Cetacean Tissue Sampling and Postmortem Investigation

Best Practice Operations Manual for Sri Lanka

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Acknowledgements

This Standard Operating Procedure for cetacean post-mortem investigation and tissue sampling in Sri Lankan waters has been developed with substantial reference to the "Best Practice on Cetacean Post Mortem Investigation and Tissue Sampling" joint ACCOBAMS and ASCOBANS document (October 2019), edited by Lonneke L. IJsseldijk (Utrecht University), Andrew C. Brownlow (Scottish Marine Animal Stranding Scheme, SRUC), and Sandro Mazzariol (University of Padua).

We are deeply grateful to Lonneke L. IJsseldijk, Andrew C. Brownlow, and Sandro Mazzariol for their excellent work in producing the original document, and to all the contributing experts who participated in the harmonization workshop held in Padua, Italy in June 2019. Their collective expertise and dedication to advancing cetacean stranding investigation protocols have provided an invaluable foundation for this adaptation to Sri Lankan waters. This document represents an application of these established international best practices to the specific context and requirements of marine mammal conservation efforts in the Indian Ocean region.

This project was funded with UK International Development from the UK Government under the Ocean Country Partnership Programme.

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1 Purpose

This protocol document establishes standardised procedures for the investigation of cetacean strandings in Sri Lankan waters, providing a comprehensive framework for data collection, post-mortem examination, tissue sampling, and diagnostic analysis. The primary objective is to ensure consistent, scientifically rigorous investigation of all cetacean mortality events occurring along Sri Lanka's extensive coastline, enabling meaningful assessment of cetacean health status, identification of threats to marine mammal populations, and evaluation of anthropogenic impacts on the marine environment.

Sri Lanka's strategic position in the Indian Ocean, with diverse cetacean populations including both resident and migratory species, necessitates robust stranding investigation capabilities. Systematic examination of stranded carcasses represents an unique opportunity to gain insights into cetacean health, population dynamics, and the threats affecting marine ecosystems. The information derived from thorough post-mortem investigations can provide crucial data on at-sea populations that would be difficult or impossible to acquire through other means, particularly for elusive species rarely observed in their natural habitat.

This protocol serves multiple interconnected purposes within Sri Lanka's marine conservation framework. It establishes clear guidance for personnel across all experience levels, from first responders conducting basic external examinations to veterinary pathologists performing comprehensive diagnostic investigations. The tiered approach accommodates varying resource availability, logistical constraints, and technical capacity whilst maintaining data quality and consistency. This flexibility proves particularly valuable in Sri Lanka's diverse coastal environments, where accessibility, infrastructure, and available expertise may vary considerably between stranding locations.

The standardised methodologies outlined herein enable reliable determination of causes of death, assessment of nutritional status, identification of disease processes, documentation of human interactions including vessel strikes and fisheries bycatch, evaluation of contaminant burdens, and collection of life history data. Such information directly supports Sri Lanka's obligations under international conservation agreements and provides essential data for marine spatial planning, fisheries management, pollution monitoring, and protected species conservation.

By adopting internationally recognised best practices adapted to Sri Lankan conditions, this protocol facilitates data comparability with regional and global stranding networks. This harmonisation enables participation in international research collaborations, disease surveillance programmes, and comparative studies of cetacean populations across the Indian Ocean region. The systematic archiving of tissues and data creates valuable resources for future research as analytical techniques advance, and new questions emerge regarding marine mammal health and conservation.

Furthermore, this protocol recognises that cetacean strandings represent sensitive events requiring careful consideration of public health, environmental protection, biosecurity, legal compliance, and cultural sensitivities. The comprehensive guidance provided ensures that investigations proceed safely, legally, and ethically whilst maximising scientific value and supporting evidence-based marine conservation policy in Sri Lanka.

2 Legal and Institutional Framework

2.1 International Legal Instruments

Sri Lanka's approach to cetacean conservation and stranding investigation operates within a comprehensive framework of international legal instruments to which the nation is party. These agreements establish baseline protections for cetacean species whilst providing mechanisms for international cooperation in conservation, research, and monitoring activities.

The International Convention for the Regulation of Whaling (ICRW), established in Washington in 1946 and administered by the International Whaling Commission (IWC), provides the primary international regulatory framework for cetacean conservation. Whilst originally focused on commercial whaling regulation, the IWC has evolved to encompass broader conservation concerns including strandings investigation, ship strikes, entanglement, pollution, and climate change impacts on cetacean populations.

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), concluded in Washington in 1973, regulates international trade in endangered species including all cetaceans. Under CITES provisions, all cetacean species are listed in either Appendix I or Appendix II, requiring specific permits for any international movement of specimens, tissues, or derivatives. This has direct implications for tissue sample exchange with international research institutions and necessitates careful documentation and permitting procedures for collaborative research projects.

The Convention on the Conservation of Migratory Species of Wild Animals (CMS), concluded in Bonn in 1979, provides framework for international cooperation in conserving migratory species throughout their range. Several cetacean species occurring in Sri Lankan waters are listed under CMS appendices, creating obligations for habitat protection, threat mitigation, and international cooperation in research and monitoring activities.

Regional agreements relevant to Sri Lankan waters include the South Asian Cooperative Environment Programme (SACEP) and various Indian Ocean regional initiatives addressing marine pollution, habitat protection, and species conservation. These include the Indian Ocean Tuna Commission (IOTC), which manages fisheries and bycatch mitigation measures affecting cetaceans; the Regional Coordination Unit for the South Asian Seas (RCU-SAS) under UNEP, which implements the South Asian Seas Action Plan; the Coordinating Body on the Seas of East Asia (COBSEA), which promotes marine environmental management in waters extending to eastern Sri Lanka; the Indian Ocean Rim Association (IORA), which facilitates maritime cooperation and marine environment protection; the SACEP Marine Protected Areas Network, which coordinates regional MPA efforts; the Memorandum of Understanding on the Conservation and Management of Marine Turtles and their Habitats of the Indian Ocean and South-East

Asia (IOSEA), which establishes marine habitat protection frameworks applicable to cetacean habitats; and relevant regional agreements under the Convention on Migratory Species (CMS) addressing migratory cetacean populations. These instruments establish cooperative frameworks for addressing transboundary conservation challenges affecting cetacean populations moving between national jurisdictions.

2.2 National Legal Framework

Sri Lanka's domestic legal framework for cetacean protection derives primarily from the Fauna and Flora Protection Ordinance (FFPO) managed by the Department of Wildlife Conservation, which provides comprehensive protection for all cetacean species occurring in Sri Lankan waters. Under FFPO provisions, all cetaceans are designated as protected species, making it illegal to kill, capture, harass, or trade in these animals or their derivatives without specific authorisation from competent authorities.

The Coast Conservation and Coastal Resources Management Act managed by the Coast Conservation and Coastal Resource Management Department provides additional protections for cetaceans through provisions regulating coastal development, pollution control, and marine habitat conservation. This legislation establishes regulatory frameworks for activities that may impact cetacean populations, including coastal construction, port development, and industrial discharge into coastal waters.

The Fisheries and Aquatic Resources Act managed by the Department of Fisheries and Aquatic Resources addresses interactions between cetaceans and fishing activities, providing legal basis for regulating fishing gear that may pose entanglement risks, establishing protected areas where fishing activities are restricted, and requiring reporting of cetacean bycatch incidents. This legislation proves particularly relevant for investigating stranding events potentially resulting from fisheries interactions.

Maritime safety legislation administered by the Marine Environment Protection Authority (MEPA) addresses vessel-related threats to cetaceans including ship strikes and marine pollution. These regulations establish vessel speed restrictions in sensitive areas, routing measures to reduce collision risks, and pollution response protocols relevant to unusual mortality events potentially linked to environmental contamination.

2.3 Permitting Requirements for Stranding Investigation

Investigation of cetacean strandings in Sri Lanka requires coordination with multiple regulatory authorities depending on the nature and scope of activities undertaken. The Department of Wildlife Conservation (DWC), as the primary authority responsible for protected species management, must be notified of all cetacean stranding events and maintains authority over decisions regarding carcass handling, sampling, and disposal.

Personnel conducting post-mortem examinations and collecting tissue samples require appropriate authorisation from DWC, typically issued through designated stranding response networks or research institutions with established relationships with regulatory authorities. Such authorisation should specify permitted activities, sampling protocols, storage locations, and arrangements for tissue archiving and potential future use in research or diagnostic investigations.

International movement of tissue samples for diagnostic analysis, collaborative research, or archival purposes requires CITES permits in addition to DWC authorisation. Export permit applications must demonstrate that sample collection and export serve legitimate scientific purposes, comply with national regulations, and do not threaten conservation of the species concerned. Import permits from receiving countries must be secured prior to export permit issuance, necessitating advance planning and coordination with international collaborators.

Veterinary authorities hold jurisdiction over aspects of stranding response relating to zoonotic disease risks, carcass disposal for public health protection, and biosecurity considerations. The Department of Animal Production and Health should be consulted regarding appropriate handling procedures, personal protective equipment requirements, and disposal methods ensuring human and animal health protection whilst complying with waste management regulations.

Local government authorities maintain responsibility for managing activities on public lands, coordinating with emergency services, and ensuring public safety during stranding response operations. Early coordination with relevant local authorities including Municipal Councils, Pradeshiya Sabhas, or District Secretariats facilitates efficient response whilst ensuring compliance with local regulations regarding beach access, equipment deployment, and carcass removal.

2.4 Institutional Responsibilities and Coordination

Effective stranding response in Sri Lanka requires close coordination between multiple institutions with distinct but complementary mandates. The Department of Wildlife Conservation serves as the primary coordinating authority, maintaining responsibility for overall protected species management whilst facilitating cooperation between research institutions, veterinary services, local authorities, and international partners.

Research institutions including universities, marine research centres, and non-governmental organisations specialising in marine mammal research should provide technical expertise for stranding investigation, post-mortem examination, and sample analysis. Formal agreements between DWC and research institutions should be developed to establish protocols for notification, response procedures, sample collection and storage, data management, and reporting requirements.

The National Aquatic Resources Research and Development Agency (NARA) contributes expertise in marine ecology, fisheries interactions, and oceanographic factors potentially relevant to stranding investigations. NARA's research vessel capabilities and laboratory facilities may support stranding response activities including offshore disposal operations, environmental sampling, and analytical procedures requiring specialised equipment.

Veterinary services provided through the Department of Animal Production and Health and veterinary faculties at Sri Lankan universities contribute essential diagnostic capabilities including pathology, microbiology, virology, and toxicology. Coordination protocols should establish clear lines of communication for consultation on unusual cases, disease outbreak investigation, and access to specialised diagnostic facilities.

Coast Guard and Navy assets may be required for strandings in remote coastal locations, offshore carcass disposal operations, or coordination of response to mass stranding events requiring substantial logistical support. Advance coordination with maritime authorities ensures rapid mobilisation of resources when required whilst clarifying jurisdictional boundaries and operational procedures.

3 Data Management and Reporting Obligations

Systematic data collection and reporting forms the foundation for effective cetacean conservation in Sri Lankan waters. All stranding events should be documented using standardised data collection forms capturing essential information on species identification, location, date, carcass condition, morphometric measurements, life history parameters, and findings from post-mortem examination when conducted.

DWC should establish and maintain a national stranding database serving as the central repository for stranding records, post-mortem findings, analytical results, and photographic documentation. Research institutions and other entities responding to strandings must submit standardised reports within specified timeframes, ensuring data completeness and facilitating timely analysis of trends that may indicate emerging conservation concerns.

DWC should consider extending reporting to relevant international bodies including the IWC, which is developing a global strandings database facilitating international comparison and collaborative research on cetacean mortality causes. Sri Lanka's contributions to international databases will enhance understanding of regional patterns whilst enabling participation in global research initiatives addressing cetacean conservation challenges.

Sensitive information regarding locations of rare species, details of ongoing criminal investigations into illegal activities affecting cetaceans, or personal information about individuals reporting strandings requires appropriate confidentiality protections. Data management protocols should establish clear policies regarding data access, information sharing with external parties, and protection of confidential information consistent with national data protection legislation.

3.1 Legal Considerations for Disposal

Carcass disposal following post-mortem investigation must comply with environmental protection legislation, public health regulations, and waste management requirements. The National Environmental Act and regulations enacted thereunder establish standards for waste disposal, pollution prevention, and environmental impact assessment for activities that may affect environmental quality. Methods of carcass disposal and their considerations are discussed in more depth in Section 9: Carcass Disposal.

4 Health & Safety

Live, dead, or decomposing cetacean tissues may harbour various potentially harmful zoonotic pathogens, and the collection, transportation, and post-mortem examination of stranded marine mammals carries several significant hazards. Sri Lankan response teams must conduct comprehensive risk assessments according to national occupational health and safety legislation and regulations established by the Department of Labour and relevant authorities. This section outlines key health and safety principles applicable to cetacean stranding response in Sri Lankan conditions, though each organisation must develop comprehensive safety protocols appropriate to their specific operating environment and local regulations.

4.1 Biological Hazards

The zoonotic risk from marine mammals, whilst generally reported as low in the scientific literature, requires serious attention and appropriate preventive measures. Infection hazards arise primarily from exposure of abraded or broken skin, or mucous membranes including the conjunctiva and respiratory tract, to fluids from stranded marine animals. Any such exposure must be reported immediately to medical professionals for assessment and appropriate treatment. Immediate disinfection of affected areas, or in case of direct eye contact thorough rinsing, proves necessary and may justify appropriate antibiotic prophylaxis depending on medical evaluation.

Several specific pathogens warrant particular attention during cetacean stranding response operations in Sri Lankan waters. Brucella species, particularly *Brucella ceti* isolated from cetaceans, can cause serious infections in humans characterised initially by symptoms including redness and swelling of hands, a condition sometimes termed 'seal finger' when associated with pinniped contact. *Mycoplasma* species present additional infection risks, as do *Neisseria* species, *Erysipelothrix rhusiopathiae*, and potentially unknown pathogens not yet characterised in Sri Lankan cetacean populations.

The tropical climate of Sri Lanka may increase certain biological risks compared to temperate regions. Warm, humid conditions favour bacterial growth and survival in carcass tissues, potentially increasing exposure risks during handling operations. High ambient temperatures accelerate decomposition, releasing aerosols containing bacteria and other microorganisms that may be inhaled during examination procedures. Insect vectors including flies are abundant in tropical coastal environments and may transmit pathogens from carcasses to response personnel.

A comprehensive hygiene protocol must be implemented for all individuals involved with retrieval, transportation, or handling of tissues derived from cetacean carcasses. All sampling and handling operations should be conducted wearing appropriate gloves and personal protective equipment. In cases involving live animals or fresh carcasses, suitable respiratory protection must be worn to guard against aerosol inhalation, particularly during procedures generating aerosols such as bone sawing or use of high-pressure water for cleaning.

Figure 1: Example of minimum PPE to be worn at all times during sampling or necropsy examinations.

Personal protective equipment (PPE) requirements vary according to the nature of activities undertaken and carcass condition. Minimum PPE for all personnel in proximity to carcasses includes disposable gloves, protective clothing that can be decontaminated or disposed of after use, and sturdy waterproof boots with good traction - waterproof trousers must not be tucked into waterproof boots (Figure 1). Anyone handling sharp objects should wear cut-resistant gloves under disposable gloves to protect from punctures. Additional protection including face shields or goggles, respiratory protection, and waterproof aprons or full-body suits may be required depending on specific circumstances and risk assessment outcomes.

Immunocompromised individuals face heightened susceptibility to zoonotic infections and should avoid direct contact with carcasses or samples derived from stranded marine mammals. This includes individuals receiving corticosteroid treatment, pregnant women, elderly persons, those with certain viral infections, and anyone with conditions affecting immune system function. Young children should similarly be excluded from direct involvement in stranding response activities due to developing immune systems and increased infection susceptibility.

Hand hygiene represents the single most important preventive measure against zoonotic disease transmission. Hands must be washed thoroughly with soap and water immediately following any contact with carcasses or tissues, before eating or drinking, and before touching the face. Where running water is unavailable during field operations, alcohol-based hand sanitisers containing minimum 60% alcohol provide effective interim hand hygiene, though hands should be washed with soap and water as soon as facilities become available.

