direct harm. Strong laws have made improvements in the management of marine mammal populations but every year, cases of injury and death warrant forensic investigation. These include vessel strikes, entanglement, drowning, shootings, mutilation, and illegal trade. Around the world, teams of stranding responders, fishery observers, veterinarians, and researchers investigate cases of human interaction with marine mammals. Often, the work is difficult as many obstacles make the full examination and forensic investigation challenging. This chapter will briefly review the laws in place to protect marine mammals, common causes of injury and death, limitations with some examinations, and recommendations in the application of forensic techniques.

Keywords

Marine mammals · Human interaction · Wildlife forensics

Laws Protecting Marine Mammals

Marine mammals are not a strict taxonomic group in the traditional sense. On the one hand, marine mammals do share some common traits; most notable, by definition, is the fact that they are all mammals, and all rely on aquatic environments for their survival. However, the term "marine mammal" includes several taxonomic groups that have converged and specialized in habitats that include not just saltwater oceanic environments but also riverine systems and estuaries that are, in fact, not strictly marine at all. Marine mammals include all seals, sea lions, walruses (all species in order Pinnipedia), marine and sea otters (select genera in the family Mustelidae), porpoises, dolphins and whales (order Cetacea), manatees and dugongs (order Sirenia), and polar bears (single species in family Ursidae). This somewhat artificial construct of assigning these taxonomically disjunct species to a group, at least in the United States, was a decision to grant them protection under the Marine Mammal Protection Act of 1972 (MMPA, most recently amended in 2015) as an ecosystem approach to conservation. It passed as a reaction to public concern over risks to marine

M. Stolen (✉)
Hubbs-SeaWorld Research Institute, Melbourne Beach, FL, USA
e-mail: mstolen@hswri.org

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mammals from human causes and a growing concern of threats to the marine environment (Lavigne et al. 1999). The result is a broad approach to conservation for all mammals that inhabit the vast marine environment as well as those species that have made their way into rivers and estuaries where they have specialized to feed and avoid predation in unique ways.

Some species of marine mammals are afforded additional protection in the United States under the Endangered Species Act (ESA) depending on their population status. Internationally, laws protecting marine mammals vary by country but may also include conservation measures under international treaties and agreements such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and regional agreements between neighboring countries (Baur et al. 1999).

In the United States, the authority to enforce the MMPA and other international treaties falls under the jurisdiction of the Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NMFS), and the Department of the Interior, United States Fish and Wildlife Service (USFWS). NMFS has the authority to protect cetaceans and pinnipeds (except walruses) and USFWS enforces laws protecting walruses, otters, polar bears, and sirenians and they have the authority to regulate trade (both legal and illegal) of all marine mammals under CITES (Baur et al. 1999).

The MMPA is primarily built on the pillar which states that it is illegal to "take" a marine mammal, which is defined in the Act as: "*to harass, hunt, capture, collect, or kill or attempt to harass, hunt, capture, or kill any marine mammals, including, without limitation, any of the following: the collection of dead animals or parts thereof; the restraint of detention of a marine mammal, no matter how temporary; tagging a marine mammal, or the negligent or intentional operation of an aircraft or vessel, or the doing of any other negligent or intentional act which results in the disturbing or molesting of a marine mammal.*" (Baur et al. 1999). This sweeping definition of take underscores the broad effects that the law has had on marine mammal protection and the vast scope of the actions needed by law enforcement to enforce it. By protecting the animals and their parts as well as any human effect that may negatively influence their behavior, the MMPA touches all aspects of human influence on these species from driving boats and dredging canals to collecting beachcast bones and shooting a marine mammal. It defines legal and illegal take in fisheries and describes how and when a marine mammal can be caught for research or public display in a zoological park. The law describes limits for approaching whales during an ecotour and hunting by indigenous communities. Thus, the MMPA protects marine mammals in all aspects from birth to death and even after death with respect to trade in their parts.

To implement the MMPA with all its pieces, like most wildlife protection plans, there must be involvement of countless people in multiple agencies including law enforcement at the local, state, national, and international levels, wildlife protection and trade agencies, tribal governments, animal health responders, and fishing industries, just to name a few. There are personnel solely dedicated to help understand and mitigate risks to marine mammals, such as observers on fishing vessels, but there are also volunteers working for stranding programs and experts looking for instances of wildlife trafficking as part of numerous global cybercrime initiatives. At each level, there are challenges of funding the work, keeping up with gear modifications and changes in fishing pressures, and understanding new threats as novel technologies such as remote operated vehicles and sonar arrays are developed. Effects on marine mammals are constantly under scrutiny as changes to the environment, economic development, and military operations have advanced significantly since the MMPA was first enacted. Amendments to the MMPA and other laws protecting marine mammals are needed to keep up with the times so that the original goals and mandates can be achieved.

Marine mammals by their very nature often travel between states, countries, and even continents as they navigate rivers, nearshore

environments, and vast expanses of open ocean. Because of this, strong international agreements and monitoring must be in place to protect marine mammals in all parts of their natural range. Likewise, these species are often the targets of wildlife traffickers, so strong import and export laws must be enforced. When a crime or violation is suspected, the investigator must notify the appropriate law enforcement agency early in the investigation so that there will be appropriate sample collection and preservation as well as interviews with appropriate people involved in the event. Often teams of scientists, forensic analysts, and on-the-ground responders are needed to fully investigate a marine mammal case. Fines may be levied, or, in very rare and egregious cases, perpetrators may be sentenced to time in probation or jail.

