RESOLUTION 4.16

GUIDELINES FOR A COORDINATED CETACEAN STRANDING RESPONSE

The Meeting of the Parties to the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area:

Taking in consideration the Recommendation of the Scientific Committee,

Recalling that the First Meeting of the Parties adopted the establishment of an "emergency task force for special mortality events" as a priority,

Recalling also Resolution 3.10 on "Guidelines to address the impact of anthropogenic noise", Resolution 3.25 on "Cetacean live stranding" and Resolution 3.29 on "Guidelines for a coordinated cetacean stranding response",

Recognizing that in recent years the ACCOBAMS area has been the scene of major cetacean mortality events, involving mass strandings over wide geographical areas, which have evoked great concern and have attracted considerable attention from the scientific community,

Convinced that in order to address new outbreaks of mortality events related to chemical, acoustic and biological pollution, as well as related to infectious agents and harmful algal blooms, affecting cetacean populations or their critical habitats, a task force should be constituted for marine mammal mortality and special events, made up of international experts,

1. *Encourages* Parties to take advantage of the two studies on "Guidelines concerning best practice and procedure for addressing cetacean mortality events related to chemical acoustic and biological pollution" and on "Guidelines for a coordinated cetacean stranding response during mortality events caused by infectious agents and harmful algal blooms", presented in Annexes 1 and 2 to the present Resolution;

2. *Urges* the Scientific Committee, in collaboration with the Secretariat and the Sub-Regional Coordination Units:
   - to update the roster of contact persons and experts from the scientific and conservation communities and from governmental environment and natural resource agencies who could contribute in appropriate fields of expertise, such as pathology, epidemiology, toxicology, biology, ecology, acoustics, and to strengthen the two emergency task forces on:
     - (i) "mass mortality", to address unusual mortality events, including epizootics and atypical mass strandings; and
     - (ii) "maritime disaster", to address oil or chemical spills affecting critical habitats of cetaceans;
   - to use existing experience to prepare contingency plans for each task force, including descriptions of administrative procedures and modalities for interventions, the decision-making processes and the management of information, communication and relations with the media;
   - to update the studies and the contingency plans periodically on the basis of past experience and new techniques and technologies;

3. *Recommends* to the Parties and invites non-Party riparian States:
   - to inform the Secretariat as rapidly as possible about unusual mortality events affecting cetacean populations or their critical habitats, so that the emergency contingency plan can be initiated; and
   - to facilitate the organization of training programmes to enhance the effectiveness of the emergency task forces;

4. *Instructs* the Secretariat:
   - in consultation with the Scientific Committee and in collaboration with States and Sub-Regional Coordination Units, to contact the relevant experts in order to initiate the emergency contingency plan; and

- to contact REMPEC and its homologous Black Sea organization under the Bucharest Convention framework in order to define a collaborative effort, as appropriate;

5. *Decides* that the present Resolution replaces Resolution 3.29.

ANNEX 1

guidelines concerning best practice and procedure for addressing cetacean mortality events related to chemical, acoustic and biological pollution [1]

1. GUIDELINES CONCERNING BEST PRACTICES AND PROCEDURES FOR ADDRESSING CETACEAN MORTALITY EVENTS RELATED TO CHEMICAL, ACOUSTIC AND BIOLOGICAL POLLUTION

1.1 Role of chemical, biological and acoustic pollution in cetacean mortalities and diseases

1.1.1 Introduction

1.1.2 Chemical pollution

1.1.2.1 Polychlorinated biphenyls

1.1.2.2 Brominated flame retardants

1.1.2.3 Polycyclic aromatic hydrocarbons

1.1.2.4 Perfluorinated compounds

1.1.2.5 Heavy metals

1.1.3 Biological pollution

1.1.4 Acoustic pollution

1.1.4.1 Anthropogenic sonar signals

1.1.4.2 Seismic surveys

1.2 Things to do in preparation for non-infectious unusual mortality events

1.2.1 Technical and administrative infrastructure needed in each Member State to best address emergencies caused by cetacean die-offs

1.2.2 Equipment list

1.2.2.1Recording material

1.2.2.2 Necropsy

1.2.2.3 Specific sampling

1.2.2.4 Minimal equipment

1.3 Actions to take during non-infectious unusual mortality events

1.3.1 Protocols for collection, transportation and storage of specimens and samples

1.3.1.1 Protocols for sample collection

1.3.1.1.1 Basic data protocol

1.3.1.1.2 Specific sample collection

1.3.1.1.2.1 Reproductive tract

1.3.1.1.2.2 Biological pollution

1.3.1.1.2.3 Chemical pollution

1.3.1.1.2.4 Acoustic pollution

1.3.2 Protocols for transportation and storage

1.4 Activities to implement after stranding

1.4.1 Debriefing meeting

1.4.2 Communication

1.4.2.1 Local government, Armed Forces, Ministry of External Affairs, Ministry of Environment, Ministry of Health

1.4.2.2 Scientists

1.4.2.3 Press

1.4.3 Preliminary report

1.4.4 Follow-up

2. CONTINGENCY PLAN DRAFT

2.1 OSCB

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[1] Document prepared by: Dr Marie-Françoise Van Bressem, Cetacean Conservation Medicine Group, CMED/CEPEC,Cra 74, 139-33, Bogota, Colombia

E-mail: mfb.cmed@gmail.com

1. GUIDELINES CONCERNING BEST PRACTICES AND PROCEDURES FOR ADDRESSING CETACEAN MORTALITY EVENTS RELATED TO CHEMICAL, ACOUSTIC AND BIOLOGICAL POLLUTION

1.1 Role of chemical, biological and acoustic pollution in cetacean mortalities and diseases

1.1.1 Introduction

Since the detection of massive mortalities in seals (Osterhaus and Vedder, 1988) and dolphins (Domingo *et al*., 1990) in the last twenty years, diseases of marine mammals have gained growing attention. Several micro- and macro-parasites that may negatively influence population growth have been identified (Van Bressem *et al*., 2009) and the role of chemical pollutants in facilitating the emergence of morbillivirus epidemics has been thoroughly investigated (Aguilar and Borrel, 1994; Ross, 2002). Evidence suggests that polychlorinated biphenyls (PCBs) and related compounds might have contributed to the severity of morbillivirus outbreaks in seals and dolphins through toxicity at the level of the immune system (Aguilar and Borrel, 1994; Ross, 2002). More recently mid-frequency sonar operations induced cetacean mass-strandings in Europe, the US and Asia following decompression and gas and fat embolic syndrome (Jepson *et al*., 2003; Fernandez *et al*., 2005; Yang *et al*., 2008). Biological pollution is also of increased concern because of the findings of terrestrial pathogens in marine mammals, of a significant increased fecal coliform count in harbour seals (*Phoca vitulina*) living near urban developments and of cutaneous disorders of miscellaneous aetiology in coastal odontocetes (Mos *et al*., 2006; Van Bressem *et al*., 2007; Miller *et al*., 2008). Chemical and biological pollution will likely increase as a result of climate change (Boxall *et al*., 2009).