IMPORTANT: Key Protocol Points

Biological Hazards

- Implement comprehensive hygiene protocols for all personnel handling carcasses or tissues
- Wear appropriate personal protective equipment including cut-resistant gloves, disposable gloves, protective clothing, and respiratory protection
- Exclude immunocompromised individuals and pregnant women from direct carcass contact
- Be aware of specific zoonotic pathogens including *Brucella*, *Mycoplasma*, *Neisseria*, and *Erysipelothrix*
- Practice thorough hand hygiene with soap and water immediately after any carcass contact
- Seek immediate medical attention for any skin puncture, cut contamination, or mucous membrane exposure
- Tropical climate increases bacterial growth and survival in tissues; take additional precautions in hot, humid conditions

4.2 Environmental Hazards

Sri Lanka's diverse coastal environments present numerous environmental hazards requiring careful assessment and management during stranding response operations. Remote locations, common for many stranding events, may have limited mobile phone reception, restricting communication capabilities and complicating emergency response should personnel require medical attention or technical assistance. Response teams should carry satellite phones or alternative communication devices ensuring connectivity even in areas lacking mobile network coverage.

The coastal environment itself presents multiple hazards requiring constant awareness. Slippery rocks, uneven surfaces, and unstable substrates increase risks of falls and injuries during carcass examination and removal operations. Tidal patterns in Sri Lankan waters can be substantial, with rising tides potentially cutting off access routes or endangering personnel working near the water's edge. Response teams must carefully monitor tide tables and maintain awareness of water levels throughout operations.

Weather conditions in tropical environments can change rapidly, presenting serious risks to field personnel. Intense solar radiation during most of the year creates risks of heat exhaustion, heat stroke, and severe sunburn, particularly during extended operations on exposed beaches without shade. Sudden thunderstorms common in Sri Lankan climate bring lightning strike risks, heavy rainfall reducing visibility and making surfaces treacherously slippery, and potential flash flooding in coastal areas with poor drainage.

Temperature extremes characteristic of tropical climates requires careful management. High temperatures and humidity create risks of heat-related illness including heat exhaustion and heat stroke, particularly for personnel wearing protective equipment that impedes normal thermoregulation. Response protocols must include regular rest breaks, provision of adequate drinking water to prevent dehydration, and monitoring of personnel for signs of heat stress. Conversely, working in water or during evening hours when temperatures drop can create hypothermia risks, requiring appropriate thermal protection.

Physical hazards associated with moving large cetacean carcasses demand careful planning and appropriate equipment. Manual handling of heavy carcasses creates significant risks of musculoskeletal injuries including back strains, pulled muscles, and other soft tissue injuries. Mechanical assistance using appropriate equipment including winches, cranes, or heavy vehicles should be employed whenever possible to minimise manual handling requirements. When manual handling proves unavoidable, proper lifting techniques must be employed and adequate numbers of personnel deployed to distribute loads safely.

Working near heavy machinery deployed for carcass removal or burial operations creates additional hazards. Personnel must maintain safe distances from operating excavators, cranes, and vehicles. Clear communication protocols and designated safety officers help prevent accidents involving mechanical equipment. High-visibility clothing ensures machinery operators can easily identify personnel positions, reducing risks of struck-by incidents.

IMPORTANT: Key Protocol Points

Environmental Hazards

- Assess tidal patterns before beginning operations; monitor water levels continuously
- Carry satellite phones or alternative communication devices for remote locations
- Protect against heat stress through regular rest breaks, adequate hydration, and shade when possible
- Monitor weather conditions; cease operations during thunderstorms or rapidly deteriorating weather
- Wear appropriate footwear with good traction on slippery coastal surfaces
- Use mechanical assistance rather than manual handling whenever possible to move large carcasses
- Maintain safe distances from operating heavy machinery; wear high-visibility clothing
- Plan for adequate numbers of personnel to distribute physical workload safely

4.3 Chemical and Residue Hazards

Chemical hazards encountered during stranding response operations require specific awareness and protective measures. Drugs administered ante-mortem or for euthanasia purposes comprise notable hazards. Most chemical euthanasia compounds used for large animals, particularly barbiturates, are highly potent and persist in tissues after death. These substances pose potential hazards to personnel conducting post-mortem investigations through dermal absorption or accidental injection, and to the environment through contamination of scavenging wildlife or groundwater.

When animals have been euthanised using chemical agents, it is imperative that complete information regarding drug name, dose, volume administered, and injection site be obtained and clearly documented. All personnel involved in subsequent handling must be informed of chemical euthanasia. Additional precautions including enhanced personal protective equipment, careful disposal procedures preventing environmental contamination, and warning markers at burial sites preventing future excavation become necessary.

Marine mammals accumulating high tissue burdens of persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), organochlorine pesticides, and other bioaccumulative contaminants present potential exposure risks to response personnel. Whilst acute toxicity from brief contact during routine handling remains unlikely, minimising exposure through appropriate protective equipment and hygiene practices remains prudent, particularly when collecting tissue samples for contaminant analysis or conducting extended examination of heavily contaminated individuals.

Environmental contamination events including oil spills may affect stranded cetaceans, introducing additional chemical hazards requiring specific handling protocols. Petroleum products may cause skin irritation and pose inhalation hazards, whilst dispersants used in oil spill response may present their own health risks. Animals affected by environmental contamination require assessment by environmental health specialists to determine appropriate handling and disposal procedures ensuring human and environmental safety.

Chemicals used during post-mortem examination, particularly 10% neutral buffered formalin widely used for tissue fixation, present significant hazards requiring careful management. Formalin, classified by international agencies as carcinogenic to humans, requires appropriate handling procedures including use in well-ventilated areas or outdoors, wearing of chemical-resistant gloves and eye protection, and prevention of skin contact and inhalation of vapours. Ethanol used for tissue preservation presents fire hazards and requires appropriate storage and handling consistent with flammable liquid protocols.

IMPORTANT: Key Protocol Points

Chemical Hazards

- Obtain complete information about any euthanasia drugs used including name, dose, and injection site
- Handle formalin in well-ventilated areas; wear chemical-resistant gloves and eye protection
- Wrap tissue samples for contaminant analysis in aluminium foil to prevent plastic contamination
- Store ethanol and other flammable chemicals according to fire safety protocols
- Dispose of chemically euthanised carcasses using methods preventing scavenger access

4.4 Post-Mortem Examination Hazards

Prior to any examination involving moving or opening a carcass, comprehensive assessment and mitigation of potential environmental and public health hazards must be conducted. Biosecurity considerations include risks from leakage of fluids that may contain infectious agents, noxious odours that may affect nearby residents or businesses, and aerosols generated during examination procedures potentially carrying microorganisms.

Aesthetic and social impacts on civil society and individual members of the public require consideration. Post-mortem examinations conducted in public view may cause distress to observers, particularly children, and may attract unwanted attention that interferes with examination procedures. Whenever possible, examinations should be conducted in secure areas with controlled access, adequate lighting, clean water supply for decontamination, and effective containment of effluent preventing environmental contamination.

Sharp instruments including scalpels, knives, bone saws, and other cutting implements present obvious injury risks during post-mortem examination. Dull blades require excessive force increasing loss of control and injury risk, so tools must be maintained in sharp condition. Proper cutting techniques directing blade movement away from the body reduce laceration risks. Cut-resistant gloves provide additional protection though must not substitute for proper technique. All sharps must be disposed of in appropriate puncture-resistant containers preventing injuries to waste handlers.

Compressed gas in decomposing carcasses presents explosion risks during initial opening. Bloated carcasses contain substantial internal pressure that may cause sudden violent release of gas and fluids when body cavities are opened. Personnel should position themselves away from potential projectile paths and make initial incisions carefully to relieve pressure gradually. Eye protection is mandatory during opening of bloated carcasses.

Decomposition gases released during examination, whilst generally not toxic at concentrations encountered during outdoor work, can cause nausea, headaches, and respiratory irritation. Work should be conducted upwind of carcasses when possible, and respiratory protection may be warranted for advanced decomposition cases. Indoor examinations require adequate mechanical ventilation ensuring gas dispersal and preventing accumulation to uncomfortable or potentially harmful levels.

IMPORTANT: Key Protocol Points

Postmortem Examination Safety

- Conduct examinations in secure areas with controlled access when possible
- Position personnel away from potential projectile paths when opening bloated carcasses
- Wear eye protection during all examination procedures, particularly when opening body cavities
- Maintain sharp cutting tools to reduce excessive force requirements
- Dispose of all sharps in appropriate puncture-resistant containers
- Work upwind of carcasses when possible to minimise exposure to decomposition gases
- Use respiratory protection for advanced decomposition cases or indoor examinations

4.5 Post-Exposure Protocols

Despite preventive measures, exposures may occur requiring prompt appropriate response. All stranding response teams should establish clear protocols for managing exposures, ensuring personnel understand reporting requirements and know how to access medical evaluation and treatment.

Skin exposures should be managed by immediate washing of affected area with soap and water for minimum fifteen minutes. For chemical exposures including formalin, continued rinsing with copious water is essential. Medical evaluation should be sought promptly, particularly if skin is broken, if exposure involves mucous membranes, or if the substance involved is unknown.

Eye exposures require immediate irrigation with clean water or sterile saline for minimum fifteen minutes, followed by prompt medical evaluation. Contact lenses should be removed if present. Even seemingly minor eye exposures warrant medical assessment due to risk of serious complications including corneal damage or infection.

Puncture wounds or cuts contaminated with biological material require thorough cleaning with soap and water, application of appropriate disinfectant, and prompt medical evaluation. Tetanus immunisation status should be verified and updated if necessary. Healthcare providers should be informed of the marine mammal exposure to enable consideration of appropriate antibiotic prophylaxis against marine bacteria.

Inhalation exposures to decomposition gases, formalin vapours, or other airborne contaminants should be managed by removing the affected individual to fresh air immediately. If respiratory symptoms develop or persist, medical evaluation should be obtained. Individuals with respiratory conditions including asthma may be particularly susceptible to airborne irritants and should take additional precautions.

Documentation of all exposures, even those seeming minor, provides important information for assessing health risks and improving safety protocols. Exposure records should include date, time, location, nature of exposure, substance involved if known, immediate symptoms, first aid provided, and medical follow-up. This information supports medical treatment decisions and helps identify patterns indicating need for protocol modifications.

IMPORTANT: Key Protocol Points

Post Exposure Response

- Wash skin exposures immediately with soap and water for minimum 15 minutes
- Irrigate eye exposures with clean water or saline for minimum 15 minutes; seek medical evaluation promptly
- Clean puncture wounds and cuts thoroughly; seek medical evaluation for antibiotic consideration
- Remove affected individuals to fresh air immediately following inhalation exposures
- Document all exposures including minor incidents to support medical treatment and protocol improvement
- Inform healthcare providers about marine mammal exposure to enable appropriate treatment decisions

4.6 Emergency Response Planning

Comprehensive emergency response plans should address potential scenarios including personnel injury, animal aggression, equipment failure, adverse weather, and other contingencies. Emergency contact information including ambulance services, coast guard, police, and local hospitals should be readily available to all response team members. Communication protocols ensure rapid emergency notification and coordinate appropriate assistance.

First aid kits appropriate for field conditions should accompany all response teams, including supplies for treating cuts, punctures, eye injuries, heat-related illness, and allergic reactions. At least one team member should hold current first aid certification and be designated as the primary first aider. For operations in remote locations distant from medical facilities, more extensive medical supplies and advanced first aid training become necessary.

Vehicle safety and mechanical reliability warrant attention for operations in remote coastal areas where vehicle breakdown could strand personnel in hazardous locations. Vehicles should be properly maintained, carry appropriate emergency equipment including spare tyres and basic tools, and have adequate fuel for return journey plus reserve. Where operations involve boat access, marine safety equipment including life jackets, communication equipment, and navigation aids must be carried and personnel must possess appropriate maritime competencies.

IMPORTANT: Key Protocol Points

General Safety Requirements

- Conduct comprehensive risk assessments before all stranding response operations
- Ensure all personnel receive appropriate training for their roles and responsibilities
- Establish clear emergency response plans including medical evacuation procedures
- Carry appropriate first aid kits; ensure at least one team member holds current first aid certification
- Maintain vehicle and equipment in good condition; carry emergency supplies for remote operations
- Establish clear communication protocols and lines of authority before field deployment
- Review and update safety protocols regularly based on lessons learned from previous responses

5 Training and Competency Requirements

Personnel involved in stranding response should receive appropriate training commensurate with their roles and responsibilities. Basic stranding response training should cover species identification, initial assessment procedures, biosecurity principles, personal protective equipment use, basic first aid, and emergency communication protocols. More advanced training for personnel conducting post-mortem examinations should include anatomical knowledge, sampling techniques, health and safety practices specific to post-mortem procedures, and recognition of zoonotic disease risks.

Regular refresher training ensures maintenance of skills and knowledge, particularly for procedures performed infrequently. Skills deteriorate without practice, and refresher sessions provide opportunities to update protocols based on new information or lessons learned from previous responses. Documentation of training completed supports competency assessment and helps identify personnel qualified for specific roles within the stranding response framework.

Supervision arrangements should ensure less experienced personnel work under guidance of experienced practitioners, enabling skill development whilst maintaining safety and data quality standards. Clear lines of authority and decision-making responsibility should be established before field deployment, preventing confusion during response operations when rapid decisions may be required.

6 Tissue Sampling and Storage

6.1 General Principles of Tissue Sampling and Storage

Cetaceans represent protected species under Sri Lankan and international legislation, necessitating careful attention to ensure all requisite local, national, and international permits are secured prior to collection, archiving, transport, or exchange of tissues. The general nature and storage requirements for various sample types are outlined in subsequent sections, though specific requirements may exist for individual testing laboratories. Close liaison with analytical facilities regarding sampling and storage protocols prior to field work is strongly advised to ensure optimal sample quality and diagnostic capability.

Several fundamental principles govern tissue collection and preservation across all sample types. Swabs or tissues designated for subsequent microbiological or virological assay must be collected as expeditiously as practical, employing aseptic technique wherever possible, with appropriate cold chain maintenance between sampling and testing. When immediate microbiological analysis proves impossible, utilisation of appropriate transport media depending on target pathogens, or freezing particularly for virological studies, represents viable alternatives. Certain viruses or bacteria may necessitate specific sampling and preservation procedures that should be established in advance with diagnostic laboratories.

Tissues intended for microscopic examination require fixation in 10% neutral buffered formalin, ideally utilising ready-mixed commercial solutions to ensure standardised quality. When commercial reagents prove unavailable, preservative characteristics should receive regular verification. Samples placed in formalin must measure no thicker than 1 cm in any dimension, with sample-to-formalin ratios maintained at minimum 1:10. Formalin should be changed after 24 hours, particularly for fatty tissues such as brain specimens. Samples designated for immunohistochemical analysis should not remain in formalin beyond 72 hours. Formalin-fixed tissues should be stored for minimal duration prior to paraffin embedding to preserve optimal diagnostic quality.

Samples collected for contaminant analysis, including persistent organic pollutants, must first be wrapped in aluminium foil to prevent sample contamination from plastic storage containers. For heavy metal analysis, samples may be placed directly in plastic containers. All such tissues should be frozen at -20°C or colder temperatures. Samples destined for DNA molecular testing may be stored frozen or in 70% ethanol; however, samples collected for genomic, metabolomic, or proteomic investigations demonstrate greater lability and require specific transport and storage media. Tissues preserved for cell culture in appropriate media should be collected as rapidly as practical using aseptic technique, with proper conservation maintained between sampling and testing.

Frozen samples may be transported internationally using dry ice (solid CO_2) or biofreeze bottles. For situations involving large numbers of field-collected samples or distance transportation, IATA-approved liquid nitrogen vapour or dry shippers are recommended to maintain sample integrity throughout transit.

6.1.1 Sample Labelling Requirements

Proper sample labelling proves essential for traceability whilst accounting for long-term storage conditions including ultra-cold freezer temperatures up to -80°C. Every individual animal must be assigned a unique reference number that appears on all data records and samples associated with each case. Labels must include as minimum the unique reference number and tissue sample identifier. Every sample container requires firmly attached labels that resist freezing or ethanol exposure as appropriate. Text must be printed or handwritten clearly using indelible ink, or pencil for formalin-exposed labels, as certain inks including standard ballpoint pen ink may fade from freeze-thaw cycles or formalin submersion. Overlying such labels with transparent tape may help prevent deterioration, though pencil remains the most reliable option for formalin-fixed tissues.

6.1.2 Tissue and Biological Specimen Archiving

Maximising tissue availability for future research necessitates creation and maintenance of comprehensive sample archives. Establishment of standardised tissue banks for Sri Lankan cetacean research assists sample identification and recovery whilst simplifying CITES accreditation procedures that facilitate sample exchanges with international research networks. Effective sample archives prove particularly essential when investigating rare species, as accumulating statistically robust sample sizes may require several years of systematic collection. Availability of diverse samples from multiple individuals and species in centralised facilities greatly facilitates long-term pathological, ecological, and population studies critical for Sri Lankan marine mammal conservation.