Classifying Anthropogenic Effects on Marine Mammals

Humans affect marine mammals in a myriad of negative ways and this may include criminal offenses in direct defiance of conservation laws such as the MMPA. These include direct lethal and nonlethal take of a marine mammal by means of shooting or other projectiles, drowning (usually associated with fishing gear), mutilation (such as a knife or gaff), vessel strike, entrapment, entanglement, and toxin exposure (e.g., oil spill). It is important to recognize that there may be chronic effects on marine mammals by all of these sources resulting in a reduction in lifespan or fecundity or an increase in pain and suffering as well as susceptibility to infectious and noninfectious disease. Other effects leading to changes in behavior or movement are more subtle and therefore fall outside the discussion of wildlife forensics that we will consider in this chapter.

Wildlife conflict and cases of anthropogenic effects on marine mammals fall into two general categories depending on the extent and type of harm. The first category involves cases with several marine mammals over time and often with some consistent input over time (chronic effects). Examples of these include post-oil spill assessments of marine mammal stocks in an affected area (part of the legal responsibility of a National Resource Damage Assessment) or chronic species level concerns for an endangered animal such as prolonged and widespread vessel strikes. These large population or species level concerns are often built upon data collected from individual cases, but the goal of managers is often to understand long-term effects on the population or species. The second category falls under what might be considered the acute result on an individual marine mammal after the interaction or crime occurs. Examples of these cases could include gunshot mortality, stabbing, or an unusual entanglement in fishing gear. In many instances, reliance on evidence and data is based on stranded animal recovery and examination.

As part of the MMPA, the United States established a National Marine Mammal Stranding Network with a responsibility to investigate causes for strandings (animals who have come ashore alive or dead or who may be injured or killed at sea) and to collect data on anthropogenic effects that may have contributed to the stranding event (Wilkinson and Worthy 1999). Often, chronic effects of long-term entanglement, toxin exposure, and nonlethal vessel strikes are also apparent during the investigation and these data likewise contribute to the understanding of harmful human–marine mammal interactions. Over time, the stranding network has developed procedures and protocols to ensure a consistent and objective examination of these cases. Currently, active and permitted stranding responders are required to complete a standardized form used by all responders in the United States which includes basic data (total length, sex, etc.) as well as more detailed information such as a description of their assessment of human interaction (HI form). Data fields on this form require a yes, no, or cannot be determined (CBD) answer to several gross examination questions and then a final determination to the main question "was there evidence of human interaction?". In order to answer yes, the examiner must have conducted an examination and must have found some evidence that is consistent with a known human interaction effect. Examples of evidence include

the presence of fishing gear or plastic ingestion but may also include evidence of interactions even in the absence of the gear. Other examples include lacerations or tissue damage consistent with a known type of trauma that can be reasonably assigned to a human interaction. Often these assessments must rely on the expertise and experience of the examiner or another expert relying on photographs and other types of evidence collected at the scene or during the examination. The question of human interaction can be placed into the "no" category when, after a full exam and based on reasonable expertise, there appears to be no evidence that a human interaction occurred with the animal. This is considered the most difficult determination to make because so many factors can affect the external or internal appearance of the evidence. These include decomposition, scavenger damage, incomplete examination as a result of the large size of the carcass or the inability to perform a complete necropsy due to weather or time constraints, lack of equipment, or proper tissue storage, etc. The third possible determination for the data field is "cannot be determined" (CBD) which is the most common answer because it accounts for all the difficulties outlined above as well as the possible inexperience of the examiner. Therefore, the evidence is deemed inconclusive (see review by Moore and Barco 2013).

Evidence of human interaction is categorized based on the type of injury (laceration, abrasion, amputation, ingestion, entanglement) and the object causing the injury (projectile, net, propeller, etc.). It is also important to note that evidence of human interaction with a stranded animal (dead or alive) does not always conclude with a determination that the interaction caused the death or stranding of the animal. For example, plastic or rope may be found inside the stomach of a whale but it may not have been the ultimate cause of the stranding. Similarly, evidence of human interaction does not necessarily imply criminal intent. However, all evidence and descriptions from human interaction cases are reviewed by teams at the regulating agency who then make determinations about the extent of the injury and make conclusions that may result in changes in maritime rules or charges being brought against a known offender. Evidence considered includes: gross examination, photographs, measurements of wounds, pathology reports, close examination of gear or other physical evidence by an expert familiar with the type, and eyewitness statements.

While a considerable amount of information regarding wildlife conflicts and marine mammals comes from stranded animal investigations, there are numerous studies analyzing data from field studies of marine mammals using techniques such as photo-identification to determine nonlethal interactions. However, these cases rarely result in a court case or investigation because the strong evidence needed is often inconclusive or missing. Exceptions to this are rare cases of harassment (considered a take and therefore a crime) that are posted on social media or the Internet and can be investigated by law enforcement.