Below are summarized information on chemical, biological and acoustic pollution in cetaceans and their role in cetacean diseases and mortalities. A special insight is given into the effects of pollution in marine mammals from European waters, especially the Mediterranean Sea that receives persistent, organic contaminants from the most contaminated regions of the world (Lelieveld *et al*., 2002).

1.1.2. Chemical pollution

During the 20th century, the global environment became contaminated with several persistent, organic contaminants, commonly referred to as 'POPs'. Contamination has resulted from deliberate discharges and applications, as well as from the inadvertent formation of byproducts of incomplete combustion or industrial processes. Classes of these POPs include the organochlorine pesticides (*e.g.,* DDT, chlordane, toxaphene), the polyhalogenated-biphenyls (PHBs; including polychlorinated biphenyls PCBs), -dibenzo-*p*-dioxins (PHDDs; including polychlorinated dibenzo-*p*-dioxins PCDDs), -dibenzofurans (PHDFs; including polychlorinated dibenzofurans PCDFs), the polychlorinated naphthalenes (PCNs), carcinogenic polycyclic aromatic hydrocarbons (PAHs) and certain brominated flame-retardants. Several POPS have 'dioxin-like' properties, i.e. they bind to the Aryl hydrocarbon receptor (*Ah*R) and initiate toxic responses. POPs are fat-soluble chemicals and are resistant to metabolic breakdown, factors that result in their bioaccumulation in aquatic food chains and persistence in the environment (see Ross, 2002; Tabuchi *et al*., 2006).

Prey items from the freshwater and marine environment, and the terrestrial food chain are the main sources of these contaminants for marine mammals. POPs may accumulate in high concentrations, affect the reproductive, immune and endocrine systems and cause cancers (Reijnders, 1986; De Swart *et al.,* 1994; Ross *et al.,* 1996). High trophic level organisms are vulnerable to accumulating high concentrations of POPs, but considerable variation exists among species. For example, cetaceans appear to be able to metabolically eliminate many dioxin-like PCBs, PCDDs and PCDFs, but are prone to accumulating the nondioxin-like (or "globular") PCBs (Tanabe *et al.*, 1988; Kannan *et al.*, 1989).
Other problematic persistent chemical contaminants not included in the POP group include the organo-metallic compounds (chemical compounds that are used in anti-foulant paints) and methyl mercury (an organic form of mercury that is highly toxic) (reviewed in Ross and Birnbaum, 2003). Mediterranean cetaceans are exposed to a cocktail of toxic compounds, some time at very high concentrations, as indicated by the data compiled here below.

1.1.2.1 Polychlorinated biphenyls

PCBs are widespread in the environment. They bio-accumulate in wildlife occupying high trophic levels as a consequence of their chemical characteristics and persistence. Pinnipeds and cetaceans accumulate high levels of PCBs in their blubber because they are at the top of the food chain, have large lipid stores, have a long life span and

a limited capacity for metabolism and excretion of compounds such as *p,p* –DDT and PCBs (Aguilar *et al*., 1999,2002; Ross *et al*., 2000). PCBs are immunotoxic causing thymus atrophy and reduced T-cell function through a common mechanism of action mediated by the cytoplasmic *Ah*R (Silkworth and Antrim, 1985; Kerkvliet *et al.*, 1990) that has been found in all mammals studied, including several marine mammal species (Hahn, 1998).

Studies carried out in seals that died during the 1988 seal epidemic and in the laboratory showed that: (1) ambient levels of environmental contaminants in the Baltic Sea herring were immunotoxic to harbor seals; (2) the pattern of effects implicated "dioxin-like" contaminants; (3) PCBs represented the major "dioxin-like" contaminant class; (4) many populations of free-ranging pinnipeds had PCB levels which exceeded those found to be immunotoxic in the captive study; and (5) environmental contaminants likely contributed to the severity of the 1988 PDV-associated mass mortality of harbor seals in northern Europe (Ross, 2002). Similarly, the striped dolphins (*Stenella coeruleoalba*) that died during the 1990-1992 epidemic had significantly higher loads of PCBs than the individuals that survived it. Given their well-known immunosuppressive effects, it was suggested that PCBs may have compromised the dolphin's immune response and increased the severity of the outbreak (Aguilar and Borrell, 1994). Though the role of environmental contaminants in the 2007 morbillivirus epidemic in the Mediterranean remains inconclusive, recent pollutant data obtained through analyses of biopsies from apparently healthy striped dolphins in 1987-2002 suggested that PCB and DDT concentrations have gradually decreased (Aguilar and Borrell, 2005). Recent studies have demonstrated a significant association between chronic PCB exposure and infectious diseases in harbour porpoises (*Phocoena phocoena*) from the British Isles. Individuals that died in poor health had a significantly higher sum of the concentrations of 25 individual chlorobiphenyl congeners ($\sum$25CBs) than those that perished by traumatic death (Jepson *et al*., 2005a, Hall *et al*., 2006).

Altogether these data suggest that contaminant-related immunosuppression likely contributed to the severity of the 1988 phocine distemper virus outbreak in harbour seals and of the 1990-1992 dolphin morbillivirus epidemic and that they may increase susceptibility of porpoises to infectious diseases.