As minimum archival protocol, lung, liver, and spleen samples for microbiological assessment should be stored below -20°C, with ecotoxicology and biomarker samples requiring storage below -70°C. Brain, lung, kidney, and spleen collected for virology, along with serum, cerebrospinal fluid, and other fluids, must all be stored below -70°C to maintain diagnostic viability.

IMPORTANT: Key Protocol Points

- Secure all necessary local, national and international permits before tissue collection
- Assign a unique reference number to every individual animal and all associated samples
- Use indelible labelling systems resistant to freezing or chemical exposure (pencil for formalin)
- Maintain cold chain integrity throughout all sample collection, storage, and transport
- Liaise with any laboratories regarding specific sampling requirements before fieldwork
- Document all sampling procedures, storage conditions, and chain of custody

7 Investigation Framework

7.1 Carcass Evaluation

7.1.1 External Assessment

The decomposition rate of stranded cetaceans cannot be accurately determined from external appearance alone or estimated solely from time since death. The process is influenced by complex factors particularly relevant in Sri Lanka's tropical coastal environment, which are crucial for accurate assessment and appropriate sampling decisions.

Environmental factors dominate decomposition rates along Sri Lankan coasts. High ambient temperatures and humidity characteristic of tropical climate significantly accelerate bacterial growth and enzymatic tissue breakdown. Intense solar radiation, severe throughout the year, dramatically affects dark-pigmented skin areas and can cause rapid blubber liquefaction. Sea temperature variations between monsoon periods create different preservation conditions for carcasses recovered from water versus beach strandings. Sand temperature variations between day and night further affect decomposition rates.

Biological factors inherent to individual animals significantly influence decomposition patterns. Body temperature at death is particularly important, as animals may be hyperthermic due to infection, stranding stress, or prolonged struggling. Blubber thickness varies between species and reflects nutritional status, with thicker layers providing insulation that retains heat whilst creating rich bacterial substrates. Body size creates differential cooling rates, with smaller cetaceans cooling faster due to higher surface area to volume ratios, affecting initial bacterial colonisation.

Post-mortem factors further complicate assessment in Sri Lankan waters. Toothed whales typically sink immediately but refloat days to weeks later when buoyed by decomposition gases, making time between death and discovery significantly longer than apparent. Scavenging by crows, gulls, and crabs may begin whilst animals are alive during stranding, meaning carcasses may show apparent post-mortem changes before death occurs. Physical damage from rocky coastlines, coral reefs, and shells accelerates bacterial invasion and decomposition.

Several physical indicators commonly used to estimate time since death in terrestrial animals are unreliable in marine mammal strandings and should not be employed for this purpose, including skin, eye, and mucous membrane dehydration. Additionally, submerged portions of floating carcasses are often better preserved than sun-exposed areas, creating inconsistent decomposition patterns that complicate assessment. Intense tropical sun exposure can rapidly accelerate decomposition and cause blubber liquefaction, further confounding external observations. More reliable indicators of decomposition state include bloating, although certain diseases like gas embolism may also cause gas accumulation and should be considered as differential diagnoses. Protruding tongue and penis also serve as useful external indicators. However, internal tissue examination provides the most reliable assessment of decomposition state and post-mortem interval in stranded cetaceans.

IMPORTANT: Key Protocol Points

External Assessment

Unreliable Indicators:

- Skin, eye, and mucous membrane dehydration should not be used to estimate time since death
- Submerged portions of floating carcasses are often better preserved than sun-exposed areas
- Intense tropical sun exposure can rapidly increase decomposition and cause blubber liquefaction

Reliable Indicators:

- Bloating (though certain diseases like gas embolism may also cause gas accumulation)
- Protruding tongue and penis
- Internal tissue examination provides the most reliable assessment

7.1.2 Internal Assessment

Internal examination provides the most reliable method for assessing decomposition state and determining tissue suitability for analytical procedures. Fresh cetacean tissues have distinctive characteristics changing in predictable patterns as decomposition progresses, though rates are accelerated in Sri Lanka's tropical climate.

Blubber examination provides one of the most useful condition indicators. Fresh blubber appears firm, ranging from white to cream-coloured, with consistency allowing clean knife sectioning. Depending on species and body condition, fresh blubber may exude clear oil when cut, indicating good preservation. Post-mortem changes begin with blood-tinged appearance through imbibition, where blood from underlying tissues penetrates the blubber matrix. Oil separation occurs through delipidation, with pooled oil eventually leaching out, leaving connective tissue matrix. This progression is typically much more rapid in Sri Lankan conditions due to consistently warm temperatures.

Muscle tissue examination is equally informative. Fresh cetacean muscle is characteristically darker than terrestrial mammals, ranging from dark red to almost black in mature deep-diving species, reflecting high myoglobin content for breath-holding. Foetal and young cetaceans display paler musculature darkening with age. Fresh muscle is firm with clearly distinguishable bundles easily separated along fascial planes. With decomposition, muscles become soft, lose bundle structure, lighten to almost translucent appearance, and lose structural integrity.

Internal organ decomposition rates are influenced by factors particularly relevant in Sri Lankan conditions. High ambient temperatures accelerate enzymatic autolysis processes. Pre-existing infections or ante-mortem hyperthermia increase apparent decomposition rates by providing optimal bacterial growth conditions. Open wounds from rocky or coral-rich coastlines provide bacterial invasion routes. Connective tissue arrangement and proteolytic enzyme content influence susceptibility. Blood promotes decomposition, so exsanguinated animals may show delayed decomposition compared to those dying with full blood volume.

Decomposition rarely occurs uniformly throughout carcasses, particularly in Sri Lanka's variable coastal environment where body regions experience different conditions. Organs most susceptible to rapid autolysis include pancreas, brain, spinal cord, liver, and thyroid due to high enzyme content and delicate tissue structure. These may show advanced decomposition whilst other tissues remain fresh, requiring careful evaluation of each tissue system individually.

IMPORTANT: Key Protocol Points

Internal Assessment – Fresh Tissue Characteristics

Blubber:

- Fresh: firm, white to cream-colored, may exude oil when cut
- Post-mortem changes: becomes blood-tinged from underlying tissues
- Advanced decomposition: oil separates and pools, leaving connective tissue matrix

Muscle:

- Fresh cetacean muscle is darker than terrestrial mammals (dark red to black in deep-diving species)
- Young animals have paler musculature
- Fresh muscle is firm with distinct bundles
- Decomposed muscle becomes soft, translucent, and loses bundle structure

7.1.3 Decomposition Condition Categories (DCC)

Standardised carcass decomposition classification is essential for determining analytical procedure reliability and providing consistent terminology. The five-category system provides assessment framework, though progression may be accelerated in Sri Lanka's tropical climate compared to temperate regions where protocols originated.

DCC 1: Extremely Fresh

Figure 2: Example of a DCC1 Extremely Fresh carcass.

DCC 1 represents recently deceased animals with minimal post-mortem change (Figure 2). These are typically live-stranded animals that died naturally or were euthanised, or sea-death animals recovered immediately. The defining characteristic is absence of significant post-mortem changes. Carcasses maintain fresh, clean smell without decomposition odour. Eyes remain clear and glassy with normal appearance. Blubber is firm, maintaining normal white colouration without blood staining or oil separation.

Muscle tissue retains characteristic firm texture and dark red to black colouration with well-defined, distinguishable bundles. All internal organs remain intact and well-defined with normal colouration and consistency. Gastrointestinal tract contains minimal gas, though some may be present from normal digestion or pathological conditions unrelated to post-mortem change. Brain maintains firm consistency with distinct surface features and can be removed intact. In Sri Lanka's hot climate, the DCC 1 window may be significantly shorter than temperate regions, requiring rapid assessment and processing.

DCC 2: Fresh

Figure 3: Example of a DCC2 Fresh carcass.

DCC 2 shows minimal decomposition whilst maintaining overall normal appearance (Figure 3). Animals retain fundamentally fresh smell, though subtle changes may begin. Minimal skin drying and wrinkling occurs, with eyes and mucous membranes showing slight drying but maintaining normal appearance. Importantly, carcasses show no bloating evidence, with tongue and penis remaining in normal positions.

Blubber remains firm and predominantly white, though occasionally showing slight blood-tinged areas where imbibition has begun. This should be distinguished from trauma-related haemorrhage by its diffuse, even distribution. Muscle tissue maintains normal characteristics though subtle softening may begin. Internal organs remain largely normal, though careful examination may reveal early changes in susceptible organs like pancreas or liver. Progression from DCC 1 to DCC 2 may occur within hours in Sri Lankan conditions, emphasising rapid initial assessment and priority sample collection importance.

DCC 3: Moderate Decomposition

Figure 4: Example of a DCC3 Moderate carcass.

DCC 3 represents a critical tissue preservation threshold where decomposition becomes evident but many analytical procedures remain viable (Figure 4). Most obvious change is carcass bloating from bacterial fermentation gas production, often accompanied by tongue and penis protrusion as internal pressure increases. Skin begins cracking and may start sloughing, though overall structure remains intact.

Characteristic mild decomposition odour becomes apparent, though not yet overwhelming. Mucous membranes appear dry and eyes become sunken as fluid is lost. Blubber examination reveals blood-tinged tissue with increasing oil separation, appearing oily. Muscle becomes noticeably softer with poorly defined bundle structure, though still identifiable and suitable for sampling.

Internal examination reveals gas-filled gut segments and organs showing uniform colouration throughout thoracic and abdominal cavities as fluids redistribute. Brain develops softer consistency but remains identifiable and usually suitable for sampling. Organs remain largely intact and distinguishable for identification and sampling, though consistency becomes soft. Kidneys and pancreas become soft and friable due to autolysis susceptibility. In tropical conditions, DCC 3 transition may be rapid, making timely assessment crucial for maximising analytical potential.

DCC 4: Advanced Decomposition

DCC4 (DAY 24)

Figure 5: Example of a DCC4 Advanced carcass.

DCC 4 shows extensive decomposition severely limiting analytical options but potentially providing valuable information for certain investigations (Figure 5). Carcasses may remain structurally intact but appear collapsed due to internal breakdown and gas loss. Skin sloughing becomes extensive with epidermis largely missing, exposing underlying blubber. Decomposition odour becomes strong and unmistakable, often detectable from considerable distance.

Blubber becomes soft, containing distinct gas pockets and pooled, separated oil areas. Normal firm texture is lost, feeling almost liquid. Muscle reaches advanced deterioration, becoming nearly liquefied and easily torn. Normal muscle-bone attachment is lost, allowing effortless separation. Remaining blood appears thin and black.

Internal organs, whilst often identifiable by location and shape, become friable and easily torn during handling. Dissection becomes extremely difficult as tissues lack structural integrity. Gastrointestinal tract is typically gas-filled and distended. Brain becomes liquefied with dark red appearance and gas pockets, approaching liquid consistency. Despite extensive changes, certain analyses remain possible, particularly involving stable structures like bones for age determination or genetic material from protected sites. Forensic investigations may still provide valuable cause-of-death or human interaction information.

DCC 5: Mummified or Skeletal

DCC5 (DAY 32)

Figure 6: Example of a DCC5 Indeterminate carcass.

DCC 5 represents final decomposition where only skeletal remains and desiccated tissues persist (Figure 6). In humid climates, complete mummification is less common than arid regions, but desiccation can occur with good air circulation and low humidity. Remaining skin appears draped over skeletal framework without underlying tissue support. Remaining soft tissues are completely desiccated and may be unrecognisable.

Organs are either absent or, if present, cannot be reliably identified. Normal anatomical relationships are lost, making individual tissue identification impossible except for resistant structures. Even at this stage, certain analyses remain possible, particularly genetic studies using bone material and morphometric studies of skeletal elements. Age determination through ear bones or teeth remains viable, and human interaction evidence like net marks or embedded foreign objects may still be detectable.

Progression to DCC 5 in Sri Lankan conditions may involve complex preservation and destruction patterns due to variable environmental exposure, scavenging pressure, and monsoon/dry season effects. Some carcasses may reach this stage rapidly during hot, dry periods, whilst others may be preserved at earlier stages if environmental conditions are favourable.

7.1.4 Tissue Sampling Recommendations by DCC

The following table (Table 1) provides guidance for tissue sampling based on decomposition condition:

Table 1: Guidance for tissue sampling based on decomposition condition.

Analysis Type	DCC 1	DCC 2	DCC 3	DCC 4	DCC 5	Sri Lankan Considerations
Genetics	✓	✓	✓	✓	✓	For DCC 4-5: use bone marrow for degraded DNA
Diet/Marine Debris	✓	✓	✓	\	X	Common plastic pollution in Sri Lankan waters
Age Determination	✓	✓	✓	\	X	
Fatty Acids/Stable Isotopes	✓	✓	✓	\	X	Important for Sri Lankan food web studies
Parasitology	✓	✓	✓	\	X	High parasite diversity in tropical waters
Morphometrics	✓	✓	✓	\	\	Bloating affects measurements in advanced DCC
Gross Pathology	✓	✓	✓	\	\	Recommended for forensic cases
Reproductive Studies	✓	✓	✓	\	X	
Toxicology	✓	✓	✓	\	X	Important given industrial pollution
Ear Investigation	✓	✓	✓	X	X	Inner ear analysis requires DCC 1

Analysis Type	DCC 1	DCC 2	DCC 3	DCC 4	DCC 5	Sri Lankan Considerations
Microbiology	✓	✓	\	\	X	Use kidney samples for septicaemia in DCC 3-4
Histopathology	✓	✓	\	\	X	
Virology	✓	✓	\	X	X	
Biotoxins	✓	✓	\	X	X	Important for harmful algal blooms
Gas Bubble Analysis	✓	✓	✓	✓	✓	Conduct before other procedures
Serology	✓	\	\	X	X	Collect cerebrospinal fluid quickly
Clinical Chemistry	✓	✓	✓	✓	✓	Vitreous humor useful in decomposed cases
Legend: ✓ = Recommended and reliable \ = Limited, may have limitations but potentially useful X = Not recommended, unreliable due to post-mortem changes						

7.1.5 Documentation and Photography

Comprehensive documentation forms the foundation of meaningful post-mortem investigations, particularly with complex decomposition patterns in Sri Lanka's diverse coastal environments. Documentation quality often determines long-term investigation value, allowing retrospective analysis and providing essential comparative study and forensic investigation information.

Detailed plain-language descriptions are acceptable and often preferable to technical terminology, particularly for teams without veterinary pathology experience. The goal is creating clear, comprehensive records interpretable by skilled professionals even when original investigators lack specialised training. Photographic documentation should accompany all written descriptions, serving as crucial evidence for later analysis and expert consultation.

Distribution and location of abnormalities must be carefully recorded with precise anatomical reference points. This includes identifying specific anatomical regions, organs, or tissues involved using standard terminology when possible but providing clear descriptive language when uncertain. Document whether abnormalities are bilateral or unilateral, as this provides crucial underlying cause clues. Distribution patterns should be characterised as diffuse (affecting large areas uniformly), focal (confined to specific locations), multifocal (present in several distinct areas), or patchy (irregularly distributed).

Physical characteristics require systematic documentation for meaningful comparative data. Size measurements should use standardised tools when available, but comparison with commonly known objects can provide valuable scale reference when unavailable. For teams with species experience, noting whether organs or body parts appear enlarged or reduced compared to normal provides important pathological insights. Three-dimensional lesion or abnormality shapes should be described using standard terminology: circular, oblong, spheroid, ovoid, target-like, wedge-shaped, irregular, papillary, pedunculated, sessile, or villous.

Lesion margins provide important diagnostic information and should be carefully characterised as indistinct (blending gradually into surrounding tissue), infiltrative (appearing to invade adjacent structures), well-demarcated (clearly defined), or specific patterns like serpiginous (snake-like), serrated, or papillary. Surface characteristics offer diagnostic clues and should be systematically described as smooth, rough, granular, crusted, ulcerated, or showing patterns like cobblestoning or corrugation.

Colour documentation requires careful attention to lighting conditions using standard descriptors. Commonly encountered colours include various red shades (bright red to dark mahogany), black, brown, grey-green, tan, white, and yellow. Colour changes often provide crucial pathological process and decomposition degree information. Consistency assessment requires careful palpation and comparison with known materials, providing essential tissue viability and post-mortem change information.