Challenges of Investigating Marine Mammal Cases

Body Size

All wildlife forensic cases pose some unique challenges often not found in human crime investigations. These have been summarized well by Cooper and Cooper (2013) and Huffman and Wallace (2012). In general, wildlife crime is difficult to monitor and respond to because of several factors including proper identification of the species, lack of resources, the difficulty of securing or processing the crime scene, and lack of adequate comparative data, just to name a few. Many additional challenges affect marine mammal investigations. The first considerable challenge is the large body size of many marine mammals, with baleen whales being one extreme, but even moderately sized marine mammals such as dolphins and large pinnipeds often weigh several hundred pounds. Large body size is problematic because it makes a full forensic necropsy difficult as the carcass often requires heavy equipment to lift and turn so that all sides of the animal can be evaluated. For example, many of the largest marine mammals require large knives, large

hooks, and several hours of work just for the removal of the blubber or fat layer. This blubber layer also contributes to the increase of internal body temperature which increases the rate of decomposition. Relatedly, large amounts of pressurized gases inside the body cavities resulting from decomposition will displace organs and can lead to difficulty when attempting to identify sources of trauma.

Due to their large size and equipment challenges, marine mammal examinations are often performed in-situ on the beach, rocky cliff, or in water. This leads to a series of additional challenges, not only with decomposition but also because these issues make it difficult to collect and preserve samples without cross-contamination. Moreover, radiographs (for ballistic or skeletal trauma analysis) and other tests used in small and moderate-size animal veterinary forensic exams are often not available to prosecutors working up marine mammal cases, even in many laboratories. In a few cases, parts of the animal such as the head or flippers may be removed and can be examined or scanned back at a specialized laboratory or veterinary hospital but again, these may require large coolers, large vehicles, and appropriate types of testing equipment not often available to local stranding program managers.

Aquatic Environments

The second consideration is the difficulty presented by the nature of the marine (or aquatic) environment itself. Carcasses often initially sink below the surface of the water making them difficult to detect until gases build up in the body cavities, whereby the carcass will begin to float (see review by Liebig et al. 2003). Once floating, the animal can then drift for several miles over several days resulting in an expansive search area. Floating animals are essentially removed from the crime scene in the traditional sense, so investigators often rely on techniques such as hindcasting (using oceanic currents and weather as a model) to place the animal back at the site where it was injured or killed (Peltier et al. 2012). This level of modeling requires expertise outside the scope of most teams and is normally only used in endangered species cases. Investigations of carcasses at sea are difficult and dangerous for the investigator so carcasses are often towed to shore requiring adequate equipment and experienced mariners. The very nature of the saltwater and shorelines that comprise the site also make examinations difficult due to saltwater intrusion into the body, effects of weather and heat/cold on the external surface, and significant opportunities for scavengers from small crabs to large terrestrial carnivores and most notably sharks (Moore and Barco 2013). All of these effects lead to difficulties in trace evidence collection. For example, traditional evidence such as fingerprint and blood splatter analysis are often not appropriate or possible to collect. It is important to note that most marine mammal deaths are never recovered as the carcasses sink, become scavenged, or float away from human detection (Wells et al. 2015; Williams et al. 2011).

Taphonomy

The third consideration for applying forensics to marine mammal cases is the lack of comparative taphonomy information such as postmortem change details and marine-specific forensic entomology and scavenger assessment. While some data do exist for proxy animals such as pigs and humans in marine environments (Anderson and Bell 2014), there are few published studies on marine mammals using experimental design to test effects such as saltwater immersion, aquatic temperature changes, or scavenger marks (Peltier et al. 2012). Carcasses in aquatic environments require an advanced understanding of aquatic (marine and freshwater) organisms that can colonize, scavenge, and live as epibionts on marine mammals. Although deepwater whale falls are well researched and continue to be a focus of researchers with advanced technology (remote and human-piloted submersibles), less research has been conducted in shallow water environments (Anderson and Bell 2014).

The best use of epibionts in marine mammal forensics has been made in large whale entanglement and ship strike cases. Whale lice (family Cyamidae) have been used as indicators of long-term, chronic injuries and serve as evidence of chronic injury and lack of vigor (Pettis et al. 2004; Sharp et al. 2019). As with terrestrial forensic cases, parasites and epibionts can also indicate the origin of the carcass which is especially important in aquatic environments as floating marine mammals can move great distances due to oceanic currents and offshore and nearshore winds.

Confounding Effects

Care should be taken in all wildlife forensic cases to understand confounding effects. One important type in marine mammal cases is the effect of scavengers on carcasses. Scavenger damage can range from a small opening such as a bird peck to complete loss of major organs, skeletal parts, and even complete consumption of the body. Understanding and identifying scavenger marks is critically important in marine mammal cases as some are often misidentified as anthropogenic mutilations and projectile cases. For example, small diameter, penetrating wounds to the body often result from bird pecks (antemortem and postmortem). In Florida, stingray barb stab wounds often present similar to bullet wounds and can include an associated path of migration. After all, a dolphin killed by a stingray is essentially a non-anthropogenic interspecies stabbing case (Fig. 1).

Other confusing external lesions that can be misinterpreted as human-made marks such as those resulting from net entanglement can occur before and after death (Moore et al. 2013) so the examiner should document all marks well with sketches and photographs in situ if possible and consult other experts when marks are present (Table 1).

Postmortem Investigation of Marine Mammal Carcasses

Marine mammal case investigations do not generally allow for a true crime scene investigation for obvious reasons, such as the aquatic environment and drift as noted earlier, although underwater investigations should be approached with traditional crime scene processing techniques if possible and warranted (Byrd and Sutton 2012). Once landed or recovered, the carcass can be treated as a typical wildlife forensic case and should be secured and treated as evidence when a crime or violation is suspected. This includes a full chain of custody for biological samples, suspected fishing gear, projectiles, photographs of the body, and standard forms with notes, measurements, and gross necropsy results.