1.1.2.2. Brominated flame retardants

Brominated flame retardants (BFRs) are a diverse group of compounds that have been extensively applied to combustible materials, such as plastics, wood, paper, and textiles to meet fire safety regulations (Alaee *et al*., 2003; de Wit, 2002). Additive flame retardants, such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), are blended with the polymers and may leach out of the products (Alaee *et al*., 2003). Being environmentally persistent compounds resistant to physical and biochemical degradation and with high production volumes, PBDEs and HBCD are among the most abundant BFRs detected in the environment (Alaee *et al*., 2003). Initially the major commercial products, the penta- and octabromodiphenylether formulations were prohibited in all applications for the European Union Market in August 2004 (European Union 2003). The deca-mix product was also banned in Europe following a ruling by the European Court of Justice in 2008. HBCD and tetrabromobisphenol-A (TBBP-A) are however still widely used. PBDEs are similar in structure to thyroxine (T4) and triiodothyronine (T3) (Hamers *et al*., 2006). Biologic effects of PBDEs in rodents are similar to those of PCBs, with increased risks for reproductive and endocrine disruption and neurodevelopmental problems (Zhou *et al*., 2002; Siddiqi *et al*., 2006; Stoker *et al*., 2004; Kuriyama *et al*., 2005; Ellis-Hutchings *et al*., 2006; Lilienthal *et al*., 2006; Talsness, 2008). BFRs negatively affect the reproductive health, immune system and development in exposed mammals including pinnipeds and cetaceans (Law et al., 2002, 2003, 2006a; Ross, 2005). They have been detected in cetaceans from Europe, the United States and Asia (Isobe *et al*., 2007; Law *et al*., 2008, Johnston-Restrepo *et al*., 2008). Rising trends in the concentrations of HBCD in the blubber have been observed in harbour porpoises stranded or dying due to physical trauma along the coasts of Bristish Isles in 1994–2003 (Law *et al*., 2008). PBDEs have also been detected in Mediterranean Sea striped dolphins, bottlenose dolphins, Risso's dolphins, a long-finned pilot whale and a fin whale (Pettersson *et al*., 2004). The impact of these contaminants on Mediterranean cetaceans is poorly known and should be further investigated (Fossi *et al*., 2006).

1.1.2.3. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a large class of molecules with condensed benzene rings. They are genotoxic and may induce cancers in humans and animals (Mastrangelo *et al*., 1996; Hakami *et al*., 2008; Topinka *et al*., 2008). Their lipophilic nature allows them to cross biological membranes and accumulate in organisms (Marsili *et al*., 2001). They are released in the environment by natural and man-made processes including combustion of wood and fossil fuels, oil plants and refineries and oil spills (Marsili *et al*., 2001). It has been estimated that an input of

635.000 tonnes of petroleum derived-hydrocarbons contaminates the Mediterranean each year (UNEP, 1988). Low molecular weight PAHs tend to remain in solution and are available to marine organisms through ingestion and respiration. Their solubility augments as temperature increases. These fat-soluble contaminants build up in fat and are mobilized with fat reserves during illnesses, reproduction and lactation and food scarcity (Marsili *et al*., 2001).

The contamination of the Saguenay River and immediate St. Lawrence estuary area by highly toxic PAHs such as the potent carcinogen benzo(a)pyrene (BaP) released massively by the local aluminum smelters over half a century and the exposure of belugas (*Delphinapterus leucas*) to these compounds were suggested as the most likely aetiology for a high prevalence of malignant tumours in belugas from the estuary (Ray *et al*., 1991; Martineau *et al*., 2002b). Total and carcinogenic PAHs were also detected in the subcutaneous blubber of fin whales (*Balaenoptera physalus*) and striped dolphins collected along the Italian coast of the Mediterranean Sea in 1993 and 1996, with naphthalene being the most ubiquitous compound (Marsili *et al*., 2001).

1.1.2.4. Perfluorinated compounds

Perfluorinated compounds (PFCs) refers to a group of man-made chemicals and their precursors, manufactured for their properties of providing resistance to heat, oil, and stains to products. Belonging to this group are subgroups of PFCs - perfluorinated carboxylic acids (PFCA) that includes perfluorooctanoic acid (PFOA) used as a polymerization aid in the manufacture of fluorinated polymers and elastomers; and perfluorinated alkyl sulfonates that includes perfuorooctane sulfonate (PFOS). Fluorotelomer alcohols are precursors to PFCAs. They are transformed in biota or in the atmosphere to produce PFCAs such as the extremely stable PFOA. They are persistent organic pollutants and are not known to degrade by any natural processes. PFCs and fluorotelomer alcohols are widely used in consumer product applications including lubricants, stain repellents (clothing and carpeting), food preparation (greaseproof packaging and non-stick cookware- Teflon), pharmaceuticals, insecticides and fire-fighting foams. They are ubiquitous and several of them have adverse effects on neuroendocrine and reproductive systems, reduce neonatal survival, are carcinogenic and immunotoxic (DeWitt *et al*., 2008, 2009a,b).
General exposure to PFOS may occur through ingestion of contaminated fish and water, or with dermal contact with PFOS containing products and direct occupational exposure at workplaces where it is manufactured. PFOA is found in the blood of the general human population (Hansen *et al*., 2001; Nakayama *et al*., 2005). Concentrations of PFOS in animals from relatively more populated and industrialized regions, such as the North American Great Lakes, Baltic Sea, and Mediterranean Sea, were greater than those in animals from remote marine locations (Giesy and Kannan, 2001). PPFOS and PFOSA were found in cetaceans from around the globe including Japan, China, Brazil, the US and the Mediterranean (Kannan *et al*., 2001, 2002; Hart *et al*., 2008; Yeung *et al*., 2009). Transplacental transfer occurred at very high levels in at least two species (Dorneles *et al*., 2008; Hart *et al*., 2008). PFOS was the most predominant fluorochemical detected in the tissues of free-ranging Mediterranean odontocetes (short-beaked common dolphins *Delphinus delphis*, common bottlenose dolphins *Tursiops truncatus*, striped dolphins and long-finned pilot whales *Globicephala melas*) analyzed and in the blood of captive bottlenose dolphins fed mackerel and herrings caught in the Mediterranean and capelin from the North Sea. The greatest PFOS concentration was observed in the liver of a common dolphin (940 ng/g, wet wt) similar to those reported for dolphins from the Florida coast (Kannan *et al*., 2002).