IMPORTANT: Key Protocol Points

- Record ambient temperature, humidity, and weather conditions
- Document time between discovery and examination
- Note any scavenging damage
- Photograph all stages of examination
- Maintain cold chain when possible, for sample preservation
- Consider heterogeneous decomposition across the carcass
- Use average DCC when decomposition varies across body regions

7.2 Tier 1 – External Examination

IMPORTANT: Key Protocol Points

- Use standardised data sheets and forms for all field documentation
- Photograph carcasses immediately upon arrival at stranding sites, even when no obvious marks are present
- Include case labels and scale bars in all photographs
- Take both close-up and wide-angle shots for proper contextualisation
- Capture lateral overviews of both sides, genital region, head with teeth/baleen, and cranio-caudal skyline views
- Measure total body length from rostrum tip to tail fluke notch with animal positioned ventrally
- Record girth measurements at dorsal fin level and optionally at axilla level
- Measure blubber thickness at dorsal, lateral, and ventral positions using perpendicular incisions
- Weigh complete carcasses when possible; clearly note when estimates are used
- Apply species-specific formulae for weight estimation when direct measurement is impossible
- Prefer chilling (0-4°C) over freezing for carcass preservation
- Only freeze carcasses when no fresh or chilled storage options exist
- Document all preservation methods used in post-mortem reports
- Establish secure digital storage systems for photographic archives
- Pay special attention to rare species requiring comprehensive documentation

7.2.1 Data Collection and Photography

Scientific value is maximised through careful documentation of systematically collected data and the use of non-ambiguous terminology. The implementation of standardised data sheets and forms is strongly recommended for all field work conducted in Sri Lankan waters and example Cetacean Basic Sampling and Cetacean Necropsy Recording forms have been provided in the Appendices. Written observations must be complemented by comprehensive photographic and video records of carcasses and their surrounding environment, as these can capture critical details including the pattern of mass strandings, evidence of predator or scavenger activity, and any markings, scars, or injuries that may disappear shortly after death or following carcass removal from the site.

Even in cases where no evident marks are visible, it remains essential to take photographs as soon as possible following arrival at the stranding site. Digital images and videos can prove extremely valuable in evaluating potential human interactions with the animal. When documenting wounds suspected to have been caused by vessel propellers, images must be taken with the camera lens positioned perpendicular to the axis of the lesion's surface to ensure accurate documentation of the injury pattern.

Photographic documentation serves multiple purposes beyond initial assessment. Images support the descriptions contained within post-mortem reports and, in more detailed tier three investigations, assist pathologists in identifying appropriate sampling areas and connecting macroscopic observations with subsequent microscopic evidence. The photographic record should encompass both general body overviews and detailed images of distinctive features.

At minimum, the photographic protocol should include lateral overviews of the entire body from both sides, detailed images of the genital slit region, photographs of the head showing exposed teeth or baleen plates, and a cranio-caudal 'skyline' image that clearly outlines the silhouette of the epaxial muscles. For cetacean species that are included in existing photo-identification catalogues, additional photographs of identifying characteristics such as colour patterns, dorsal fin configuration, and fluke markings should be captured. Rare species or specimens require particular attention and should receive comprehensive photographic documentation to ensure a complete scientific record is maintained.

When photographing specimens, it is recommended to include a case label and ruler or scale bar within each image. The label should ideally contain the animal identification number, the date of stranding, species identification, investigating organisation details, and specific information about the lesion or body part being documented. Close-up images should be accompanied by wider-angle shots to provide contextual information for viewers. Photographers must take care to minimise shadows, reflections, and glare whilst ensuring that fingers, instruments, or equipment do not obstruct important areas of the specimen. Scale bars and labels should be positioned so they do not occlude significant features.

Particularly noteworthy lesions or distinctive features should also be photographed without any scale or label present, as these images may be suitable for future publication purposes. When tissues or organs have been removed from the carcass, best practice involves placing them on an absorbent background that minimises blood visibility within the field of view.

Given the substantial storage requirements of high-resolution digital media, investigators should consider establishing secure archival systems, such as cloud-based storage solutions, to ensure long-term preservation and accessibility of photographic records.

IMPORTANT: Key Protocol Points

Key Photographs

- Position of animal on beach
- Left lateral (entire body, head to tail)
- Right lateral (entire body, head to tail)
- Dorsal view (overhead, entire body)
- Ventral view (belly up, entire body)
- Dorsal fin (each side)
- Genital area
- Cranio-caudal skyline view (to assess body condition)
- Head – left lateral
- Head – right lateral
- Head – dorsal (top of head, blowhole visible)
- Head – ventral (underside, jaw/throat)
- Blowhole – close-up
- Mouth – open (teeth/baleen visible)
- Eye – close-up (both eyes if possible)
- Any external lesions/injuries/scars, etc.

7.2.2 Body Measurements

Two principal measurements form the foundation of morphometric data collection: total body length and body girth. Length measurement requires placing the animal on its ventral surface when possible, then holding a measuring tape or ruler in a straight line parallel to the longitudinal body axis. The measurement spans from the notch present in the tail fluke to the tip of the rostrum.

Girth measurements should be taken for carcasses in decomposition condition codes DCC1-3, recorded in centimetres by placing the measuring tape around the carcass immediately cranial to the dorsal fin without compressing the body tissues (Figure 7). A secondary girth measurement may be obtained at the level of the axilla, positioned immediately caudal to the pectoral fin. In cases where complete encircling measurement is not feasible, such as when examining large whale species, investigators should take a half-girth measurement and double the result. Any instances where the carcass exhibits bloating, incompleteness, or other factors that may compromise measurement reliability must be clearly documented.

Blubber thickness measurement requires making a dorsoventral incision along the girth measurement line at the level of the cranial insertion of the dorsal fin. Blubber thickness should be recorded in millimetres at three specific locations: dorsal, lateral, and ventral positions. It is crucial to ensure that incisions are made perpendicular to the skin surface to obtain accurate measurements. Epidermal thickness is not routinely measured as part of standard protocols, though additional measurements may be taken when circumstances warrant, particularly when dealing with rare or scientifically significant species.

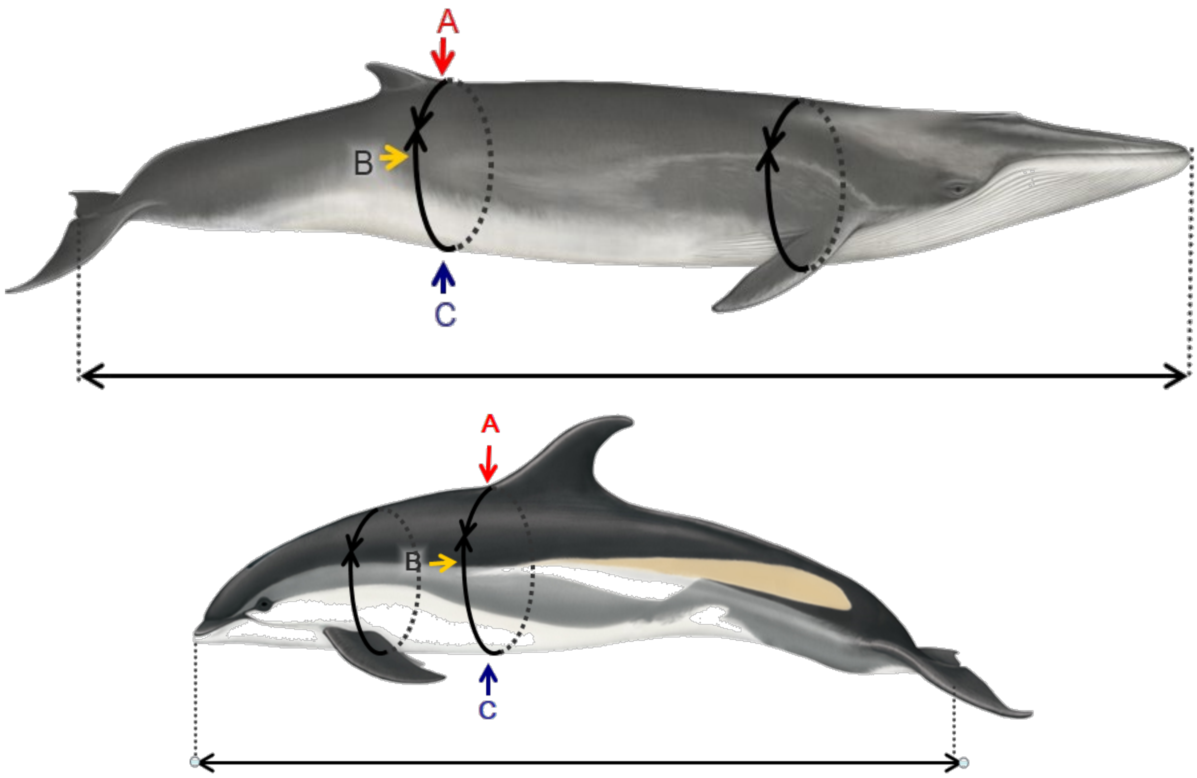

Figure 7: Images indicating the location of necessary measurements to be taken of stranded cetaceans, on the example of a fin whale (*Balaenoptera physalus*) and an Atlantic white-sided dolphin (*Leucopleurus acutus*). The letters indicate the location of blubber thickness measurement sites: A (red) = dorsal; B (yellow) = lateral; C (blue) = ventral. Images © CSIP/Lucy Molleson (top image) and WDC/Lucy Molleson (bottom image).

Complete carcasses should be weighed in kilograms whenever possible. When animals are not intact, this should be clearly noted and an estimated weight recorded. In situations where direct weighing is not feasible, weight can be estimated using established relationships between total length and body mass. These estimations must account for the carcass condition, as decomposition states can significantly affect the accuracy of weight estimates.

For more precise weight estimations, investigators may apply the functional relationship formula developed by Trites & Pauly (1998), where the maximum body length of a given species relates to mean population mass through the equation:

$M = a L^b$

with M representing mass in kilograms and L representing length in centimetres. The coefficients vary between Odontocetes and Mysticetes and between sexes:

- Mysticetes: males use a = -7.347 and b = 2.329, whilst females use a = -7.503 and b = 2.347
- Odontocetes: males use a = -8.702 and b = 2.382, whilst females use a = -9.003 and b = 2.432
- Sperm whales, due to their unique anatomical characteristics, require a specialised formula developed by Lockyer (1991): $M = 0.218 \times L^{2.74}$.

7.2.3 Freezing Carcasses Prior to Post-Mortem Examination

In circumstances where immediate post-mortem investigation capacity is unavailable, freezing the carcass represents a possible alternative preservation method. However, chilling the carcass at temperatures between 0–4°C for periods up to 5–10 days is strongly preferable to freezing, particularly for small cetaceans, due to the artefacts that are unavoidably induced by freeze-thaw processes.

These freeze-thaw artefacts can mask or completely obliterate critical pathological indicators, potentially compromising the scientific and diagnostic value of subsequent examinations. Therefore, freezing should only be employed as a last resort when no possibility exists for examining the carcass in fresh condition or storing it under chilled conditions. Any decision to freeze a carcass prior to examination must be clearly documented and stated in the final post-mortem report to ensure proper interpretation of findings.

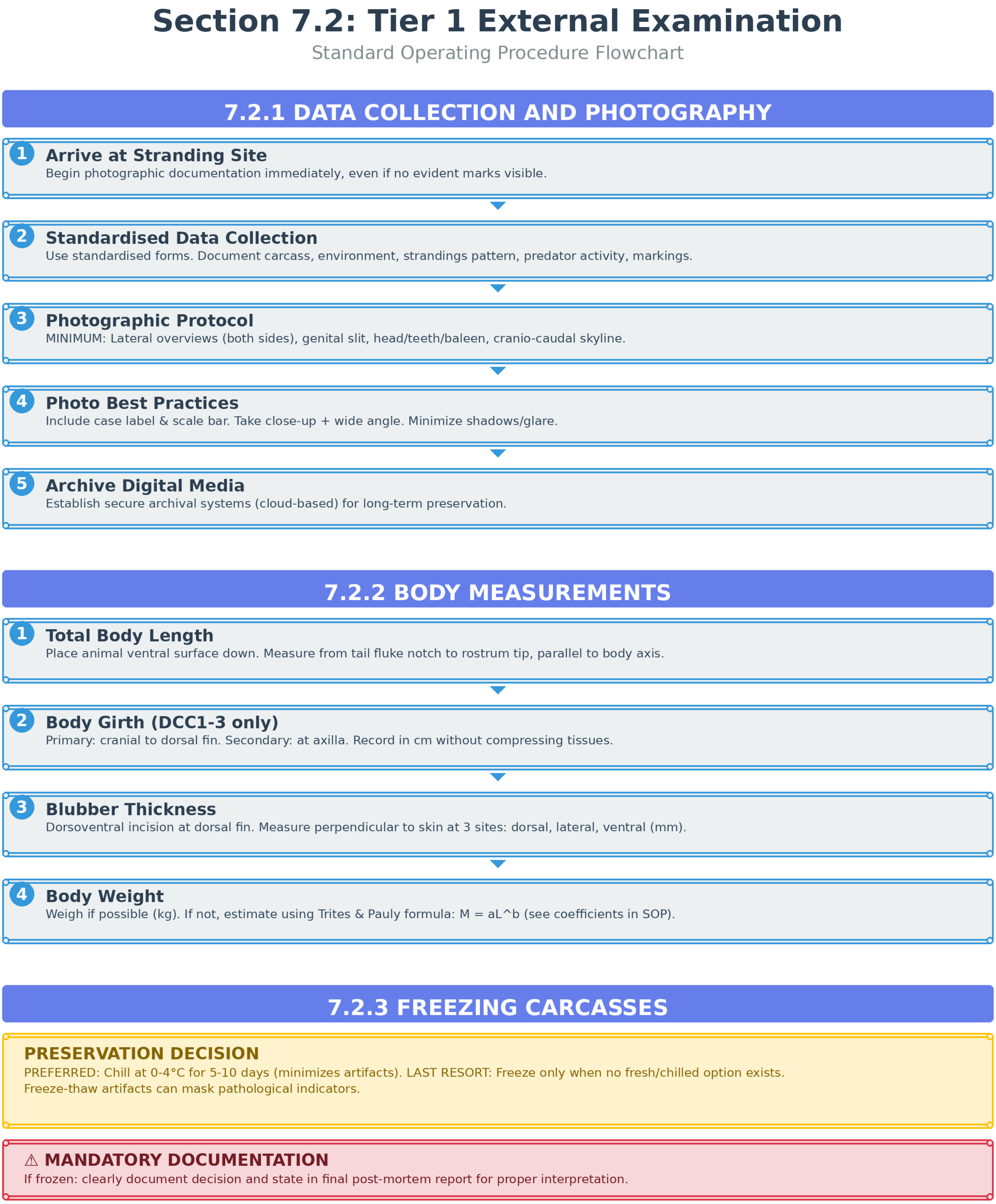

Figure 8: Flow Chart outlining Tier 1 External Examination Process.

7.3 Tier 2 – Tissue Sampling and Post-mortem Investigations

This section provides comprehensive protocols for postmortem investigations and tissue sampling of cetaceans found in Sri Lankan waters, modified from established international guidelines. These protocols are designed to ensure thorough examination and appropriate sample collection for histological, microbiological, virological, and parasitological investigations.

7.3.1 General Examination Principles

During any postmortem examination, all anatomical structures must be examined visually in their natural position, including the vascular system, through careful palpation and systematic incision into organs. All findings must be meticulously recorded, including notation of 'no abnormalities detected' (NAD) for normal structures and any organ systems that remain unexamined (NE). The presence and sampling of gas bubbles within the cardiovascular system requires particular attention and should be carried out following specific established protocols.

When lesions are identified in any organs, comprehensive descriptions must include their size, location, colour, texture, shape, and margins, as well as the nature of the transition from normal to abnormal tissue. This includes documenting how well or poorly demarcated the lesion appears. Such objective assessment allows for accurate description of observed changes compared to normal anatomical conditions. For less experienced personnel, this systematic approach, combined with comprehensive photography during examination, enables consultation with skilled experts for guidance and confirmation of findings.

All lesions should be photographed with a ruler or scale bar for accurate documentation. Representative tissue samples must be placed into appropriate fixatives, with 10% neutral buffered formalin being the most commonly used and widely available fixative for histological examination. Sample collection should ensure inclusion of the transition zone from normal to abnormal tissue, whilst also including tissue samples showing no gross lesions for comparative purposes. Additional samples should be collected for specific testing and stored appropriately according to the suspected aetiology of any identified lesions.

7.3.2 Examination Sequence and Timing

The procedures for dissecting and examining cetacean carcasses depend significantly on the size and species involved but should follow established guidelines as outlined below. Initially, all procedures described in tier one protocols should be completed before proceeding to tier two examinations. Gas examination with possible subsequent sampling and ear collection for inner ear analysis, along with samples for microbiological and virological analyses, must be taken as early as possible in the examination process to avoid artefacts caused by sectioning, decomposition, contamination, or microbial genome degradation through proteolysis.