Standardized forms and protocols are easily accessible for use in marine mammal necropsies. These include standard data such as total length and sex (Level A data in the United States) and human interaction investigation forms. Standard procedures and protocols are readily available (Geraci and Lounsbury 2005). Carcasses are categorized by decomposition code from 1 (alive) to 5 (mummified, skeletal). This allows investigators to gauge the need and applicability of the sample modality and to inform the pathologist and other consulting scientists about the limitations of the investigation at the time of recovery. For example, it is important to note if the carcass had been frozen prior to necropsy because freezing may produce artifacts that change an interpretation during examination both grossly and in histopathology. Condition codes and appropriate sample collection methods are provided by Geraci and Lounsbury (2005) and Moore et al. (2013). Level B data are collected upon exam. These include photographs, sketches, and morphometric data. Level C data include pathology interpretations, advanced sample results such as DNA analyses, viral screenings, toxicology screening, etc.

Marine mammal forensic necropsies rely on universal techniques common to veterinary forensic science (Cooper and Cooper 2013, Huffman

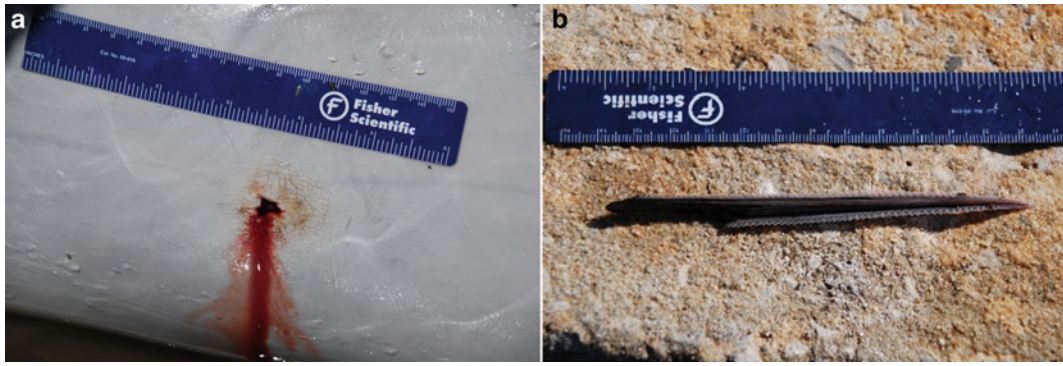

Fig. 1 (**a**) Ventral body of a bottlenose dolphin (Hubbs-0903-Tt) with stab wound resulting from a stingray. (**b**) Barb from the stingray found inside the dolphin. Photo credit: Hubbs-SeaWorld Research Institute

and Wallace 2012). The recent review of marine mammal cases by Moore et al. (2013) best summarized the appropriate approach to many forensic marine mammal case investigations including sharp and blunt force trauma, entrapment, gear entanglement, and gunshot cases.

Table 1 Types of marks commonly found on marine mammals with potential anthropogenic sources and confounding factors

Gross description	Common anthropogenic source	Confounding causes
Open circular wound of small diameter	Bullet hole	Antemortem stingray barb sharp injury
	Sharp instrument (e.g., Screwdriver)	Bird peck
		Other scavengers (invertebrates etc.)
		Postmortem carcass collection (e.g., Gaff use)
		Cookie-cutter shark (*Isistius*) bites
Parallel impressions, abrasions, lacerations	Net or line entanglement	Conspecific tooth "rake" marks
	Debris entanglement	Scavenger marks
		Predation tooth marks (sharks, other marine mammals)
		Freezer artifacts
Cross-hatching or cross-shaped marks	Net entanglement	Postmortem artifacts (e.g., Truck bed liners, body bags)
		Scavenger marks
Encircling marks around head and appendages	Rope or debris entanglement	Marks resulting from securing and towing carcass
Head or body part missing including teeth	Mutilation (e.g., trophy collection)	Antemortem predation
	Entanglement	Postmortem scavenging (shark, alligator)
		Decomposition
Diffuse subdermal blood staining	Blunt force trauma from watercraft	Conspecific aggression
	Entanglement or entrapment	Predation
Broken and luxated bones	Watercraft strike	Conspecific aggression
		Predation and large scavengers
		Mechanical disruption during stranding (alive or dead)

Likewise, individual case reports such as Byard et al. (2001, 2013) provide details for conducting thorough necropsies when an anthropogenic injury or criminal case is suspected. Generally, marine mammal forensic cases fall into several common types: watercraft strike, underwater entrapment, entanglement in gear or debris, ingestion of gear or debris, projectiles/bullets, mutilation (often postmortem) including to obtain trophies and parts, collection/sale crimes including cybercrime, toxic spills, and underwater sound emissions. Brief reviews of each are provided here as a summary.

Watercraft Strike

Virtually any type of vessel moving at speed on the water has the potential to injure or kill a marine mammal. Naturally, the larger the vessel and the faster the speed, the more potential for harm to marine mammals. Large ships are known to kill and seriously injure large whales at sea and even small vessels such as personal watercraft can injure and kill smaller marine mammals. Ships and smaller vessels cause harm in two ways: sharp injuries from propellers and blunt force trauma from a strike from the hull or skeg. In some cases, the animal can suffer from both types of injury. Sharp injuries have the potential to cause acute death by exsanguination, lethal mutilation and amputation, and fatal laceration and puncture to vital organs, as well as chronic mortality through infection, loss of mobility leading to reduction in feeding, swimming ability, or predator avoidance, issues with buoyancy and effects on the natural physiology of the animal. Examples of watercraft injury and death are well documented but perhaps two stand out more than others as they continue to significantly contribute to mortality in two ESA-listed species: North Atlantic right whales (*Eubalaena glacialis*) and West Indian manatees (*Trichechus manatus*).