A recent study in bottlenose dolphin epidermal cell cultures suggests that exposure to PFOS significantly alters normal gene expression patterns and causes a cellular stress response, a decreased cell cycle progression and cellular proliferation and reduced protein translation (Mollenhauer *et al*., 2009). Though no direct mortalities due to these compounds were reported their ubiquitous presence, high concentration in several species, maternal transfer and toxicity are cause for concern.

1.1.2.5. Heavy metals

Marine mammals accumulate high levels of mercury (Hg) and cadmium (Cd) (Wagemann and Muir, 1984; Aguilar *et al*., 1999). The natural occurrence of these elements in seawater has involved detoxification capacities to support elevated exposure to toxic metals in their environment (reviewed in Das *et al*., 2000). Cd can be stored over long periods in the kidneys of marine mammals (Lahaye *et al*., 2006). In odontocetes the demethylation of organic Hg occurs in the liver and leads to the production of non-toxic granules of tiemannite that are not excreted (Martoja and Berry, 1980). Since these granules are not excreted, inorganic Hg would be stored in the liver for the whole life resulting in elevated concentrations of Hg in this organ (Nigro and Leonzio, 1996; Lahaye *et al*., 2006). The immune system is susceptible to long-term mercury exposure. A reduced viability, metabolic activity as well as DNA and RNA

synthesis were observed *in vitro* in stimulated lymphocytes from harbour seals following exposition to more than 1µM concentration of methylmercury (Das *et al*., 2008). In addition to immunosuppression, metal pollutants may induce immunoenhancement leading to hypersensitivity and autoimmunity **(**Kakuschke and Prange, 2007**).**

High Hg concentrations in harbour porpoises from the German Waters of the North and Baltic Seas were significantly associated with prevalence of parasitic infections and pneumonia (Siebert *et al*., 1999). The mean liver concentrations of Hg, Se, the Hg:Se molar ratio and Zn in harbor porpoises found dead along the coasts of the British Isles were significantly higher in those that died of infectious diseases than in those that died of a physical traumas (Bennett *et al*., 2001). Hg and Cd were also detected in the liver and kidneys of Mediterranean bottlenose dolphins and striped dolphins, respectively, at high concentrations in some individuals (Lahaye *et al*., 2006).

1.1.3. Biological pollution

Coastal ecosystems are continuously invaded by microorganisms from ballast waters, aquaculture waste and untreated run-off waters (Weber *et al*., 1994; Rhodes *et al*., 2000; Cabello, 2004, 2006; Drake *et al*., 2007). The discharge of water, sediments and biofilms from ships' ballast water tanks is a prominent vector of aquatic invasive species (Ruiz *et al*., 2000; Drake *et al*., 2007). The use in aquaculture of a wide variety of antibiotics in large amounts, including non-biodegradable antibiotics useful in human medicine, ensures that these remain in the aquatic environment, exerting their selective pressure for long periods of time. This has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments (including the Mediterranean Sea), in the increase of antibiotic resistance in fish pathogens and in alterations of the bacterial flora both in sediments and in the water column (Rigos *et al*., 2004; Cabello, 2006). Increasing water temperatures, a consequence of global warming, likely enhance the survival of some marine bacterial pathogens such as *Vibrio* spp. and increase exposure (Pascual *et al*., 2002). An increased pathogen exposure due to biological pollution has been detected in harbour seals inhabiting urban sites along the coast of Washington State and British Columbia (Mos *et al*., 2006). Biological contamination is also thought to have played a role in the emergence of miscellaneous skin diseases observed in cetaceans from the Americas and the Indian Ocean (Van Bressem *et al*., 2007; Flach *et al*., 2008; Kiszka *et al*., 2009).

1.1.4. *Acoustic pollution*

Cetaceans depend on sound to find food, communicate, detect predators and navigate. Escalating mechanized use of the sea, such as for shipping, military activities, oil and gas exploration and recreation, is increasing the amount of noise that humans introduce into the oceans, sometimes over very large distances. Anthropogenic underwater noise is a relatively novel environmental element for cetaceans and they may not be able to cope with it (Simmonds *et al*., 2004; Wright *et al*., 2007).

Powerful underwater sounds cause damage to the hearing systems, which can result in: (1) disorientation, (2) disconnection from school, pod or community, (3) internal bleeding; ruptured tissues, deafness and strandings as well as physiological harm. For example, exposure to an unexpected and unnatural loud noise could startle a deep-diving whale, causing it to bolt for the surface in a panic – such a rapid ascent could lead to bubbles forming in the tissues (a condition known in human divers as "the bends") and then to a stranding (Weilgart, 2007).

Anthropogenic sound sources vary in space and time but may be grouped into general categories: (1) explosions, (2) large commercial ships, (3) airguns and other seismic exploration devices, (4) military sonars, (5) navigation and depth-finding sonars, (6) research sound sources, (7) acoustic harassment devices (AHDs) and pingers, (8) polar icebreakers, (9) offshore drilling and other industrial activity, and (10) small ships, boats, and personal watercraft (Hildbrand, 2005). The following paragraphs summarize data on military sonars and seismic explorations.

1.1.4.1. Anthropogenic sonar signals

Sonar is an acronym for Sound Navigation and Ranging. A wide range of sonar systems is in use for both civilian and military applications. They intentionally create acoustic energy to probe the ocean. They can be categorized as low-frequency (<1 kHz), mid-frequency (1–20 kHz), and high-frequency (> 20 kHz). Low-frequency active (LFA) sonars are used for broadscale surveillance. Mid-frequency tactical antisubmarine warfare (ASW) sonars are designed to detect submarines over several tens of kilometers. They are incorporated into the hulls of submarine-hunting surface

vessels (Hildbrand, 2005). All active sonars emit a noise pulse or "ping". These sound pulses bounce off a target (such as a submarine) and return as echoes that are detected by hydrophones.