Particular attention must be paid to the brain and central nervous system, ears, pancreas, thyroid gland, and liver, which should be fixed in 10% neutral buffered formalin as soon as possible for histopathological investigations due to rapid postmortem autolysis affecting these tissues. Care must be taken throughout the examination to prevent cross-contamination with enteric micro-organisms. For this reason, examination of the gastrointestinal tract should be performed last in the examination sequence, unless gross pathology is evident in the gastrointestinal system. When gastrointestinal pathology is present, it is recommended to remove the entire tract and investigate it on a separate table or area to enable immediate sample collection.

7.3.3 External Examination

Following the photographic documentation, body measurements, and assessment of carcass condition described in tier one protocols, the animal should be thoroughly examined for external lesions. This examination should include identification of signs of intra-species and inter-species interactions, as well as evidence of anthropogenic interactions. Particular note should be made of any penetrating wounds, and systematic collection of ectoparasites should be undertaken with appropriate sampling. Ectoparasites are most commonly found in or near body openings including wounds, in natural crevices, or adjacent to and on the fins and flukes.

A 2 cm^2 piece of full-thickness skin should be collected where possible, excluding the blubber layer, and muscle samples should be taken for DNA studies. Additional samples of skin, blubber, and muscles should be obtained for various postmortem examinations including ecotoxicological studies, histopathology, and stable isotope analyses. The oral cavity requires thorough examination, including teeth or baleen, tongue, gingiva, and lymphatic tissue. The eyes should be examined for bubbles, evidence of intraocular haemorrhage, asymmetry, or swelling. Similarly, the blowhole, anus, genital slit, and mammary slits when present should be inspected for lesions, discolorations, and discharges.

In examining the mammary region, pressure should be applied to the skin cranial to the mammary slits in a caudal direction to express any content present in the mammary glands. If liquid can be extruded, a sample should be collected for analyses such as toxicology, with careful recording of the volume, colour, and consistency of the liquid. Any abnormalities identified should be sampled to facilitate identification of aetiological agents.

7.3.4 Nutritional Condition Assessment

Assessing the body condition state provides a crucial indicator of the animal's ante mortem health status. This assessment should be based on blubber thickness, lipid composition, and back muscle mass. Physiological blubber thickness is naturally influenced by numerous factors including species, season, geographic region, sex, age, reproductive status, and environmental temperature, making isolated assessment challenging. However, emaciated animals characteristically lose both fat reserves and muscle mass, most notably visible in the blubber and lumbar muscles dorsal to the spine.

Accurate assessment of abnormal blubber thickness requires experience with the specific species in relation to its environment, as blubber thickness varies physiologically according to season, age, and sex. Additionally, measurement of lipid percentages in the blubber layer can provide informative descriptors of nutritional condition in fresh cases. Based on the state of blubber and skeletal muscle, nutritional condition can be characterised using a five-point scale ranging from very good to emaciated, with specific criteria for each category relating to body outline, presence of internal fat deposits, and blubber thickness measurements.

NCC 1: Very Good

Figure 9: Example of a NCC1 Very Good nutritional condition.

Animals in NCC1 Very Good nutritional condition exhibit a convex outline when viewed from a cranial perspective, with a rounded appearance visible caudal to the skull and lateral to the dorsal fin (Figure 9). Subcutaneous pleural, and other visceral fat deposits are present, and blubber layers are thick throughout the body.

NCC 2: Good

Figure 10: Example of a NCC2 Good nutritional condition.

Animals in NCC2 Good nutritional condition exhibit a convex outline when viewed from a cranial perspective, with no hollow appearance visible caudal to the skull and lateral to the dorsal fin (Figure 10). There is possibly some subcutaneous pleural, and other visceral fat present.

NCC 3: Suboptimal

Figure 11: Example of a NCC3 Suboptimal nutritional condition.

Animals in NCC3 Suboptimal nutritional condition exhibit an outline that is not fully round when viewed from a cranial perspective, with a slight hollow or almost flat appearance visible caudal to the skull and lateral to the dorsal fin (Figure 11). No internal fat deposits are observed.

NCC 4: Poor

Figure 12: Example of a NCC4 Poor nutritional condition.

Animals in NCC4 Poor nutritional condition exhibit a moderately concave outline when viewed from a cranial perspective, with the lateral aspects of the vertebrae visible (Figure 12). A hollow appearance is evident caudal to the skull and lateral to the dorsal fin, and the scapulas can be observed protruding noticeably.

NCC 5: Emaciated

Figure 13: Example of a NCC5 Emaciated nutritional condition.

Animals in NCC5 Emaciated nutritional condition exhibit a very concave outline when viewed from a cranial perspective, with the lateral aspects of the vertebrae easily palpable (Figure 13). An extremely hollow appearance is visible caudal to the skull and lateral to the dorsal fin, and the scapulas protrude prominently. Blubber layers are minimal, measuring less than 1 cm in small odontocetes.

IMPORTANT: Key Protocol Points

- Document everything: Record all findings including 'no abnormalities detected' and photograph all lesions with scale bars
- Systematic approach: Follow established sequence - external examination, subcutaneous examination, abdominal organs, thoracic organs, head examination, skeletal system
- Sample preservation: Use appropriate fixatives and storage temperatures (-20°C for biotoxins, room temperature for some samples, 10% formalin for histology)
- Early sample collection: Gas examination, ear collection, and microbiological samples must be taken immediately to prevent decomposition artefacts
- Brain, pancreas, thyroid, liver, and ears: Fix in 10% neutral buffered formalin as soon as possible due to rapid postmortem autolysis
- Nutritional condition: Assess body condition based on blubber thickness, lipid composition, and muscle mass using five-point scale

7.3.5 Subcutaneous Examination

Blubber thickness should be measured as described in tier one protocols, with careful recording of blubber colour variations including white, yellow, or pink coloration. Particular attention should be paid to the melon and acoustic fat bodies both externally and internally to the lower jaw. Multiple incisions should be made into these tissues to check for haemorrhages, with appropriate samples of blubber and muscle collected following established sampling procedures.

The animal should be positioned in right lateral recumbency when possible, with a mid-line ventral incision made from the symphysis of the mandible to a short distance posterior of the anus, carefully circumventing the umbilical region, genital slit, and anus. A second incision should be made from the posterior end of the ventral incision extending almost to the dorsal mid-line. Using natural tissue plane weaknesses, the skin and blubber should be separated from underlying muscle to remove the integument from the upper side.

During this phase, particular attention must be paid to subcutaneous veins to detect and quantify any evidence of gas bubbles according to established protocols. The blubber layer should be examined as extensively as possible by cutting strips and noting colour and presence of any discolouration such as haemorrhages. Any parasites or lesions in the blubber should be recorded with representative samples collected. *Cestoda* parasites may appear as white cysts less than 1 cm in diameter, often in the ano-genital region or dorsal aspect of the chest wall, whilst nematodes may also be found in subcutaneous tissue as a result of larval migration patterns.

7.3.6 Internal Organ Examination

When opening body cavities, any abnormal liquid or lesions must be noted and carefully assessed to establish origin and aetiology. The anatomical position of organs should be verified, paying attention to any displacement, ruptures, or herniation. Any free fluid in thoracic and abdominal cavities should be collected for analysis. The presence of gas bubbles, particularly in the thorax, mediastinum, and peri-renal location, in mesenteric veins and lumbo-sacral plexus should be evaluated and quantified according to established protocols.

The abdominal cavity should be opened prior to the thoracic cavity when possible to observe proper diaphragm position and assess the presence of gas in the chest. After removing the left abdominal wall and gross evaluation of pre-existing punctures to the thoracic wall, physiological negative pressure can be confirmed by incising the diaphragm and observing small caudal displacement accompanied by the sound of air influx.

7.3.7 Abdominal Organ Examination

Care must be taken to avoid contamination of the carcass with gastrointestinal tract contents, preferably by tying off the oesophagus and rectum to prevent content leakage and removing the stomachs, mesenteric lymph nodes with intestines, pancreas, and spleen as a unit. The pancreas should be removed, macroscopically examined, checked for parasites, and fixed in 10% neutral buffered formalin as soon as possible due to rapid decomposition from chemical and enzymatic postmortem autolysis.

The spleen should be assessed with splenic tissue sampled for microbiological and virological examination. Any accessory spleens and presence of gas bubbles in mesenteric vasculature or spleen should be noted. The urinary bladder should be examined in situ with any urine present sampled using a syringe, noting volume, colour, turbidity, and nature of contents. This sample proves useful for microbiology, with urine frozen at -20°C for biotoxin examination. Any uroliths detected should be frozen at -20°C prior to analysis.

For female specimens, the entire reproductive tract should be removed and the vagina and uterus opened to examine and sample any stones, mucosal alterations, or discharge. Reproductive history can be informed by ovarian tissue analysis, with recording of any corpora lutea, albicantia, or follicles on each ovary before storage in 10% neutral buffered formalin with transverse incisions to allow proper fixation.

In males, the testes should be removed, checked for symmetry, and weighed with notation of whether epididymal tissue is included, measuring the long axis length as testes size correlates with sexual maturity and activity. Cross-sectional incisions at 1 cm intervals should be made for examination, with appropriate storage for reproductive studies. The genital opening, penis, epididymis, and accessory glands should be examined with additional sampling for histology, virology, and microbiology conducted when abnormalities are observed.

7.3.8 Gastrointestinal Tract (GIT) Examination

To avoid contamination from gastrointestinal content, both ends should be tied before extracting the tract from the abdominal cavity. When micro- and nano-plastic studies are being considered, environmental contamination through airborne particulates, tools, clothing, or other plastic objects should be minimised. Microbiological sampling should be considered as the first step and carried out as soon as possible before other procedures.

Mesenteric lymph nodes should be located and examined with a 0.5 cm thick cross-sectional slice taken from halfway along the length for histological examination. The stomachs should be separated from the intestine by ligating the gastrointestinal tract after the ampulla duodenalis, noting that cetaceans generally have three stomach compartments with species-specific variation in compartment numbers.

The presence and amount of ingested prey species, digesta, otoliths, and parasites should be recorded with appropriate sampling. Evidence of marine litter should be noted by recording and weighing the presence and type of any macro-plastics or other ingested marine debris in the stomachs, retaining material for future studies. Better evaluation for marine litter ingestion should be carried out by washing gastric surfaces and stomach contents using appropriate mesh sizes to separate diet and parasites from foreign bodies.

7.3.9 Cranial Region and Thoracic Examination

Before opening the thorax, the presence of negative pressure in the chest should be assessed. The thorax should be opened by cutting ribs and checking for possible bone fractures. The larynx should be located with its position noted, and the oral cavity checked for foreign bodies. The presence and colour of any foam or obstruction should be noted.

The heart and lungs should be freed from the thoracic cavity, checking for adhesions between lungs and thoracic wall or diaphragm and the presence of any anomalous gas presence in the mediastinum or visceral pleura. Any free fluids in the pericardial sac and thoracic cavity should be noted and sampled, as these fluids can serve as serum substitutes in cases of moderate decomposition when serum cannot be obtained directly.

Both lungs should be examined for profound asymmetry in size or congestion, with multiple incisions made into the parenchyma. The level and distribution of any parasite burden in pulmonary parenchyma should be noted with representative samples taken. A minimum of two pieces from each lung should be collected for histological examination, including one from the hilus and one from near the surface including pleura.

7.3.10 Head Examination

The eyes should be examined and collected for histology with the optical nerve attached. Each ear bone should be removed carefully as soon as possible, noting any signs of bleeding or trauma in surrounding tissue and the external ear canal. Ear sinuses, Eustachian tubes, nasal sacs, and sinus cavities should be examined thoroughly.

For carcasses in good condition, ears should be removed for examination of infectious diseases, degenerative and traumatic changes, with comprehensive microbiological, parasitological, virological, and histological investigations conducted. The inner ear should be fixed following specific protocols including removal of the stapes, perforation of round and oval window membranes, and slow perfusion of fixative into inner ear structures.

The melon should be examined through serial sections to assess trauma or inflammatory lesions, including assessment of upper airways for foreign bodies, parasites, or discharge. For odontocetes, teeth should be examined noting tooth wear, dentition integrity, gingival state, and tooth number and position. At least four intact teeth should be removed from the middle of the lower jaw for age determination. For mysticetes, baleens should be examined for gross lesions, parasites, and trauma, with up to six baleen plates collected.

Cerebrospinal fluid should be sampled before removing the head and opening the skull to examine the brain. The brain should be removed from the skull with systematic examination and appropriate sampling for various analytical purposes including histopathological, microbiological, virological, and ecotoxicological investigations.

7.3.11 Skeletal System Examination

Representative samples from different muscle locations and diaphragm should be collected for histology, toxicology, and parasitology. All bones should be investigated macroscopically for changes including fractures, inflammations, and degenerations. Samples from humerus, lumbar vertebrae, ribs, and any lesions should be preserved for histological and infectious disease investigations as well as toxicology. The rete mirabilis should be sampled for histological examination.

IMPORTANT: Key Protocol Points

- Representative sampling: Collect samples for histology, microbiology, virology, toxicology, and parasitology from all major organ systems
- Prevent cross-contamination: Examine gastrointestinal tract last unless gross pathology is present, then examine on separate table
- Reproductive assessment: Complete examination of reproductive organs with appropriate sampling, including ovarian scarring assessment in females
- Gas bubble assessment: Check for and quantify gas bubbles in cardiovascular system, particularly thorax, mediastinum, and peri-renal locations
- Marine debris documentation: Record and weigh all plastic debris and marine litter found in gastrointestinal tract
- Cerebrospinal fluid: Collect before opening skull using sterile technique with flame sterilisation of sampling site

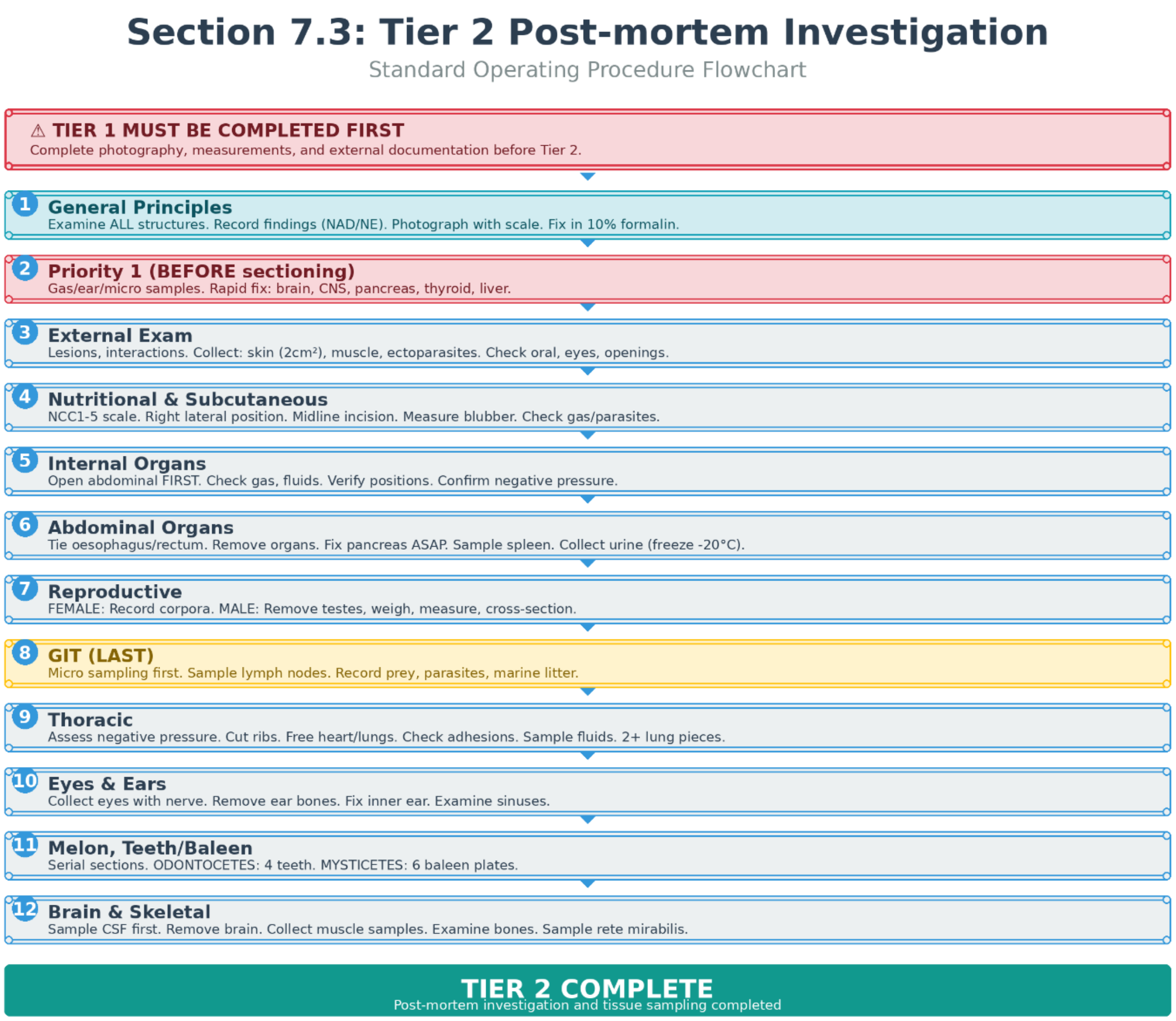

Figure 14: Flow Chart outlining Tier 2 External Examination Process.