North American right whales (NARWs) are the most endangered large whales on the planet and are protected by both the MMPA and the ESA in the United States. These whales were nearly extinct by the early 1900s as a result of several hundred years of commercial whaling (Kenney 2009). A recent study by Sharp et al. (2019) of right whale mortalities found that 42% of deaths during the study period could be attributed to vessel strikes. Right whales migrate from northern latitudes to Florida, close to shore, and straight through many commercial shipping areas. Despite large-scale efforts to decrease their susceptibility (e.g., mariner warning systems, protected status, education campaigns) NARWs continue to be killed by vessels. Gross descriptions and pathology and a review of cases by Sharp et al. (2019) are illustrative of the type of injuries, both acute and chronic, that result from vessel strikes.

Similarly, it is well known that boat strikes are a common source of mortality for manatees, another species at risk in the southeastern United States. These slow-moving sirenians have difficulty maneuvering away from approaching vessels especially when boats are moving quickly. Speed limits for boats have been in place for a number of years and failure of boaters to comply with these “manatee zone” enforcement regulations can result in fines, probation, and imprisonment for repeat offenders. Because of the high mortality rate from this trauma and the legal requirements of correctly identifying the cause of death in these cases, manatee experts with the Florida Fish and Wildlife Conservation Commission in Florida have developed detailed protocols for each manatee examined by the agency. Fresh carcasses are transported to a laboratory where they are systematically examined, and detailed notes and photographs are collected. Highly decomposed animals may be examined by regional field biologists but again, using a stepwise protocol that is built upon a strong foundation of forensic evidence collection. This allows the agency to reasonably assign carcasses, even when decomposed, to the watercraft death category (Fig. 2). Results from Rommel et al. (2007) show the benefits of taking a stepwise forensics approach to these cases resulting in accurate and defendable cause of death determinations.

Fig. 2 (**a**) Decomposed body of a Florida manatee (MNW18064) with sloughing epidermis. (**b**) Reconstruction of the epidermis from the manatee showing marks consistent with propeller strike. (**c**) Dissected thoracic area of the manatee showing hemorrhage. (**d**) Dissected body of the manatee showing a broken rib and large blood clot. Photo credit: Florida Fish and Wildlife Conservation Commission

Important features of these sharp and blunt force trauma watercraft cases include broken or luxated ribs, broken vertebrae and skulls, propeller lacerations, large blood clots in body cavities, evidence of hemothorax and pneumothorax, contusions, organ rupture and emaciation (Lightsey et al. 2006; Rommel et al. 2007; Sharp et al. 2019; Moore and Barco 2013). Examiners involved in vessel strike cases should familiarize themselves with associated gross lesions and take adequate histological samples to confirm injury and cause of death. As always, photographs showing the location of the wound and close-up images of wound/lesion sampling should be included as well as sketches, measurements, and trace evidence collection (e.g., hull/skeg paint on carcass).

Underwater Entrapment/Drowning

While all marine mammals are dependent on and spend much of their time in or around the water and are, in fact, the most aquatically adapted mammals in the world, nevertheless they are still air breathers and have limited survival time when trapped underwater. Entrapment is typically a result of being caught in fishing gear but can also result from human-made structures such as construction equipment, underwater machinery, or loch gates (Osinga and Morick 2008; O'Shea et al. 1985; Reynolds et al. 2018). While entrapment in fishing gear and entanglement are linked, we single out this type of entanglement here to highlight the cause of death in these cases as asphyxiation or capture myopathy rather than death as a result of gear wounds, which we discuss in the following section on entanglement. Drowning in marine mammals is often difficult

to interpret without expert veterinary pathology opinion, especially in cases where there is no direct evidence of gear on the animal. Some evidence of drowning in marine mammals includes edematous lungs, froth or large amounts of fluid in the airways, dark red lungs, and gas bubbles in tissues (Jepson et al. 2013). Examiners in these cases should make detailed descriptions of opened airways and photograph evidence as early in the necropsy as possible. Appropriate histopathology samples of the entire body with emphasis on the respiratory system are important.

Entanglement

Entanglement in gear and debris is a leading cause of anthropogenic mortality in marine mammals and occurs in virtually all areas where marine mammals are found, including shallow bays, estuaries, and rivers as well as pelagic environments (Read 2008). International laws and treaties include regulations for fishing gear to mitigate the effects of bycatch on marine mammals. Entanglement in human-made materials results in both acute and chronic health effects and is caused by the obvious sources such as fishing gear (Cassoff et al. 2011; Moore and Van der Hoop 2012; Moore et al. 2013) as well as debris not always easily associated with marine waters such as plastic waste, clothing, and other household items (Laist 1987; Moore and Barco 2013). Injuries from entanglement vary by the associated species but generally result in lacerations, abrasions, mutilation, and amputations of appendages and impressions from gear or debris (Moore and Barco 2013; Moore et al. 2013). Examiners should be familiar with fishing practices in their study area and spend some time understanding the signs of entanglement in such gear for local species (Moore and Barco 2013). Marine mammals are susceptible to entanglement as they swim through a three-dimensional space where floating debris is often essentially invisible to them and accumulates throughout foraging areas. Gear marks are commonly found around appendages especially the insertion or leading edge of the dorsal fins, flippers, and flukes of pinnipeds and cetaceans and the mouth, cervical regions, and peduncles of cetaceans and pinnipeds (Moore et al. 2013). Large whales may carry gear from several interactions and gear may remain embedded for years often leading to death years after the initial entanglement event (Fig. 3).