Multiple mass strandings of beaked whales have been documented over the last decade following acoustic exposure to anthropogenic sounds, especially mid-frequency sonars, in Europe, the US and Asia (see Cox *et al*., 2006 for a review). These strandings affected Cuvier's beaked whale (*Ziphius cavirostris*), Blainville's beaked whale (*Mesoplodon densirostris*), northern bottlenose whale *Hyperoodon ampullatus* and Gervais' beaked whale *Mesoplodon europaeus* (see Cox *et al*., 2006 and Simmonds *et al*., 2004 for reviews). Affected whales had a condition called gas and fat embolic syndrome (GFES) characterized by extensive fat and gas bubble emboli, an ensemble of lesions most similar to decompression sickness (DCS) in human divers (Jepson *et al*., 2003, 2005b; Fernandez *et al*., 2005). The prevalent hypothesis is that GFES is induced through a precondition of tissue N2 supersaturation coupled with a behavioural response (increased or decreased surface interval, ascent rate, or dive duration, leading to increased supersaturation, thereby increasing DCS risk) to acoustic exposure (Jepson *et al*., 2003; Cox *et al*., 2006). Other suggestions include an acoustic signal that could (1) activate existing stabilized bubble nuclei allowing them to grow by passive diffusion, and/or (2) drive activated bubbles to expand through rectified diffusion (Cox *et al*., 2006). Each of these hypotheses assumed that these beaked whales live with significantly elevated blood and tissue tension N2 levels, a fact supported by a recent mathematical model (Hooker *et al*., 2009). In the Mediterranean strandings related to acoustic testing occurred in Greece in May 1996 (Frantzis, 1998).

1.1.4.2. Seismic surveys

Seismic airguns, used by the petroleum industry to detect pockets of oil or natural gas within the ocean floor and by researchers to locate sub-surface geological features, sound like underwater gun blasts and at times can be heard throughout entire ocean basins. Such impulsive sounds can be acutely harmful to nearby animals, but may also disturb (repeatedly startle) marine mammals to the point where they abandon important habitat (Nieukirk *et al*., 2004; Simmonds *et al*., 2004). The possibility that seismic noise can lead to strandings and/or death in marine mammals exists. Indeed, two Cuvier's beaked whales stranded in the Gulf of California in September 2002 coincidently with seismic reflections (Hildebrand, 2005). During the 2002 breeding season, three seismic surveys conducted in the Southern portion of Abrolhos Bank, Bahia and Espírito Santo States, Brazil may have been responsible for an increase in the strandings rate of adult humpback whales (*Megaptera novaeangliae*) (Engel *et al*., 2004). Hearing damage may also have indirectly killed humpback whales by compromising their navigation or sensory system (Todd *et al*., 1996).

1.2 Things to do in preparation for non-infectious unusual mortality events

Marine mammal strandings attract a lot of public attention. Several dolphins may beach over weeks along thousands of kilometres. The degree of response of each country will depend on the existence of active stranding networks and marine mammal research groups as well as on its economic and logistic possibilities. Some countries may be able to provide most of the scientific, technical and administrative infrastructure needed to face a massive stranding while others may only offer a more reduced support or none at all. Collaboration between Member States will be a plus to effectively attend these events. The foundation of an expert Sub-Committee on Cetacean Unusual Mortalities (CEUM) within the ACCOBAMS Scientific Committee would optimise the answer to die-offs in the Agreement Zone. The CEUM Sub-Committee should ideally have the equipment described in 1.2.2.1- 1.2.2.3. Nevertheless, much can be done with a more reduced infrastructure and equipment (1.2.2.4).

1.2.1. Technical and administrative infrastructure needed in each Member State to best address emergencies caused by cetacean die-offs

All Member States should at least have an on-scene coordinator body (OSCB) that would contact the CEUM Sub-Committee and any other relevant institution in the case of a suspected mass-mortality, send data to the Mediterranean Database of Cetacean Strandings (MEDACES- http://medaces.uv.es/home_eng.htm), deal with the public and media, ensure that the proper samples are taken, be responsible to obtain all necessary permits and deal with the carcasses. The OSCB should ideally depend on an existing stranding network, a natural science museum, a university or a ministry (Agriculture, Environment, Fisheries). It should collaborate with existing national entities related to marine mammal stranding such as active stranding networks and marine mammal research groups, wildlife conservation and rescue centres, aquaria and oceanaria, coastguards, park officials and local authorities. It should also establish Memoranda of Agreement (MOA) with the Navy that could be directly involved in sonar

activities as well as with Oil and Gas Companies involved in seismic surveys. Ideally, the Navy MOA should permit collaboration between the Naval Forces and the OSCB during stranding events possibly related to sonar activities by allowing the use of their planes, helicopters, boats and/or, trucks for transport of stranding responders or animals or assistance with aerial surveys to discern the extent of such an event. The MOA with the Oil and Gas Companies should facilitate access to OSCB marine mammal observers to their boats. The OSCB should also launch an agreement with universities or medical institutions willing to offer free tomographic examination of the cetacean's head stranded during acoustic operations and with universities or research institutes interested to collaborate on chemical and biological contamination. The OSCB should have all necessary addresses and phone numbers in the case of an emergency as well as a precise protocol to collect samples for research.

The OSCB basic technical and administrative infrastructure should include:

- A stranding hotline telephone, dedicated to record any stranding occurring along the coast and operating 24 hours, seven days a week;
- A computer with permanent internet access;
- A printer;
- Portable telephones;
- A GPS to register stranding locations;
- Digital cameras;
- DVD reader;
- Educative material;
- A specialized marine mammal library;
- A website describing the activities of the OSCB as well as the names of the persons in charge and to be contacted in the event of a die-off;
- A database on cetacean mortality events
- A centrifuge to spin blood samples;
- A large fridge to keep samples at 4°C;
- A –80°C freezer to store samples for longer periods of time.

1.2.2. Equipment list

The optimal and complete equipment list to face stranding of live and dead animals has been presented in another ACCOBAMS document (Van Bressem, 2009). A checklist for recording material, necropsy and sampling for chemical, acoustic and biological pollution is provided here below.