7.4 Tier 3 – Advanced Diagnostics

This tier encompasses advanced diagnostic procedures and specialised sampling protocols for comprehensive investigation of cetacean strandings in Sri Lankan waters. These protocols build upon the foundational and systematic examination procedures established in Tiers One and Two, focusing on sophisticated analytical techniques that require specific sample collection, preservation, and storage methodologies.

7.4.1 Morphometric Studies and Advanced Imaging

When facilities and resources permit, assessment of carcasses through advanced imaging technologies prior to post-mortem examination provides invaluable data for morphometric analysis and pathology identification. Computed Tomography (CT) scanning enables visualisation of complete skeletal anatomy and lesions including trauma evidence and projectile injuries, whilst also facilitating bone density assessment and evaluation of joint structures and intervertebral discs. Magnetic Resonance Imaging (MRI) permits comprehensive assessment of internal anatomy including organ positioning, all soft tissue structures, and numerous potential pathologies that may not be visible through external examination alone.

At more fundamental levels, collection and maceration of skulls, specific bones, or complete skeletons provides substantial value for stable isotope analysis, morphometric studies, and accession to museum collections throughout Sri Lanka and internationally. However, pathological investigations must always take precedence over maintaining skeletal integrity for museum collection purposes. Histology samples should be systematically collected from any bone abnormalities present in examined animals to ensure comprehensive diagnostic assessment.

7.4.2 Age Determination Procedures

Longitudinal cross-sections through odontocete cetacean teeth reveal annual growth rings enabling accurate age determination. To ensure complete ring visibility on cross-section, minimum four whole undamaged or least worn teeth should be removed from middle lower jaw regions of each individual, as these teeth typically demonstrate straightest configuration facilitating age determination. Small odontocete teeth may be extracted easily through careful insertion of sharp knives or scalpels on either side of gums to cut periodontal ligaments. Species possessing larger, more conical teeth such as delphinids require initial loosening by levering spaces between teeth and sockets using sharp pointed instruments, with dental elevators providing useful extraction tools. When tooth extraction proves impossible, mandibular sections containing minimum four teeth should be collected. In baleen whales, age estimation may be conducted through analysis of baleen plates or earplugs.

Loose teeth, jaw sections, baleen, and earplugs may be stored frozen, with storage temperature not proving critical. Alternatively, for short-term storage, specimens may be fixed in 70% ethanol. Dry storage must be avoided as dehydration may cause tooth cracking compromising subsequent analysis.

7.4.3 Diet Analysis, Marine Litter, and Micro-/Nano-Plastics Investigation

Minimising contamination of gastrointestinal tract contents from environmental microplastic sources requires oesophageal and rectal ligation followed by removal of entire unopened GIT for examination in clean areas. Alternatively, components including oesophagus, gastric chambers, and intestine may be separated using strings or cable ties and removed piecemeal. All components may be stored frozen at -20°C for subsequent analysis when immediate tract examination proves unfeasible.

The GIT should be placed on non-plastic surfaces or grids prior to opening and examination. Gross abnormalities and presence of ingesta, digesta, parasites, and marine debris require thorough recording. Gut contents and mucosal lining should be gently rinsed and filtered through meshes of decreasing size to separate macro and meso-plastics (useful samples up to 1 mm), micro-plastics (up to 50 µm when possible), dietary components (up to 500 µm), and parasites (up to 250 µm). Remaining fluid may be preserved for nano-plastic investigations.

Due to substantial environmental contamination risks, microplastic analysis protocols typically require samples collected from air and any plastic materials used or present during sampling and analysis. These serve as negative controls and include gloves, clothing, instruments, cleaning equipment, and water pipes. Plastic materials recovered from GIT should be categorised as rope, net, floats, monofilament, braided line, hooks, packaging, cigarette butts, plastics, and other anthropogenic materials. Size, shape through image analysis of digital photographs, mass, volume, and polymer type for plastics using Raman spectroscopy or Fourier transform infrared spectroscopy (FT-IR) should all be recorded, with all evidence identified as to source using established techniques. Marine litter classification should employ MSFD master list protocols.

7.4.4 Genomic Studies and Molecular Diagnostics

DNA recovery proceeds most readily from skin or muscle samples. These specimens may be stored frozen at -20°C or in 70% ethanol. When facilities permit, storage of duplicate samples in RNAlater® at -80°C enables more advanced genetic sequencing techniques. RNAlater® solution should only be used with fresh tissue; tissues must not be frozen before immersion, and samples should be cut to ≤ 5 mm in any single dimension. Fresh tissue should be placed in five to ten times the volume of RNAlater® solution. Most tissues in RNAlater® solution may be stored at room temperature for one week without compromising RNA quality, or at -20°C or -80°C indefinitely. Immediate freezing should be avoided; allow minimum 12 hours at 4°C to permit thorough solution penetration into tissue, remove supernatant, then freeze at -20°C or -80°C for long-term storage.

7.4.5 Reproduction Studies

Female gonads require placement of both ovaries whole in 10% neutral buffered formalin. Differentiation between left and right ovaries proves important, achievable through separate containers or small incisions in one ovary. Male gonads necessitate separate weighing of each testis following epididymis removal. Subsequently, cross-sectional slices approximately 1 cm thick from midway along organ length should be placed in 10% neutral buffered formalin. As with all formalin-fixed tissues, tissue-to-fixative ratios should exceed 1:10 to enable adequate preservation. Once fixed after 24-48 hours, samples may be stored in smaller fixative volumes.

7.4.6 Histopathology and Immunohistochemistry Sampling

In grossly normal organs, representative cross-sectional samples measuring 3-5mm, and no more than 10mm thick, should be collected. All focal gross lesions require sampling to include adjacent normal tissue pieces. Sharp scalpel use and gentle handling prove essential to avoid crush artefacts, particularly in soft and delicate tissues including brain, spinal cord, pancreas, lymphoid organs, and GIT mucosa. Individually labelled containers or tissue fixation in permeable bags such as tea bags may facilitate differentiation and sample identification when multiple samples are fixed from morphologically or microscopically similar organs such as lymphoid tissues.

Ten percent neutral buffered formalin (4% formaldehyde) represents the most common fixative. Other potentially less hazardous fixatives are emerging commercially, though experience regarding their use for immunohistochemistry or in-situ hybridisation examined tissues remains limited. Formalin penetrates at approximately 1 cm per day, hence the requirement to reduce samples to 1–1.5 cm diameter avoiding inner tissue portion autolysis. Tissues should remain in fixative at room temperature for minimum 24–48 hours, except brain specimens. Longer immersion may affect immunohistochemical studies. Tissues with high blood content should have fixative changed every 24 hours until no longer discoloured. Formalin injection into internal spaces ensures proper fixation for structures such as ears, eyes, and intestine.

Brain specimens should be placed in minimum ten times as much formalin as tissue for initial fixation, remaining in this fluid for minimum one week. To facilitate faster fixation, multiple transverse or coronal incisions may be made through frontal lobes to expose lateral ventricles. An alternative method involves chilling brain for one hour at -20°C prior to making complete coronal cuts 1 cm wide, separating slices with paper sheets and immersing in 10% neutral buffered formalin. This facilitates more rapid fixative penetration into all brain areas. In freshly deceased cases, ensure brain cooling to near room temperature prior to fixative immersion to avoid fixation artefacts such as dark neurons or arteriolar contraction.

Samples may be stored at room temperature. Once fixation completes, samples may be stored in smaller volumes of 10% neutral buffered formalin. When processing time exceeds three months, change to formol saline at this point to avoid saponification. When long-period preservation of formalin-fixed samples proves necessary, paraffin embedding represents the superior option. When immunohistochemistry is required, tissues may subsequently be stored in 70% ethanol. To minimise fixation artefacts, neural tissue sample processing within three months is recommended. Samples stored for years likely possess limited diagnostic value for immunohistochemistry.

7.4.7 Microbiological Sampling and Analysis

Microbiological diagnostic sampling should be conducted in collaboration with laboratories undertaking sample processing. Preferably, routine microbiological examination should be conducted on standard tissue suites comprising lung, liver, spleen, kidney, brain, cerebrospinal fluid, and any organs or tissues with gross lesions of suspected microbiological aetiology.

Samples should be collected using aseptic technique whenever possible. Tissue samples whether solid or liquid, or swabs such as nylon flocked swabs, both prove appropriate for microbiological examination. Each approach possesses advantages and disadvantages, with choice depending on laboratory performing microbiological analysis and target microorganisms.

For solid tissue samples, blocks approximately 4 cm^3 should be cut using sterile scalpels and forceps and placed into sterile containers. Samples should be kept chilled below 4°C and processed within 24 hours of collection. When analysis proves impossible within that timeframe, samples may be frozen at -20°C to allow subsequent growth of fastidious organisms.

For swab preparation, tissue surfaces should be seared with hot blades to remove surface contamination, then incised with sterile scalpels and sterile swabs introduced into incisions, avoiding contact with surrounding tissues. Direct inoculation onto culture medium or alternative storage in tubes containing transport medium and storage at 4°C for up to 24 hours prior to processing may be employed. When longer storage proves likely, solid tissue samples are preferred.

For neurobrucellosis (*Brucella ceti*) diagnosis, cerebrospinal fluid represents the sample of choice, collected aseptically through foramen magnum using sterile Pasteur pipettes or syringes and sterile needles of appropriate length (1.3 x 88 mm).

Sample culture should be undertaken by specialist pathologists or microbiologists, as different media and methods are required depending on suspected target microorganisms. Many pathogens require selective growth media or specialised containment due to zoonotic potential, for example *Brucella* or *Mycobacterium* species.

7.4.8 Virological Sampling and Analysis

Sampling choices for virological diagnostics should be made in collaboration with processing laboratories, considering tissue tropisms of suspected viruses, pathological indicators, and carcass conditions. Tissues including lung, spleen, brain, and kidney represent ranges of samples required for several known viruses including morbillivirus, influenza, poxvirus, and *herpesviridae* detection. Any lesions of suspected viral aetiology should be sampled similarly. For virus isolation, samples of $1cm^3$ from relevant organs should be collected aseptically and placed into sterile containers with viral transport medium.

When samples are collected for molecular diagnostics such as PCR and submitted to laboratories within 24 hours of sampling, they may be stored chilled at 0–4°C; otherwise freezing at -80°C proves necessary. When ultra-cold freezing proves impossible, samples may be placed in RNAlater® and stored chilled or frozen at -20°C.

Sero-epidemiological investigations against viral and other infectious agents ideally require approximately 7 ml of fresh, non-haemolysed blood, centrifuged to obtain serum. Serum should be separated from whole blood as rapidly as possible and stored at -80°C. Serum demonstrating some erythrocytic haemolysis evidence possesses limited diagnostic value. When blood samples prove unobtainable, pericardial fluid, vitreous humour, or even lung or muscle tissue juices offer suitable substitutes. In fresh cases, cerebrospinal fluid sample collection is highly recommended to screen for antibodies against ranges of neurotropic pathogens including Cetacean morbillivirus, herpesvirus, West Nile virus, Brucella spp., and *Toxoplasma gondii*.

7.4.9 Parasitological Sampling and Analysis

Parasite specimens should be gently removed or dissected from tissues and cleaned in tap water using soft paintbrushes. Specimens may be held submerged in petri dishes of water for immediate analysis, or stored in 70% ethanol, alcohol with 10% glycerine, or frozen at -20°C.

When total parasite burden cannot be recorded, semi-quantitative assessment of infestation degree provides valuable information, for example none equals no macroscopically visible parasites, mild, moderate, or severe infestation, recorded for each organ. Overall burden and associated lesion documentation using photographs and histopathology is recommended. Furthermore, macroscopically detectable severity of parasite-associated lesions should be recorded as no lesion, mild, moderate, or severe, with tissue sampled in 10% neutral buffered formalin for histopathological examination.

7.4.10 Toxicological Sampling and Analysis

Sampling choices for toxicological assessment should be made in collaboration with laboratories undertaking sample processing. Archiving duplicate samples of blubber, muscle, liver, kidneys, and brain for subsequent persistent organic pollutant (POP), plastic additive, trace element, and fatty acid analysis is recommended. Due to potential for contaminant traces in samples to adsorb or absorb onto plastic and vice versa, tissues destined for POP analysis should only contact stainless steel, aluminium, glass, or Teflon. Samples are most conveniently wrapped in standard catering-grade aluminium foil with shiny side outward – avoiding recycled foil which might contain plastic particles – before storage in standard plastic containers.

Milk samples should be collected from any lactating females and stored in glass containers prior to POP analysis. When containers possess plastic caps, covering openings with aluminium foil with shiny side outward prevents sample contact with plastic caps for reasons outlined above.

Brain, muscle, liver, and kidney samples may be collected for trace element analysis. These samples should not contact any metals other than stainless steel and thus may be stored in plastic containers. Bone and blood samples also prove suitable for heavy metal analysis.

When foetuses are present but too small for full post-mortem examination, whole foetuses and placental portions may be wrapped in aluminium foil with shiny side outward for POP analysis. For foetuses permitting dissection, full post-mortem investigation with individual tissue sampling is recommended.

Minimum sample sizes for trace elements and organochlorine analysis equal 10 g of solid tissue or 10 ml for milk. Samples should be stored frozen at -20°C until analysis. For DCC1-2 specimens, samples should be stored at -80°C in liquid nitrogen or RNAlater® for Real-Time PCR.

7.4.11 Biotoxin Sampling and Analysis

Biotoxin sample collection proves highly recommended particularly in cases of unusual mortality events, mass mortalities, individuals exhibiting neurological symptoms whilst alive, or when harmful algal blooms are suspected in surrounding areas. Biotoxin samples include liver, kidney, brain, muscle, serum, aqueous humour, stomach contents, intestinal contents, faeces, and urine. Tissue samples may be stored in plastic bags. Stomach and intestinal contents, faeces, and urine may be collected in appropriately sized vials, usually 10–20 ml. Approximately 5–10 ml of urine and 1–2 ml of aqueous humour should be collected using sterile syringes and needles and stored in appropriately sized vials.

Samples should be stored at -80°C unless being shipped immediately on dry ice to maintain biotoxin stability and diagnostic capability.

IMPORTANT: Key Protocol Points

- Store lung, liver, spleen for microbiology at -20°C minimum
- Store brain, lung, kidney, spleen, serum, and CSF for virology at -70°C minimum
- Collect minimum four intact teeth from middle lower jaw for age determination
- Never store teeth, baleen, or earplugs dry; use freezing or 70% ethanol
- Ligate oesophagus and rectum before GIT removal to prevent contamination
- Filter gut contents through decreasing mesh sizes (1 mm, 500 µm, 250 µm, 50 µm)
- Collect negative control samples from environment for microplastic studies
- Store DNA samples frozen at -20°C or in 70% ethanol
- Use RNAlater® at -80°C for advanced genetic sequencing when facilities permit
- Fix histopathology samples in 10% neutral buffered formalin at 1:10 ratio minimum
- Keep tissue samples ≤ 1 cm thick in any dimension for proper formalin penetration
- Change formalin after 24 hours for fatty tissues and high blood content samples
- Store immunohistochemistry samples in formalin ≤ 72 hours maximum
- Collect microbiological samples aseptically within 24 hours; freeze at -20°C if delayed
- Use viral transport medium for virus isolation samples
- Store virology samples at -80°C; use RNAlater® if ultra-cold storage unavailable
- Collect 7ml fresh blood for sero-epidemiological studies; separate serum immediately
- Use pericardial fluid, vitreous humour, or tissue juices as serum substitutes when necessary
- Wrap persistent organic pollutant samples in aluminium foil (shiny side out) before plastic storage
- Avoid contact between POP samples and any materials except stainless steel, aluminium, glass, or Teflon
- Store toxicology samples at -20°C minimum; -80°C for DCC1-2 specimens
- Collect biotoxin samples particularly for unusual mortality events and harmful algal bloom cases
- Store biotoxin samples at -80°C unless shipping immediately on dry ice
- Process neural tissue samples within three months to minimise fixation artefacts

8 Suggested Sampling Protocols

The following section provides comprehensive sampling protocols for cetacean post-mortem investigations in Sri Lankan waters. These protocols should be adapted according to specific scientific and policy objectives of individual stranding response networks. Two complementary approaches are presented: Table 2 organises sampling requirements by diagnostic investigation type, whilst Table 3 arranges the same information by organ system. This dual approach facilitates both targeted diagnostic planning and systematic organ-by-organ sample collection during field procedures.