The cause of death in these cases may include hypoxia if the drag of the gear prevents the animal from surfacing adequately, exsanguination when gear severs major blood vessels, systemic infection, emaciation, and shock. Important sampling considerations for suspected entanglements include a full suite of histological samples with lesions and associated photographs, general tissue collection to identify any underlying health effects, frozen samples to identify potential toxicology that may be involved, sketches and photographs of gear and photographs of identifiable areas of the body such as dorsal fin and flukes to individually identify the animal for prior history in areas with ongoing population studies.

Ingestion of Gear and Marine Debris

Just as marine mammals become entangled in gear and debris so will they ingest pieces of these items. Plastic debris ingestion cases are becoming more common (Butterworth 2016; Jacobsen et al. 2010; Laist 1997) but other items such as recreational and commercial fishing gear ingestion are also well described in the literature. While ingestion of gear and debris does not always lead to death in many cases (Beck and Barros 1991; Stolen et al. 2013), the effects of indigestible trash are not well understood or studied (Simmonds 2012). Some cetaceans, such as dolphins, are unfortunately uniquely maladapted to the ingestion of fishing gear by their anatomy. Modified laryngeal cartilages (goosebeak) allow for the passage of air between the external nares (blowhole) and the trachea leading to the lungs. Ingested gear (often with a hook or lure attached) may become lodged around the cartilages and may encircle the structure. Multiple wraps of line around the goosebeak may lead to

Fig. 3 (**a**) At sea photo of decomposed North Atlantic right whale (VAQS20081005) showing rope entanglement. (**b**) Pectoral flipper of right whale showing multiple wraps of rope. (**c**) Open oral cavity of right whale showing intertwined rope through the baleen. Photo credit: Virginia Aquarium and Marine Science Center

asphyxiation and aspiration of prey items into the respiratory system (Stolen et al. 2013). While some cases of debris ingestion are a result of non-targeted prey selection, some types of plastic debris mimic normal prey for some marine mammals such as beaked whales and other pelagic squid eaters. Large baleen whales that skim the surface of the water are also susceptible to the ingestion of floating debris. Examiners of all marine mammal carcasses should take care to open the stomachs and intestines of all carcasses, even of decomposed animals, to look for debris and should attempt to understand the proximate effects of the ingested items on the area of the body. Ingested gear and debris should be photographed in situ and collected using appropriate forensic techniques including chain of custody if warranted.

Projectiles

Domestic, agricultural, and wildlife veterinary forensic science commonly includes the study of bullets and other projectiles in cases of animal cruelty and death. Marine mammals are no exception, but such cases are less likely to be: first, identified, and second, reasonably proven to have contributed to the death of the animal. For reasons that we have already discussed, finding a projectile in a large marine mammal, especially one that is decomposed, is difficult. The victim is often too large to be easily examined using scanning equipment and small wounds are often difficult to identify or may be misinterpreted as from another source such as a bird peck. Following the path of a projectile through a large carcass is also challenging. However, in some populations where death from gunshot is more common,

researchers have been able to develop systems for identifying and describing such cases. For example, in the western United States, sea lions are often the victims of aggression from humans as they are competitors for the same prey species (Goldstein et al. 1999; Stroud and Roffe 1979; Würsig and Gailey 2002). However, small cetaceans have also been the targets of shootings using firearms as well as other projectiles and sharp weapons (Byard et al. 2001; Vail 2016). Examiners of such cases should, if possible, use scanning equipment such as CT and radiography, even if only for a portion of the body (the suspected area of concern); this portion must be removed and scanned separately. Careful examination of external wounds and the track of the projectile or weapon and a detailed collection of photographs and histology samples should be made (Fig. 4). Prosecutors unfamiliar with these types of cases should refer to published protocols in standard forensic references (Cooper and Cooper 2013; Moore et al. 2013; Moore and Barco 2013). Of course, law enforcement should be notified immediately and should help coordinate chain of custody and evidence handling.

Illegal Mutilation and Trophy Collection

Governments and regulating agencies around the world including the United States recognize the long history of indigenous peoples' interaction with several marine mammal species especially in the high arctic. As such, the MMPA and other international treaties allow for exemptions for such groups and provide a framework for the legal collection and use of marine mammal parts and have set up safeguards to ensure regulations are followed (Burn 1998; Hovelsrud et al. 2008). While hunting or harvest of a marine mammal is legal in such circumstances, it remains a controversial subject as many marine mammals that are targeted are still declining. In this forensic context, we are referring to the illegal collection of marine mammal parts. Several marine mammal species are commonly involved in mutilation and trophy collection either to retain the artifact in its original form (e.g., teeth) or for it to be modified for art or souvenirs such as figurines or scrimshaw.