1.2.2.1. Recording material

- Waterproof pencils;
- Metal clipboards, waterproof labels;
- Data forms, necropsy and collection protocol forms;
- Camera and film, extra batteries, video camera with additional memory cards;
- Tape measure (metric), at least 20 meters long (plastic and metallic);
- Hoist/crane, scales to record organ weights (0,1-10kg);

1.2.2.2. Necropsy

- Rope, at least 20 meters, blankets, stretchers to move carcasses, if necessary;
- Gloves (non-powdered, vinyl)
- Necropsy instruments: multiple stainless steel scalpel handles, stainless steel scalpel blades, stainless steel scissors, stainless steel forceps forceps and knives;
- Stainless steel surgical scissors;
- Knife sharpener, if possible in secure pack;
- Stainless steel flensing knives and hooks with appropriate sharpening tools, chain saw, axe, or reciprocating saw to cut through the cranium, chest or vertebrae;
- Hammers, chisels and handsaws;
- Retractors of various sizes and shapes. Self-retaining retractors with one or two movable arms mounted on a slide bar are most useful;
- Sterile instruments for culture collection;
- Whirlpacks;

- Jars, vials;
- Buckets;
- Flashlights with extra batteries and light bulbs;
- Containers (from vials to garbage cans) for sample collection, including ice chest, dry ice and, if possible, liquid nitrogen;
- Gas generator and flood lights with extra bulbs and gasoline;
- Lights;
- Portable or electric circular saw;
- Accessible water supply with hose;
- Buckets;
- Garbage bags, dish soap, paper towels for clean-up.

1.2.2.3. Specific sampling (chemical, biologic and acoustic pollution)

- 10% neutral buffered formalin;
- 2.5% buffered glutaraldehyde and/or 4% paraformaldehyde (for transmission and scanning electron microscopy);
- Dimethyl sulfoxide (DMSO)
- methylene chloride or methanol
- Isopropanol alcohol for contaminant sampling;
- clean and sealed glass containers for contaminant sampling
- Teflon bags for contaminant sampling (precleaned)
- Needles and syringes;
- Heparinized syringes;
- ethylenediaminetetraacetic acid- and heparin-containing tubes
- Culture vials fro microbiology;
- Transport medium for microbiology and cell culture;
- Sterile swabs;
- Sterile urine cups;
- Glass slides;
- Serum tubes for blood and urine collection and gas burner to sear organ surfaces and sterilize scalpel blades;
- Coolers for samples refrigeration;
- Liquid nitrogen (if possible)

1.2.2.4. Minimal equipment

The following minimal equipment also permits to document the event and take valuable samples from freshly dead dolphins. In this case, all samples for toxicology should be large to allow further processing with stainless steel instruments.

- Recording material (waterproof pencils, metal clipboards, waterproof labels, data forms, necropsy and collection protocol forms);
- Camera;
- Mobile phone;
- Buckets;
- Water sprayer;
- Gloves, plastic boots and masks;
- Wide plastic sheets;
- Butcher knives;
- Butcher saws;
- Scalpel and scalpel blades;
- Vials and jars;
- Plastic bags (whirlpacks);
- Aluminium foils;
- Ropes.

1.3 Actions to take during non-infectious unusual mortality events

Several situations may occur during non-infectious unusual mortality events:

- Single stranded dolphins found dead or agonizing on different beaches;
- Several dead dolphins stranded together on the shore;
- Dead and live cetaceans stranded simultaneously on a beach.

In all cases, excellent coordination between the OSCB staff, the proposed CEUM Sub-Committee, other organizations specialised in these events and military institutions will be the key for a successful answer. The protocols given below are broadly based on Geraci and Lounsbury (2005). The second edition of '*Marine Mammal Ashore: A Field Guide for Strandings*' provides extensive information on how to deal with stranded, live or dead dolphins and whales and one or more copies should be in the library of all bodies involved with cetacean strandings. It would be wise to carry one copy to the field. Several papers cited in the present document are available online or upon request to the authors and would be worth to have in the library for more in-depth information.

1.3.1. Protocols for collection, transportation and storage of specimens and samples

1.3.1.1. Protocols for sample collection

Prior to sample collection, basic data should be collected in order to get crucial biological parameters. Recording the whale/dolphin condition is important to determine which samples should be given priority. Only the animals considered fresh or slightly decomposed are worth sampling for microbiology, toxicology and histopathology. All samples collected for microbiology and toxicology should be taken as aseptically as possible. The necropsy should be carried out by an experienced scientist. Notes should be taken by an assistant.

After collection of the basic data, the body should be opened, preferably on a wide plastic sheet or on a necropsy table. All instruments necessary for collecting biological samples such as bags, jars and vials with or without liquids should be clean, sterile and at hand before making the first incision. An assistant should label the containers and take notes and pictures.

Glass containers and Teflon bags are recommended for both organic compound and heavy metal analysis. Although glass containers should have a teflon-lined cap, foil-lined caps are acceptable for organic compound analysis. Sample jars should be cleaned with detergent, rinsed with tap water, soaked in 1:1 acid, rinsed with metal-free water, and rinsed again with high purity methylene chloride or methanol (PSEP 1989a,b). Containers should be kept capped and sealed after cleaning and prior to sample collection. Handling of containers should be kept to a minimum and the inside of the container should not be touched by anything other than the sample. Cross-contamination between tissues should be avoided. The scalpel and forceps should be cleaned after taking each sample. All tissue surfaces that come into contact with implements that were not cleaned (e.g., blubber when the body was opened) should be cut away with clean implements. The sample should not come into contact with the outside of the sampling container or the ground. When conditions are not ideal and sterility is not guarantee, remove a large slice (300-400 grs of the required tissue as hygienically as possible. Record whether the knife is ferrous or stainless or metal steel. The large samples may be collected in aluminium foil, plastic bags or buckets. They should be sealed, labelled with a waterproof pen, placed in a cooler with ice and transported to the laboratory quickly.

Skin samples for cell culture should be collected in culture medium with antibiotic and anti-fungi and kept on ice. They should be processed within 24h. These skin samples should be collected only in the case of an existing agreement with a university or research institute.

Small (1 cm^3) and representative samples of all organs and tissues from fresh cetaceans should be promptly fixed in 10% neutral buffered formalin solution for histopathology. The pancreas should be fixed as soon as possible, given the enhanced susceptibility of this organ to *post mortem* autolysis. The fixative containing the above tissue samples should be replaced with fresh formalin solution after 24 hours.

If there is suspicion of sonar-related stranding, if there is possibility to carry out tomography and if the specimens are fresh enough, the whole head should be collected and kept at on ice or in a 4°C till examination is carried out.