Sample collection priorities must consider carcass condition, available storage facilities, and analytical capacity. The maximum Decomposition Condition Code (DCC) indicated for each sample type represents the threshold beyond which diagnostic value significantly diminishes. Fresh carcasses in DCC1-2 condition permit the broadest range of analyses, whilst more decomposed specimens in DCC3-5 remain suitable for selected investigations such as genetics, life history studies, and museum collections.

Storage requirements vary substantially across diagnostic applications. Samples for microbiological culture require refrigeration at 0–4°C and processing within 24 hours. Virological and biomarker samples demand ultra-cold storage at -70°C to -80°C. Toxicological and dietary samples may be stored at -20°C. Histopathological samples require fixation in 10% neutral buffered formalin, whilst genetic material may be preserved in 70% ethanol or RNAlater® depending on analytical requirements.

Aseptic technique proves essential for microbiological and virological sampling to prevent contamination and ensure diagnostic accuracy. When collecting samples for persistent organic pollutant analysis, contact with plastic materials must be avoided; samples should be wrapped in aluminium foil with the shiny side facing outward before placement in storage containers. For heavy metal analysis, only stainless steel instruments should contact samples, which may then be stored in plastic containers.

The protocols emphasise duplicate sampling where feasible, particularly for toxicological analyses, to enable multiple analytical approaches and provide archived material for future investigations. This practice proves especially valuable for rare species or unusual mortality events where comprehensive retrospective analysis may be required.

Table 2: Suggested Minimum Sampling Protocol by Diagnostic Investigation.

Diagnostic Investigation	Tissue or Organ	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
(Algal) Biotoxins	Faeces	3		✓					10–20 ml	
	Stomach content	3		✓					10–20 ml	
	Urine	3		✓					5–10 ml	
Biomarkers	Skin	1			✓			✓	2 cm^3 aseptic	
	Brain	1			✓			✓		Paramedian cut, 1/3 frozen
	CSF	1			✓				2 ml	
	Liver	2			✓			✓	2 cm^3 aseptic	
	Lung	2			✓			✓		
	Pericardial fluid	2			✓				> 5 ml supernatant	Centrifuged
Cell Culture	Skin	1							1 cm^3 aseptic	
	Liver	1								
Contaminants	Brain	3		✓	✓				2 x > 10 g	1x aluminium foil, 1x plastic; paramedian cut, 1/3 frozen
	Kidney	3		✓						
	Liver	3		✓						

Diagnostic Investigation	Tissue or Organ	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Contaminants (continued)	Blubber	3		✓						
	Blood	3		✓					10 ml glass vial	
	Placenta/foetal tissue	3			✓				Whole or samples	Whole foetus if small; 2 x > 10 g tissue, 1x aluminium foil, 1x plastic
	Milk	3			✓				10 ml glass vial	
	Muscle	3		✓	✓				2 x > 10 g	1x aluminium foil, 1x plastic
Diet and Marine Debris	Stomach content	4		✓		✓			Collect whole	
Genetics	Muscle	5		✓				✓	2 cm^3 aseptic	
	Skin	4		✓		✓		✓	2 cm^3 aseptic	
Histopathology	Brain	3					✓			Paramedian cut, 2/3 in formalin
	Cochlea	3					✓		Whole complex	Immersed for histopathology
	GIT	3					✓			2–3 regions including abnormal margins; open intestines before fixing
	Kidney	3					✓		1 x 1 cm sections	Including abnormality margins
	Liver	3					✓			
	Lung	4					✓			
	Spleen	3					✓			

Diagnostic Investigation	Tissue or Organ	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Histopathology (continued)	Skin	4					✓			
	Adrenals	3					✓			Measure cortico-medullary ratio; weigh
	Lymph nodes	3					✓			
	Pancreas	2					✓			Fix quickly; rapidly autolyses
	Skeletal muscle	3					✓			Fixed under tension; epaxial and rectus abdominus
	Cardiac muscle	3					✓			2-3 heart regions including valvular margins
	Reproductive tract	3					✓			Including abnormal margins
Life History	Teeth	5		✓		✓	✓		4-5 teeth	10% formalin or frozen
	Gonadal tissue	4					✓			Ovaries whole; testes cross-section; measure length/weight; note asymmetry
	Baleen	5		✓					Whole plate	Freeze -20°C
Microbiology	Lung	3	✓						Aseptic or swab	Refrigerate 0–4°C before culture; DCC4 possible with specific methods
	Brain	3	✓						Aseptic or swab	
	CSF	3	✓						Aseptic or swab	
	Heart	3	✓						Aseptic or swab	
	Kidney	3	✓						Aseptic or swab	

Diagnostic Investigation	Tissue or Organ	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Microbiology (continued)	Liver	3	✓						Aseptic or swab	
	Spleen	3	✓						Aseptic or swab	
	Reproductive tract	3	✓						Aseptic or swab	
	Blood clots	2	✓						>5ml	Do not centrifuge
Museum Collections	Scapula	5		✓					Whole	Freeze -20°C
	Skeleton	5		✓					Whole	
	Skull	5		✓					Whole	
Parasitology	Stomach contents	4		✓		✓			Whole	Dissect parasite head attachments; freeze -20°C
	Intestine	4		✓		✓				
	Lung	4		✓		✓				
	Liver	4		✓		✓				
	Subcutaneous tissue	4		✓		✓				
	Renal vasculature/kidneys	4		✓		✓				Particularly fin and beaked whales
	Cochlea/Eustachian tube	4		✓		✓				
	Other tissues	4		✓		✓			Whole	70% ethanol; dissect parasite attachments

Diagnostic Investigation	Tissue or Organ	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Parasitology (continued)	Faeces	4		✓					5 g	
	Cardiac/skeletal muscle	3	✓							Toxoplasma identification; store -80°C
SEM/Ultrastructure	Cochlea	1					✓			Perfuse with 10% formalin or 4% paraformaldehyde per protocol
Serology	Blood	2			✓				From right ventricle	Centrifuge 1000-1500 rpm/8-10 min; store -70/80°C
Stable Isotopes/Fatty Acids	Skin	4		✓					2 cm^3 aseptic	Freeze -70/80°C
	Muscle	4		✓						
	Blubber	4		✓						
	Baleen	5		✓						
	Teeth	5		✓						
	Liver	4		✓						
Virology	Brain	3			✓				2 cm^3 aseptic	Freeze -70/80°C
	Liver	3			✓				2 cm^3 aseptic	
	Lung	3			✓				2 cm^3 aseptic	
	Placenta/foetal tissue	3			✓					
	Spleen	3			✓				2 cm^3 aseptic	
	Lymph nodes	3			✓				2 cm^3 aseptic	

Table 3: Suggested Minimum Sampling Protocol by Organ System.

Tissue or Organ	Diagnostic Investigation	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Adrenals	Histopathology	3					✓			Measure cortico-medullary ratio across middle; weigh
Baleen	Stable isotopes/fatty acids	5		✓						
	Life history	5		✓						
Blood	Serology	2			✓				From right ventricle	Centrifuge 3,000 rpm; freeze serum -20°C
	Microbiology	2	✓						> 5 ml supernatant	Centrifuged for serology
	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
Blubber	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
	Stable isotopes/fatty acids	4		✓					2 cm^3 aseptic	Freeze -70/80°C
Brain	Biomarkers	1			✓			✓	2 cm^3 aseptic	Paramedian cut, 1/3 frozen
	Virology	3			✓				2 cm^3 aseptic, multiple sites	Freeze -70/80°C
	Contaminants	3		✓	✓				> 10 g	Wrapped in aluminium foil; paramedian cut, 1/3 frozen
	Histopathology	3					✓			Paramedian cut, 2/3 in formalin
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture
Cardiac Muscle	Histopathology	3					✓			2–3 heart regions including valvular margins
	Parasitology	3	✓						Aseptic or swab	Toxoplasma identification; store -80°C
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture

Tissue or Organ	Diagnostic Investigation	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Cochlea	SEM/ultrastructure	1					✓			Perfuse with 10% formalin or 4% paraformaldehyde
	Histopathology	3					✓		Whole complex	Immerse for histopathology
	Parasitology	4		✓		✓				
CSF	Biomarkers	1			✓				2 cm^3 aseptic	
	Microbiology	3	✓						Aseptic or swab	Best for Brucella spp. culture
Faeces	(Algal) biotoxins	3		✓					50 g	
	Parasitology	4		✓					5 g	
GIT	Histopathology	3					✓			2–3 regions including abnormal margins; open intestines before fixing
Gonadal Tissue	Life history	4					✓			Ovaries whole; testes cross-section; measure length/weight; record asymmetry; include epididymis
Intestine	Microbiology	2	✓						≥ 3 samples	Different tract locations
	Parasitology	4		✓		✓			Whole	Dissect parasite attachments; freeze -20°C
Kidney	Histopathology	2					✓		1 cm sections	Over normal/abnormal border
	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture

Tissue or Organ	Diagnostic Investigation	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Liver	Biomarkers	2			✓			✓		
	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
	Histopathology	3					✓		1 cm sections	Over normal/abnormal border
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture
	Virology	3			✓				2 cm^3 aseptic	Freeze -70/80°C
	Parasitology	4		✓		✓			Whole	Dissect parasite attachments; freeze -20°C
	Stable isotopes/fatty acids	4		✓						
Lung	Biomarkers	2			✓			✓		
	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture
	Virology	3			✓				2 cm^3 aseptic	Freeze -70/80°C
	Histopathology	4					✓		1 cm sections	Over normal/abnormal border
	Parasitology	4		✓		✓			Whole	Dissect parasite attachments; freeze -20°C
Lymph Nodes	Virology	3			✓				2 cm^3 aseptic	Freeze -70/80°C
	Histopathology	3					✓		1cm sections	Over normal/abnormal border

Tissue or Organ	Diagnostic Investigation	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Muscle	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
	Stable isotopes/fatty acids	4		✓					2 cm^3 aseptic	Freeze -70/80°C
	Genetics	5		✓				✓	2 cm^3 aseptic	
	Parasitology	3		✓						Toxoplasma identification; store -80°C
Other Tissues	Parasitology	4		✓		✓			Whole	Dissect parasite attachments; 70% ethanol
Pancreas	Histopathology	2					✓		1 cm sections	Fix quickly; rapidly autolyses
Pericardial Fluid	Biomarkers	2			✓				> 5 ml supernatant	Centrifuged
Placenta/Foetal Tissue	Contaminants	3			✓				2 cm^3 aseptic	Freeze -70/80°C
	Virology	3			✓				2 cm^3 aseptic	Freeze -70/80°C
Renal Vasculature	Parasitology	4		✓		✓				
Reproductive Tract	Microbiology	3	✓				✓		Aseptic or swab	Refrigerate +1°C before culture
	Histopathology	3					✓		1 cm sections	Over normal/abnormal border
Scapula	Museum collections	5		✓					Whole	Freeze -20°C
Skeletal Muscle	Histopathology	3					✓			Fixed under tension; epaxial and rectus abdominus
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture

Tissue or Organ	Diagnostic Investigation	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Skeleton	Museum collections	5		✓					Whole	Freeze -20°C
Skin	Biomarkers	1			✓			✓	2 cm^3 aseptic	
	Contaminants	3		✓					> 10 g	Wrapped in aluminium foil
	Genetics	4		✓		✓		✓	2 cm^3 aseptic	
	Histopathology	4					✓			
	Stable isotopes/fatty acids	4		✓					2 cm^3 aseptic	Freeze -70/80°C
Skull	Museum collections	5		✓					Whole	Freeze -20°C
Spleen	Histopathology	3					✓		1 cm sections	Over normal/abnormal border
	Microbiology	3	✓						Aseptic or swab	Refrigerate +1°C before culture
	Virology	3			✓				2 cm^3 aseptic	Freeze -70/80°C
Stomach Content	(Algal) biotoxins	3		✓					Whole	
	Diet/marine debris	4		✓		✓			Whole	
	Parasitology	4		✓		✓			Whole	Dissect parasite attachments; freeze -20°C
Subcutaneous Tissue	Parasitology	4		✓		✓				

Tissue or Organ	Diagnostic Investigation	Max DCC	Aseptic Fresh	Frozen -20°C	Frozen -80°C	Ethanol	10% Formalin	RNAlater	Quantity	Comment
Teeth	Life history	5		✓		✓	✓		4–5 teeth	10% formalin or frozen
	Stable isotopes/fatty acids	5		✓						
Urine	(Algal) biotoxins	3		✓					5 ml	

IMPORTANT: Key Protocol Points

- Adapt protocols according to specific scientific and policy objectives
- Consider maximum DCC thresholds; diagnostic value diminishes beyond stated limits
- DCC1-2 carcasses permit broadest range of analyses
- Store microbiological samples refrigerated at 0-4°C; process within 24 hours
- Store virology and biomarker samples at -70°C to -80°C
- Store toxicology and dietary samples at -20°C minimum
- Fix histopathological samples in 10% neutral buffered formalin
- Preserve genetic material in 70% ethanol or RNAlater® as appropriate
- Maintain aseptic technique for microbiological and virological sampling
- Wrap persistent organic pollutant samples in aluminium foil (shiny side out) before plastic storage
- Use only stainless steel instruments for heavy metal analysis samples
- Collect duplicate samples for toxicological analyses when feasible
- Archive material for rare species and unusual mortality events
- Label all samples with unique reference numbers and tissue identifiers
- Document storage temperatures and preservation methods comprehensively
- Coordinate with laboratories regarding specific requirements before field work
- Prioritise time-sensitive samples: microbiology, virology, rapidly autolysing tissues
- Maintain complete chain of custody documentation for all samples

9 Carcass Disposal

Following completion of post-mortem investigations of cetacean strandings in Sri Lankan waters, proper carcass disposal must be conducted in accordance with existing national legal framework regulations to ensure human safety and prevent disease transmission to both human and animal populations. In many jurisdictions, responsibility for disposal lies with local authorities; however, advice and technical guidance may be sought from stranding investigation teams who possess specialised knowledge of appropriate disposal methodologies for marine mammals.

Sri Lankan authorities should establish comprehensive national protocols adapted to local environmental conditions, available infrastructure, and regulatory requirements. Local veterinary authorities and qualified technicians should be contacted prior to moving carcasses to initiate postmortem procedures, ensuring coordinated response and appropriate disposal planning from the outset.

9.1 Natural Decomposition

When post-mortem examinations are conducted on remote, difficult-to-access, or isolated shorelines, it may prove possible to obtain permission to leave carcasses exposed for natural scavenger activity. This approach permits natural ecological processes to proceed whilst minimising disposal costs and logistical challenges. However, this method is not recommended following euthanasia procedures involving chemical agents, as residual chemicals may pose risks to scavenging wildlife. Decomposition rates vary substantially depending on environmental factors including temperature, humidity, and seasonal conditions, as well as species-specific characteristics such as body size and blubber thickness. Comprehensive photographic documentation of carcass condition when left on site proves essential for subsequent identification should the carcass re-strand at different locations along the coastline.

9.2 Burial On Site

When ecological conditions and local regulations permit, beach burial often represents the most practical and affordable disposal option for larger cetaceans, particularly in locations where transport infrastructure proves inadequate for moving substantial carcasses. Local permissions must be obtained prior to burial to avoid contamination or disruption of water supplies, groundwater resources, and other environmentally sensitive areas. This approach requires suitable excavation equipment and heavy machinery to ensure carcasses are buried at sufficient depth to prevent re-exposure during monsoon conditions or storms.

Ideally, excavation should be completed in close proximity to the carcass prior to commencing post-mortem examination, allowing immediate disposal of soft tissues during dissection procedures. This approach minimises handling requirements and reduces potential for environmental contamination during the disposal process. When carcasses may be subsequently exhumed for accession of skeletal remains to museum collections for scientific or educational purposes, burial sites must be marked precisely using GPS coordinates or ferrous material markers to facilitate location using metal detectors. Detailed burial site documentation including depth measurements, surrounding landmarks, and photographic records should be maintained in centralised databases to enable future recovery when required.

