

## **Field and Laboratory Procedures for Hawaiian Monk Seal Deaths and Description of Cause-of-Death Types Used in the Expert Panel Review**

### **1 | Standard field and laboratory protocols for assessment of Hawaiian monk seal deaths**

All reports of seal strandings are investigated as soon as possible by NMFS staff or designated personnel. Depending on accessibility and level of decomposition, carcasses are either examined in the field or, as is more often the case, transported to the NMFS laboratory in Honolulu for necropsy. Carcasses are covered in ice for transport whenever possible, and if transport durations are prolonged (i.e., air cargo transport from another island to Honolulu) carcasses are both covered in ice and kept refrigerated. Carcasses may also be frozen prior to examination.

Each carcass is examined externally and, whenever possible, all internal organs and body systems, including the placenta, are visually examined by trained biologists and veterinarians. Normal and abnormal findings are described in a gross necropsy report. In fresh dead carcasses, blood samples are collected and whole blood, plasma and serum are archived for future biomedical surveillance or ancillary diagnostic evaluation. Representative samples of all identifiable organs are fixed in 10% neutral buffered formalin. Conjunctival, oral, nasal, rectal and genital swabs are collected with sterile cotton-tipped swabs and frozen at  $-80^{\circ}\text{C}$ . Samples of brain, heart, lung, kidney, adrenal gland, lymph nodes (mandibular, axillary, prescapular, tracheobronchial, mesenteric) are frozen at  $-80^{\circ}\text{C}$ ; additional samples of lesions or organs may be collected and frozen at veterinary discretion.

Fixed samples are examined by board certified veterinary pathologists according to standard methods. Histopathology results are used to guide ancillary diagnostic tests such as serology, immunohistochemistry or molecular diagnostics. Nasal swabs are screened for morbillivirus by PCR (methods described in Barbieri et al., 2018). Mortalities directly attributed to protozoal disease are classified according to established case definitions (Barbieri et al., 2016). Ancillary information that may be related to the cause of death, such as presence of natural or man-made hazards are documented. In cases where anthropogenic factors are suspected, seal deaths are referred to agency or local law enforcement for further investigation.

### **2 | Description of Cause-of-Death types used in the Expert Panel Review**

*Protozoal Disease:* Lethal protozoal infections in Hawaiian monk seals are dominated by *Toxoplasma gondii*; *Sarcocystis* infections are typically incidental findings. These cases of toxoplasmosis in Hawaiian monk seals directly led to death through inflammation and multiple organ dysfunction (including the adipose tissue, heart, liver and brain). Histopathology and immunohistochemistry are the primary diagnostic modalities used to define cases, given that these analyses establish a direct link between disease and pathogen presence. When feasible, serology and molecular data provide additional confirmatory evidence. A case definition for protozoal mortalities in Hawaiian monk seals is described in Barbieri et al. 2016.

*Other Disease:* This category includes infectious and non-infectious/inflammatory diseases (non-protozoal) that are not observed frequently enough to be grouped individually. In some cases, postmortem examination identified lesions sufficient to cause death but lacked thorough diagnostic information to definitively identify the root cause, largely due to advanced decomposition. Examples include cardiovascular disease, intussusception, and renal enlargement (with suspected leptospirosis).

*Malnutrition* (including senescent-related): Diagnostics include externally visible evidence of emaciation (pronounced loss of nuchal fat (“peanut” head), low muscle/blubber mass, visible ribs, protruding pelvic girdle) and may be supported by histopathology findings such as fat, liver and muscle atrophy, pancreatic atrophy and zymogen depletion, lymphoid depletion, gastrointestinal parasitism and lack of ingesta/digesta.

*Natural Drowning* (peracute underwater entrapment or PUE) due to natural causes: There are no pathognomonic signs or diagnostic tests that can be used to definitively attribute death to drowning in marine mammals. Moore et al. (2013) identify a suite of characteristics that are consistent with PUE, a term used to encompass both wet and dry drowning in marine mammals. Diagnostics include: seal sighting history (e.g., last seen apparently healthy, in robust nutritional condition), hemorrhage (head, neck, shoulders/ chest, ocular, or other organs, scleral injection), congestion of lungs or other organs, pulmonary edema, and froth in airways. This is also a diagnosis of exclusion, which requires ruling out

other detectable causes of morbidity or death. Natural PUE can include entrapment in natural features (reefs or other), and pups carried away from shore due to currents or tides. Distinguishing between natural and anthropogenic PUE depends on such factors as location, ancillary observations, and other forensic evidence (see below).