Mutilation may be a secondary human interaction when the primary injury/death was caused by a fishery interaction (entrapment or entanglement). In some cases, once the discovery of the animal with attached gear is made, there may be an attempt by the fishery personnel to sink the body or otherwise disguise the evidence. In these cases, the ventral body may be incised with a knife or appendages may be removed (Moore and Barco 2013). Mutilation may also occur when someone removes a portion of the body for fishing bait or to collect the body part as a "souvenir." In some of these cases, the person may not know that doing so violates the MMPA so public education is paramount. Examiners of carcasses that have been mutilated should recognize the possibilities for such in the context of local fishery interactions and both legal and illegal collection of parts. Careful collection of samples including the remainder of skeletal parts is important in the forensic investigation to determine how and when (antemortem or postmortem) the part was removed.

Illegal Sale of Parts

While some legal collection of marine mammal parts is permitted both by the United States and international law, illegal collection, and sale of parts continues to be a significant issue. There are generally two categories of marine mammal part sales/trades. The first is collection and trade for art or handicrafts and household goods. Prior to 1972 when the MMPA was enacted, marine mammal parts could be collected and used legally in such industries. It is therefore important to identify the proper documentation such as sale receipts and identifying marks on the piece to provide the best evidence of a legal take and sale. Likewise, some parts (or derivations of parts) may be legally sold by indigenous tradespeople provided they follow the requirements of the laws in the countries where the animal was killed and laws regarding the trade of the pieces

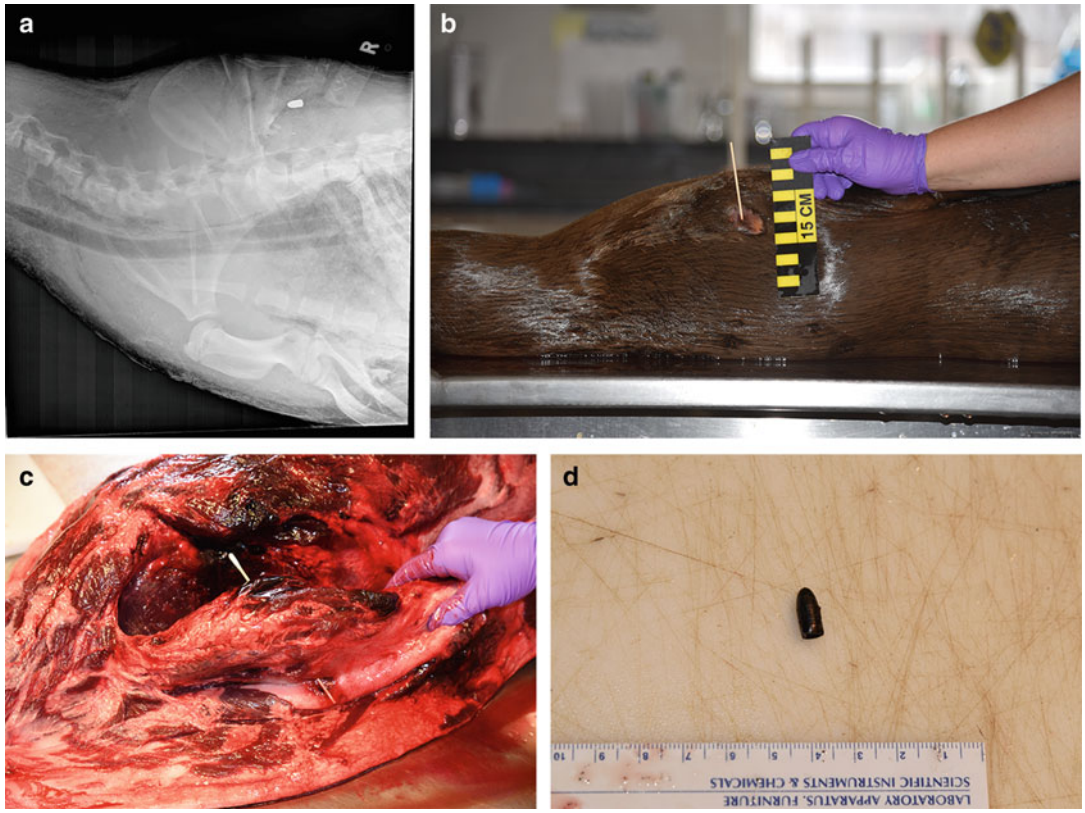

Fig. 4 (**a**) Radiograph of California sea lion (LMLZC2018OCT01-2) showing bullet lodged near the scapula. (**b**) Penetrating wound on the lateral body of a sea lion with a swab inserted to show wound track. (**c**) Dissection of sea lion showing internal wound track from bullet. (**d**) Bullet removed from the body of sea lion during necropsy. Photo credit: UCSC Long Marine Laboratory

through international laws and agreements such as CITES (Baur et al. 1999).

The second category refers to soft tissues (usually blubber or meat) from harvested marine mammals. In some countries, it is legal to kill and sell marine mammals for consumption. This trade takes place under the oversight of the International Whaling Commission (IWC) with international agreements (Gambell 1993). Several investigations and published research have shown how difficult it is to regulate such trade due to the difficulty in correctly identifying the tissues/meat at the market or in a restaurant. Breakthroughs in DNA collection and analyses including portable test kits have provided researchers and investigators invaluable devices in the forensic toolbox (Baker et al. 2007, 2010; Palumbi and Cipriano 1998). This genetic expertise remains a vital piece of forensic investigations and the continual effort to conserve cetaceans and other marine mammals through surveillance of products sold in markets and through Internet suppliers (Baker et al. 2003). Note that although technology has vastly improved the efficiency and accuracy of parts identification, the ability to do so remains with genetic and morphology experts such as those in dedicated forensic laboratories at NOAA/NMFS, USFWS, and academic institutions.