Samples for microbiology (skin lesions, blood, etc...) should be only taken from freshly dead cetaceans, collected in a sealed container previously cleaned and sterilized containing transport medium, identified and kept on ice or at 4°C. If laboratory tests are not planned within the next days, then freeze at –80°C.

1.3.1.1.1. Basic Data Protocol

- Investigator
  - Name:
  - telephone:

    - e-mail:
- Date:
- Location of stranding:
- Presence of other dead aquatic animals:
    - Species:
    - Number (estimation):
- Field number:
- Species[2]:
- Sex[3]:
- Standard body length[4]:
- Condition:
    - alive
    - fresh
    - early decomposition
    - advanced decomposition
    - mummified
- Fatness stage: fat, normal, thin, emaciated
- Indications for acoustic testing manoeuvres[5]:
    - presence of naval exercises YES/NO
    - number of boats:
    - distance from coast:
    - extension of the area:
    - frequency used, date and time of the exercises:
    - characteristic of the vessel (vessel length, speed and heading):
    - identify key characteristics of sound (e.g. frequency, amplitude, energy, directional transmission pattern, use of arrays vs. single sources, etc.)
    - characteristics of environmental parameters that may influence sound propagation
    - behaviour of cetaceans before stranding:
        ** continually circling or moving haphazardly in a tightly packed group – with or without a member occasionally breaking away and swimming towards the beach: YES/NO.*
        ** abnormal respiration including increased or decreased rate or volume of breathing, abnormal content or odour: YES/NO*
        ** presence of an individual or group of a species that has not historically been seen in a particular habitat, for example a pelagic species in a shallow bay when historic records indicate that it is a rare event: YES/NO.*
        ** abnormal behaviour for that species, such as abnormal surfacing or swimming pattern, listing, and abnormal appearance: YES/NO*
    - presence of external abnormalities (especially bleeding from the eyes and ears): YES/NO
        - Description - pictures
- Indication for an algal bloom: YES/NO
- Evidence for human interactions: YES/NO
    - Net marks
    - Knife cuts
    - Wounds caused by vessel strikes
    - Description-pictures

- Presence of skin lesions and wounds: YES/NO.

[2] Species identification should be done by qualified persons. Ideally a picture of each specimen with its field number should be taken.
[3] A picture of the genital region with field number will help to confirm the sex.
[4] Precise how it was taken (measurements should be parallel to the dolphin body, e.g. total length from snout to fluke notch).
[5] This checklist should be filled by an assistant or an experienced volunteer while the principal researcher carries on with the rest of the protocol.

- Description – pictures

- Collect samples in 10% neutral buffered formalin solution, DMSO and, if possible, keep some unfixed samples at –80°C

- Lactating: YES/NO

1.3.1.1.2 Specific sample collection[6]

1.3.1.1.2.1. Reproductive tract

Ovaries and testes should always be examined, weighed, photographed and collected in 10% formalin (4% end concentration) to assess sexual maturity. The presence/absence of corpora albicantia and a corpus luteum should be recorded. Uterus should be opened to check for a foetus. The latter should be measured, weighed and sexed and, if small, conserved in formalin. Presence of sperm in the epidydimis should be evaluated. A piece of at least 1 cm^3 of both testes should be collected in formalin. The following questions may be answered in the field if time permits otherwise in the lab after addressing the mortality event.

- Ovaries:
  - presence of corpus albicans: NO, YES
  - presence of corpus luteum: YES, NO

- Foetus in uterus: YES, NO
  - sex
  - length
  - weight

- Testes: YES/NO
  - Right:

    presence of seminal fluid
    length
    weight
  - Left:

    presence of seminal fluid
    length
    weight

1.3.1.1.2.2. Biological pollution

- Document, describe and take pictures of any change in organ gross morphology.

- Collect cutaneous lesions and subcutaneous abscesses in 10% formalin (histology) and in containers with cell culture medium (microbiology);
- Collect 5-10grs samples from the kidneys, testes, uterus, placenta and foetus (if available), mammary glands and spleen, keep on ice and refrigerate at 4°C or freeze at –80°C if long delays are unavoidable (> 24h) before

[6] Basic and advanced data protocols are also available at the Medaces website: http://medaces.uv.es/home_eng.htm

further analysis. When no freezing facilities are available, smaller samples should be kept in DMSO. Preserve 1 cm^3 samples of the same organs in formalin.
- Collect pleural and peritoneal fluids, urine and pus from abscesses and store half in aerobic containers and half in anaerobic containers. Keep on ice and then freeze at –80°C if a laboratory is not at hand.
- Extract 5-10 ml blood directly from the heart or major blood vessels after disinfecting the surface with alcohol and put on ice. You may attempt to centrifuge the blood and take the supernatant before freezing to avoid further hemolysis;
- Collect water around the site of stranding (preferably before massive arrival of people) in a sterile container, seal and put on ice before freezing;

1.3.1.1.2.3. Chemical pollution

The following organs are useful to evaluate the burden of contaminants in cetaceans.
- Blubber: take a large sample (300-400 grs minimum) of blubber about 10 cm caudal to the blowhole or directly below the dorsal fin on the mid-lateral line, place in an aluminium foil, then in an sealed plastic bag with field number and store on ice;
- Skin: take a 10 cm^2 sample of clean skin, preserve in a container with culture medium containing antibiotics and anti-fungi, seal, identify and keep on ice;
- Liver: slice 300-400 grs from the caudal end of the liver, place in an aluminium foil, then in an sealed plastic bag with field number and place on ice;
- Kidney: take 500 grs of from the caudal end of the left kidney, place in an aluminium foil, then in an sealed plastic bag with field number and place on ice;
- Blood: collect 50 ml blood in a tube, seal, identify and keep on ice;

1.3.1.1.2.4. Acoustic pollution

With suspect sonar-related strandings, arrangements should be made for computerized tomography (CT) of the entire head or ears and close evaluation of the larynx should be undertaken for evidence of submucosal hemorrhage. Samples of peribullar adipose tissue should also be collected for histopathology. Tissues from all organs should be collected, if feasible.