9.3 At-Sea Disposal

Towing carcass remains offshore for open ocean disposal represents a viable option for larger specimens, particularly when beach burial proves impractical due to substrate conditions or regulatory restrictions. However, considerable care must be taken to ensure carcasses sink adequately to prevent them becoming hazards to shipping traffic or washing back ashore on populated beaches. This may necessitate weighting carcasses or making strategic incisions to release gas accumulation that provides buoyancy. Disposal locations must be selected to account for prevailing currents, tidal patterns, and proximity to shipping lanes, fishing grounds, and popular beach areas. Environmental impact assessments should consider potential effects on benthic ecosystems and local fisheries before implementing at-sea disposal programmes.

9.4 Transport to Landfill

Transportation of carcasses for post-mortem examination and subsequent disposal at licensed landfill facilities represents the most commonly employed method in more densely populated regions where beach burial or natural decomposition prove impractical. However, this approach often requires landfill operators to obtain additional permissions and permits specifically for cetacean disposal, as these carcasses may not fall under standard waste management categories. The transport of large specimens through urban areas or populated regions poses substantial logistical challenges including road access limitations, traffic disruption, and public health concerns. Transport is strongly recommended to be conducted in closed containers to prevent leakage, control odours, and maintain public health standards during transit through populated areas.

9.5 Incineration

Transportation of post-mortem remains to commercial incineration facilities represents an alternative disposal solution that may be considered environmentally conscious waste management when properly regulated. High-temperature incineration ensures complete destruction of potentially infectious materials whilst reducing carcass volume substantially. However, this approach requires access to facilities capable of handling large organic loads and may prove cost-prohibitive for routine disposal of cetacean carcasses. Environmental impact assessments should consider air quality implications and emissions standards when evaluating incineration as a disposal methodology for Sri Lankan cetacean stranding response programmes.

9.6 Composting and Rendering

Increasing interest has developed internationally in composting stranded marine mammals using methodologies similar to those employed successfully for livestock carcass disposal. These systems utilise controlled biological decomposition processes to convert organic materials into stable compost products whilst managing odours and preventing environmental contamination. General principles of natural rendering composting systems are available through agricultural extension services and academic resources.

However, many countries including those signatories to CITES prohibit the sale of products derived from cetaceans, potentially including compost or biodiesel from rendering processes. Sri Lankan authorities must carefully evaluate national and international regulatory frameworks before implementing composting programmes, as commercial rendering plants may prove reluctant to accept cetacean carcasses due to these restrictions and potential complications with product marketability.

Within this regulatory context, emphasis must be placed on regulations enforced to minimise human and animal exposure risks to prion agents, exemplified by Bovine Spongiform Encephalopathy (BSE) causative agents. Current international protocols prohibit the use of meat and bone meal derived from any species, both mammalian and avian, for animal feeding purposes. These restrictions apply regardless of source species and must be incorporated into disposal planning to ensure compliance with both national food safety regulations and international best practices for disease prevention.

IMPORTANT: Key Protocol Points

- Conduct all disposal in accordance with Sri Lankan national legal framework regulations
- Ensure human safety and prevent disease transmission throughout disposal processes
- • Coordinate with local veterinary authorities before moving carcasses
- Contact qualified technicians prior to initiating post-mortem procedures Plan disposal methodology before commencing post-mortem examination
- Consider environmental impacts including groundwater, benthic ecosystems, and air quality
- Maintain comprehensive disposal records including locations, methods, and dates
- Assess logistical requirements including equipment, transport, and personnel
- Evaluate cost implications of various disposal options
- Ensure public health protection throughout all disposal operations
- Document disposal sites precisely for potential future reference or skeletal recovery

- **Natural Decomposition**: Only on remote, isolated shores; not after chemical euthanasia; document thoroughly with photographs

- **Beach Burial**: Most practical for large cetaceans; obtain local permissions; avoid water supplies and sensitive areas; excavate holes prior to post-mortem examination; bury at sufficient depth for storm protection; mark sites with GPS or ferrous markers for potential future skeletal recovery

- **At-Sea Disposal**: Weight carcasses adequately to ensure sinking; avoid shipping lanes; prevent wash-back to shore; consider currents and tidal patterns

- **Landfill Transport**: Requires additional permissions for facilities; use closed containers through populated areas; plan routes avoiding peak traffic

- **Incineration**: Environmentally conscious option; requires facilities capable of large organic loads; assess air quality implications

- **Composting/Rendering**: Follow livestock composting methodologies; verify CITES compliance before implementation; commercial plants may refuse cetacean carcasses due to product sale restrictions; meat and bone meal cannot be used for animal feeding under prion prevention regulations

Glossary

Anthropogenic – Originating from or caused by human activity. In cetacean stranding investigations, this term commonly refers to injuries, mortality, or impacts resulting from human actions such as vessel strikes, entanglement, pollution, or noise disturbance.

Aseptic Technique – A set of procedures used to prevent contamination by microorganisms. In tissue sampling, this involves using sterile instruments, avoiding contact with non-sterile surfaces, and minimising microbial contamination to ensure sample validity for microbiological and virological analyses.

Autolysis – The self-digestion of cells and tissues by their own enzymes after death. Autolysis proceeds at different rates in different tissues, with brain, pancreas, and liver being particularly susceptible to rapid post-mortem breakdown. Warm tropical conditions accelerate autolytic processes.

Baleen – Keratinous plates that hang from the upper jaw of mysticete whales, used to filter food from seawater. Baleen can be used for age determination and stable isotope analysis to understand diet and migration patterns.

Biomarkers – Measurable indicators of biological processes, exposure to contaminants, or disease states. In cetacean health assessment, biomarkers may include enzyme levels, stress hormones, or cellular changes indicating exposure to pollutants or pathogens.

Biotoxins – Toxic substances produced by living organisms, particularly algae. Harmful algal blooms can produce biotoxins that accumulate in marine food webs and cause mortality in cetaceans. Common marine biotoxins include saxitoxin, brevetoxin, and domoic acid.

Blubber – The thick subcutaneous layer of fat found in marine mammals that provides insulation, energy storage, and buoyancy. Blubber thickness and composition provide important indicators of nutritional status and are commonly sampled for contaminant analysis.

Bycatch – The unintentional capture of non-target species during fishing operations. Cetacean bycatch in fishing gear represents a significant source of anthropogenic mortality globally and may be evident in stranded animals showing characteristic net marks or injuries.

Carcass – The dead body of an animal. In this protocol, the term encompasses cetaceans found dead, those that die following live stranding, and those euthanised due to poor prognosis.

Cerebrospinal Fluid (CSF) – The clear fluid surrounding the brain and spinal cord. CSF samples are valuable for diagnosing neurological diseases and detecting antibodies to neurotropic pathogens.

Cetacean – Any member of the mammalian order Cetacea, which includes whales, dolphins, and porpoises. Cetaceans are divided into two suborders: Mysticeti (baleen whales) and Odontoceti (toothed whales).

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora. An international agreement regulating trade in endangered species, including all cetaceans. CITES permits are required for international movement of cetacean specimens and tissues.

Crush Artefact – Physical distortion or damage to tissue caused by excessive pressure or rough handling during sample collection or processing.

Decomposition Condition Code (DCC) – A standardised classification system assessing the degree of post-mortem decomposition in stranded cetaceans. The five-category system (DCC1-5) ranges from extremely fresh (DCC1) to mummified or skeletal remains (DCC5) and determines the suitability of tissues for various analyses.

Delipidation – The process of oil or fat separation from tissues during decomposition. In cetacean carcasses, delipidation of blubber is characterised by pooling and leaching of oil, leaving behind a matrix of connective tissue fibres.

Delphinids – Members of the family Delphinidae, the oceanic dolphins. This is the largest and most diverse cetacean family, including species such as bottlenose dolphins, spinner dolphins, and pilot whales commonly found in Sri Lankan waters.

Ectoparasites – Parasites that live on the external surface of their host. In cetaceans, ectoparasites include whale lice (cyamids), barnacles, and copepods, typically found near body openings, in skin folds, or on fins and flukes.

Entanglement – The condition of being caught or wrapped in marine debris or fishing gear. Entanglement is a significant cause of cetacean mortality and morbidity, often identifiable by characteristic marks, embedded line, or associated injuries.

Epidermis – The outermost layer of skin. In cetaceans, the epidermis is relatively thin and not routinely measured during morphometric assessments, though it may be examined histologically for disease or injury.

Euthanasia – The act of humanely ending the life of an animal to prevent suffering. In stranding response, euthanasia may be necessary for animals with injuries or conditions incompatible with survival. Chemical euthanasia introduces specific disposal considerations due to tissue persistence of euthanasia agents.

Exsanguinated – The condition of having lost a significant proportion or all of the blood volume from the circulatory system, typically as a result of severe haemorrhage or blood loss prior to death.

Fixative – A chemical solution used to preserve biological tissues and prevent decomposition. The most commonly used fixative is 10% neutral buffered formalin, which preserves tissue architecture for histopathological examination.

Foetus – An unborn offspring developing in the uterus. Foetal examination provides valuable reproductive and developmental information, and foetal tissues may be sampled for contaminant analysis and disease investigation.

Formalin – An aqueous solution of formaldehyde, typically used at 10% concentration (containing 4% formaldehyde) for tissue fixation. Formalin is classified as carcinogenic and requires careful handling with appropriate personal protective equipment.

Gastrointestinal Tract (GIT) – The digestive system extending from mouth to anus, including oesophagus, stomachs, and intestines. GIT examination provides information on diet, parasite burden, disease, and ingestion of marine debris.

Girth – The circumference of the body, typically measured at the level of the dorsal fin insertion. Girth measurements, combined with length, provide important morphometric data and may be used to estimate body condition and mass.

Harmful Algal Bloom (HAB) – A proliferation of algae that produces toxins harmful to marine life, humans, or the environment. HABs can cause cetacean mortality through biotoxin exposure via contaminated prey.

Histopathology – The microscopic examination of tissue to study disease manifestations. Histopathological analysis of formalin-fixed tissues provides detailed information about disease processes, inflammatory responses, and tissue damage.

Imbibition – The post-mortem discolouration of tissues, particularly blubber, caused by penetration of blood from adjacent tissues. Imbibition represents an early decomposition change and should be distinguished from ante-mortem haemorrhage.

Immunohistochemistry – A laboratory technique using antibodies to detect specific proteins or antigens in tissue sections. This method helps identify infectious agents, characterise cell types, and diagnose specific diseases.

In Situ – In the original place or position. During post-mortem examination, organs are first examined in situ before removal to assess anatomical relationships and identify displacements, herniation, or ruptures.

Life History – Biological characteristics including age, growth rates, sexual maturity, reproductive status, and mortality patterns. Life history data from stranded cetaceans inform population assessments and conservation management.

Mass Stranding – An event involving two or more cetaceans (excluding mother-calf pairs) stranding at the same time and place. Mass strandings may result from various causes including social cohesion, disease, environmental factors, or human activities.

Melon – The fatty structure in the forehead of odontocetes involved in sound production and echolocation. The melon should be examined for trauma or inflammatory lesions that might affect acoustic function.

Microplastics – Small plastic particles less than 5mm in size resulting from breakdown of larger plastic items or manufactured as microbeads. Microplastic ingestion by cetaceans is an emerging conservation concern requiring specialised sampling protocols.

Morphometrics – The measurement and analysis of body form and structure. Standard cetacean morphometric measurements include total length, girth, flipper length, and various cranial dimensions.

Mysticetes – Baleen whales, the suborder Mysticeti. These cetaceans possess baleen plates rather than teeth and include the largest animals on Earth. Mysticetes occurring in Sri Lankan waters include blue whales, Bryde's whales, and humpback whales.

Necropsy – Synonymous with post-mortem examination or autopsy; the systematic examination of a dead animal to determine cause of death, assess health status, and collect scientific data.

Neonatal – Relating to newborn animals. Neonatal cetaceans show distinctive features including foetal folds, soft flukes, presence of body hair, and pale colouration, which may persist for days to weeks after birth.

Nutritional Condition Code (NCC) – A classification system assessing the nutritional status of cetaceans based on blubber thickness, muscle mass, and visible body condition. NCC ranges from very good (robust, well-nourished) to emaciated (severe depletion of fat and muscle reserves).

Odontocetes – Toothed whales, the suborder Odontoceti. This diverse group includes dolphins, porpoises, and sperm whales, all possessing teeth rather than baleen. Odontocetes use echolocation for navigation and prey detection.

Otoliths – Calcified structures in the inner ear of fish, commonly found in cetacean stomach contents. Otoliths can be identified to species and used to reconstruct prey composition and feeding ecology.

Palpation – Examination by touch or pressure to assess tissue texture, organ size, presence of masses, or other abnormalities. Palpation is an essential component of thorough post-mortem examination.

Parasite – An organism living in or on another organism (the host) and deriving nutrients at the host's expense. Cetaceans harbour diverse parasite communities including nematodes, cestodes, trematodes, and protozoans, which may cause disease when burdens are high.

Pathogen – A disease-causing microorganism including bacteria, viruses, fungi, or parasites. Identification of pathogens in stranded cetaceans contributes to understanding disease ecology and monitoring emerging infectious diseases.

Persistent Organic Pollutants (POPs) – Chemical substances that persist in the environment, bioaccumulate in food webs, and pose risks to human health and ecosystems. Common POPs in marine mammals include PCBs, DDT, and related compounds.

Petechiae – Small pinpoint haemorrhages visible on organ surfaces or in tissues, often indicating acute distress, trauma, septicaemia, or specific disease processes.

Post-Mortem – After death. Post-mortem changes include autolysis, decomposition, and other alterations that occur following death and may complicate interpretation of findings.

Prion – An infectious protein that causes fatal neurodegenerative diseases. Whilst prion diseases have not been documented in cetaceans, regulations addressing prion risks in livestock apply to cetacean carcass disposal in many jurisdictions.

Prosection – Systematic dissection and examination of a body according to established protocols, typically for educational or investigative purposes rather than diagnostic determination of cause of death.

Proteolysis – The breakdown of proteins into smaller polypeptides or amino acids through enzymatic action. In post-mortem tissue, proteolysis is a key component of autolysis (self-digestion), where the body's own enzymes cause tissue degradation.

Renule – The functional unit of cetacean kidneys. Cetaceans possess reniculate kidneys composed of multiple renules rather than the smooth kidneys of most terrestrial mammals. Histological samples should include at least one complete renule.

Rete Mirabilis – A complex network of blood vessels providing oxygen storage and thermoregulation in diving mammals. The thoracic rete mirabilis should be examined for evidence of haemorrhage, which may indicate trauma or certain disease processes.

RNAlater – A commercial solution that stabilises and protects RNA in tissue samples. RNAlater enables storage of samples at moderate temperatures whilst preserving RNA integrity for advanced genetic and molecular analyses.

Rostrum – The anterior projection of the skull forming the upper jaw and snout. Total body length is measured from the rostrum tip to the tail fluke notch.

Sentinel Species – A species whose health status provides early warning of environmental changes or contamination affecting entire ecosystems. As apex predators with long lifespans, cetaceans serve as important sentinel species for marine ecosystem health.

Septicaemia – A serious bloodstream infection causing systemic inflammatory response. Septicaemia may be evident at post-mortem through characteristic petechial haemorrhages, organ congestion, and bacterial isolation from normally sterile sites.

Stable Isotopes – Variant forms of chemical elements with different numbers of neutrons. Analysis of stable isotope ratios in cetacean tissues provides information about diet, trophic level, and geographic origin.

Stranding – An event in which a cetacean is on land or in shallow water and unable to return to deeper water without assistance. Strandings include both dead animals and live animals in distressed condition.

Subcutaneous – Beneath the skin. In cetaceans, the subcutaneous region contains the blubber layer, which should be examined for thickness, colour, parasites, haemorrhages, and other abnormalities.

Tympano-Periotic Complex – The ear bones of cetaceans, comprising the tympanic bulla and periotic bone. This complex should be carefully removed for examination of hearing structures, age determination, and assessment of acoustic trauma.

Unusual Mortality Event (UME) – An unexpected mortality event involving significant numbers of marine mammals, demanding immediate response. UMEs are characterised by marked increases in mortality, unusual species composition, similar pathological findings, or occurrence in vulnerable populations.

Zoonosis (Zoonotic Disease) – A disease that can be transmitted from animals to humans. Several zoonotic pathogens including Brucella, Erysipelothrix, and various other bacteria may be present in cetacean tissues, necessitating appropriate biosafety precautions during handling.

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