Toxin Exposure

It is well-known that marine mammals are exposed to toxins throughout their lifetime. Many of these toxins bioaccumulate in their tissues over time and cause harm in various ways. While these findings are anthropogenic in nature, the long-term nature of the exposure does not often lead to forensic investigations. However, acute toxic exposure, most notably in the form of oil spills are indeed some of the most important types of forensic investigations as the effects often take hold on multiple species or populations and can be traced using modern chemistry even when the effects persist for many years. The full extent of harmful effects of oil spills on marine mammals is still being investigated. Unfortunately, we do not have to go back in time very far to see this play out on a large scale; it can be seen as recently as the Deepwater Horizon Oil Spill (DWH) of 2010 in the Gulf of Mexico. Scientists continue to monitor the populations of marine mammals in the Gulf to determine what long-term effects are being experienced by populations of bottlenose dolphins and other pelagic species (Takeshita et al. 2017; Schwacke et al. 2013). Effects of oil spills on pinnipeds and sea otters (as well as cetaceans) are also well documented from the 1989 Exxon Valdez Spill in Alaska (Peterson et al. 2003).

The DWH spill investigation continues as part of the Natural Resource Damage Assessment, which is the legal framework for identifying impacts to natural areas and their biological components. Thus, it could reasonably be argued that the resulting investigation including collection and sampling of dead and sick marine mammals during and since the spill constitutes one of the largest and most comprehensive forensic investigations in history (Helm et al. 2015; Wallace et al. 2017). Examiners and investigators involved in oil spills (and other toxic spills) must be familiar with standard protocols for collecting, archiving, and analyzing samples and data. Often this type of investigation requires large teams working together (Takeshita et al. 2017; Wilkin et al. 2017). Workers on site must be properly trained as hazardous waste operators/responders and chain of custody for collected samples must be followed. Therefore, prior training and drills are critically important. When at all possible, caches of supplies for the initial collection of samples and response should be ready for deployment in staging areas near possible spill sites.

Underwater Sound Exposure

Threats to marine mammals from underwater sound sources are relatively new and it is only recently that researchers have begun to understand the impact that sound can have on the behavior and bodies of marine mammals. There are numerous types of anthropogenic sounds now ubiquitous in the marine environment including ship noise, military sonar, pile driving associated with industry and construction, seismic surveys, and even noise from underwater wind turbines and other energy exploration. All of these have the potential to disrupt the behavior of marine mammals for short and long periods of time but many also have the potential to cause hearing damage (Ketten 1995; Madsen et al. 2006; Moore et al. 2012) and there is mounting evidence that sonar events can change dive behavior and lead to serious, even fatal physiological changes such as fat embolism and gas bubble lesions (Fernández et al. 2005; Jepson et al. 2003). Generally, gross descriptions and examinations of suspect sound trauma in marine mammals will not be conclusive. Advanced sampling, specialized clinical equipment, and expert opinion are necessary in such cases (De Quirós et al. 2011; Ketten 2014; Van Bonn et al. 2011). It is not reasonable to expect that most stranding responders or even pathologists will have all the tools and supplies needed to follow exact protocols for sound-related trauma events, but examiners are encouraged to have protocols in hand and to be familiar with in situ sampling procedures. As with any forensic investigation, photographs are essential as well as histology, blood samples, and frozen tissues. Notification should be made to the proper regulating body so

that potential sources of sound or other factors can be investigated with the potential to suspend operations if warranted.

Final Thoughts

Perhaps no marine mammal on the planet epitomizes the need for more attention on conservation and the focus of law enforcement, regulators, forensic investigators, and the public than the vaquita (*Phocoena sinus*). The vaquita is the most endangered marine mammal in the world. A small porpoise with a very limited range in waters of the Gulf of California in Mexico, the species has been in decline since the 1950s. The population has been decimated as bycatch in gillnets and trawls set for fish and shrimp (D'Agrosa et al. 2000). The most recent and deadliest threat to this endemic cetacean is the illegal gillnet fishery for one particular fish (*Totoaba macdonaldi*) and the trade of their swim bladders which are sent to China and Hong Kong as a food delicacy and for their perceived medicinal properties (Martínez and Martínez 2018; Rojas-Bracho et al. 2006). Recent efforts to capture and breed these porpoises in an ex situ facility failed with one animal dying in the attempt to save the species. International laws and enforcement have proven insufficient to curb the high price that fishermen can demand for the illegal product (Rojas-Bracho et al. 2019). This case illustrates the role of forensic science, conservation biology, law enforcement, local people, and multilateral efforts in a global conservation crisis.

Conservation measures, forensic investigations, technology, and field efforts must be improved if we are going to help the remaining vaquitas, North Atlantic right whales, Hawaiian monk seals, and all other marine mammals at risk. Unfortunately, we are running out of time as the effects of climate change and political and cultural unrest only fuel the conflicts, illegal trade, and poverty that is at the heart of many conservation crises. One part of a global strategy to halt the decimation of marine mammal populations should include an increase in the number of qualified forensic investigators, advances in portable field equipment, and wider dissemination of forensic approaches to wildlife conservation.

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