*Reproductive Complications:* Includes a range of conditions that only apply to fetuses and pups. This includes aborted fetuses and stillborn pups for which no proximate cause (e.g., disease) of death was identified. Reproductive complications encompass perinatal mortalities due to dystocia, which is typically diagnosed by observing aspirated meconium, an excess of aspirated squamous cells or evidence of hypoxia on histopathology, and may be paired with gross findings of hemorrhage around the head and neck, and in some cases, sighting information documenting recent fetal abdominal movement. While not common, some abortions have been attributed to placentitis and ascending infections in the fetus, such as that resulting from cervical incompetence, without any impact on the health of the dam. The reproductive complications group also includes perinatal pups that succumbed due to failure to thrive (e.g., due to agalactia, weakness) or abandonment and thus emaciation from maternal neglect.

*Maternal trauma:* Pertains to pups that are crushed, suffocated or otherwise accidentally killed by the maternal female

*Natural Trauma:* Trauma (skeletal, musculature, or internal) which cannot be definitively assigned to any anthropogenic source and which may have resulted (or for which there is strong evidence) that the injury resulted from natural causes. This includes impact with any natural feature or injury inflicted by predators or other seals. These cases are also typically average to robust in nutritional condition. Sighting history, if available, generally does not indicate any reason for a pre-existing health concern and there is no other evidence of underlying disease or other morbidity.

*Anthropogenic Trauma:* Includes intentional or unintentional human-caused injury by shooting, clubbing, vessel or vehicle strike, or any other means of inflicting blunt or sharp force trauma. Diagnostic evidence includes external wounds, skeletal fractures, hemorrhage, and damage to muscles or internal organs detected during necropsy; these are supported by microscopic evidence (e.g., extravasation of blood cells, cardiac arrest, and pulmonary edema) that is indicative of ante- or perimortem trauma. Observations of the instrument used to inflict injury, investigations by law enforcement, and witness reports may provide corroborating evidence supporting the conclusions.

*Anthropogenic Drowning (nets or other):* Primary diagnostics of PUE are same as for Natural Drowning and may include additional evidence of observations/recent seal sighting history with a hook and trailing line, history of seal in areas known to have frequent net use, presence of net or line marks on seal, undigested or partially digested prey in the stomach, or net marks on prey items. As in natural drowning, this is a diagnosis of exclusion, which requires ruling out other detectable causes of morbidity or death.

*Hooking:* Mortalities attributable to ingestion or snagging by fishing hooks and similar gear. Pertains to both direct injury to organs or tissues and inability to forage due to reduced mobility or obstruction of the oral cavity or digestive system.

*Research-enhancement activities:* Unintentional mortalities associated with any research-handling event, including trauma, adverse reaction to sedative or handling, thermal stress, metabolic stress, or other.

## REFERENCES

- Barbieri, M. M., Duncan, C., Harting, A. L., Pabilonia, K. L., Johanos, T. C., Goldstein, T., Robinson, S. J., & Littnan, C. L. (2018). Survey for placental disease and reproductive pathogens in the endangered Hawaiian monk seal (*Neomonachus schauinslandi*). *Journal of Wildlife Diseases*, *54*, 564–568.
- Barbieri, M. M., Kashinsky, L., Rotstein, D. S., Colegrove, K. M., Haman, K. H., Magargal, S. L., ... Littnan, C. L. (2016). Protozoal-related mortalities in endangered Hawaiian monk seal *Neomonachus schauinslandi*. *Diseases of Aquatic Organisms*, *121*, 85–95.
- Moore, M. J., van der Hoop, J., Barco, S. G., Costidis, A. M., Gulland, F. M., Jepson, P. D., ... McLellan, W. A. (2013). Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma. *Diseases of Aquatic Organisms*, *103*, 229–264.