- Live animal
  - blood
  - diagnostics such as auditory evoked potential (AEP) computerized tomography (CT) or ultrasound
  - rehabilitation
- Dead animal
  - When possible collect head for diagnostic imaging including CT/MRI scans or ultrasound of entire head;
  - Collect tissues (1 cm^3) from all organs and preserve in formalin 10%, with emphasis on the brain, peribullar adipose tissue, hypophysis, choroid plexus, cervical spinal cord, liver, lung, kidney, heart, lymph nodes, digestive tracts, reproductive tracts, and perilaryngeal tissues, including the trachea and thyroid and eyes. All sampled should be collected in separate bags (whirlpacks) and clearly identified.

1.3.2 Protocols for transportation and storage

Contact the local CITES Management Authority (http://www.cites.org/common/directy/e_directy.html) to know the requirements to obtain permits to export cetacean samples. Contact the laboratories that will analyse the samples and coordinate for sample dispatch according to the airline procedures. Make sure that somebody will collect the samples at their arrival and that the person in charge is not on holidays at the time you send the samples. Keep telephone and e-mail contact until you are assured that the samples arrived and were properly stored.

Microbiology: All fresh samples should be kept on ice or cold packs, away from the sun while waiting for further processing. Upon arrival in the laboratory, they should be kept at 4°C and immediately dispatched to the laboratory, if possible. If long delays are expected they should be frozen at –20°C or –80° C. Storage should be organized in a way that samples are easily found when the freezer is full. Records should be kept of any sample location.

Toxicology

Chemical analysis: samples en route to the analytical laboratory should be packed in dry ice. However, if delivery time is short (less than 6 hours, depending on ambient temperatures), then samples could be delivered in coolers filled with ice. All samples for toxicology should be stored in a freezer at –20°C or below until analysis. Storage time and temperature records should be recorded. The maximum holding times for tissues recommended by PSEP guidelines are 1 year for organics (with the exception of volatile organic compounds, which have a maximum holding time of 14 days), 28 days for mercury, and 2 years for all other metals. Samples held for longer periods may be suitable for analysis of some contaminants, but suitability should be evaluated based on the contaminants being tested and then described in a report presenting results for these samples.

Skin culture: skin samples to be used for cell culture should be maintained on cool packs and send as soon as possible to the laboratory. They should never be frozen nor left without ice.

Acoustic pollution

With suspect sonar related strandings, arrangements should be made for CT of the entire head or ears and close evaluation of the larynx should be undertaken for evidence of submucosal haemorrhage. Samples of peribullar adipose tissue should be collected for histopathology.

1.4Activities to implement after stranding

1.4.1. Debriefing meeting

Organize a debriefing meeting with all the people involved in the stranding and ask them their opinion on the event, the number of cetaceans they counted and attended, the presence of other dead aquatic animals on the beach, if live dolphins and whales were observed in waters close to the beach where the event happened, if the response to the stranding was adequate in their opinion, what material was missing. Thank all volunteers for their help and distribute any new information material and stickers. Speak with fishermen, members of the military and local people and ask if they have observed the occurrence of unusual species during the days preceding the stranding, if free-ranging cetaceans known to occur in the region exhibited an unusual behaviour, if military operations had taken place during the last days, or if there were reports of seismic surveys in neighbour waters.

1.4.2. Communication

1.4.2.1. Local government, Armed Forces, Ministry of External Affairs, Ministry of Environment and Ministry of Health

Call or write the local government, the Ministries o Health and Environment as well as the Navy and the Oil and Gas Companies if there are strong indications for strandings related to acoustic pollution.

1.4.2.2. Scientists

E-mail or call scientists that have signed a MOA. Ask for their comments and help. Send data to the Mediterranean Database of Cetacean Strandings.
(MEDACES- http://medaces.uv.es/home_eng.htm).

1.4.2.3. Press

Write a brief note on the event for the media. Alert the media and public for the possibility of more cetacean strandings on every beach and encourage them to report.

1.4.3. Preliminary report

Write an initial report as soon as possible. Points to summarize in the report should include the following (Geraci and Lounsbury, 2005):

- Date and location of the stranding
- Type of beach;
- Nature, timing, effectiveness of the initial response;
- Account of the scene as described by the team:

    - species involved and number of specimens per species,
    - pattern of stranding,
    - presence of other dead or sick aquatic animals,
    - presence of live cetaceans exhibiting an unusual behaviour in adjacent waters,
    - evidence for the use of mid-frequency sonar,
    - cetacean condition,
    - indication for an epidemic,
    - environmental conditions.
- Necropsy findings;
- Specimens collected, place where they are stored, condition for storage;
- Actions taken and reason for decisions:
    - intended response plan,
    - impediments to implementation,
    - eventual action.
- Additional information:
    - photographs, maps, drawings,
    - reports from independent groups (police, coastguards, stranding networks, rehabilitation facility, Navy, fishermen),
    - Things to be improved.

1.4.4. Follow-up

Ask for a follow-up of the analysis and prepare a manuscript on the findings together with all involved institutions.

2. CONTINGENCY PLAN DRAFT

Cetaceans from the Mediterranean harbour a cocktail of chemical, toxic pollutants, some likely to have increased the severity of disease epidemics. Mid-frequency sonar operations have caused the stranding of beaked whales in Greece (Frantzis, 1998). Biological contamination is of concern because of the release of untreated freshwater run-off, aquaculture, maritime traffic and discharge of ballast waters in Mediterranean waters. Thus, Member States should be ready for the eventuality of cetacean strandings, diseases and mortalities related to these agents. The development and strengthening of existing national and regional stranding networks will be key to properly address these events. Importantly, data on strandings along the coasts of the Black and Mediterranean Sea as well as the contiguous Atlantic waters should be sent to MEDACES (http://medaces.uv.es/home_eng.htm) set-up in 2001 to co-ordinate all national and regional efforts for riparian countries. The establishment of a CEUM Sub-Committee within the ACCOBAMS Scientific Committee would further improve answer to strandings by facilitating coordination between Member States and helping with infrastructure and capacity building. The foundation of CEUM Working Group that would communicate by e-mail would facilitate information diffusion. Memoranda of Agreement with the Naval Forces as well as with Oil and Gas Companies would improve answer to cetacean die-offs related to acoustic pollution.

2.1. OSCB

An efficient contingency plan will be based on the foundation of a national OSCB that will be responsible for the activities and decisions related to unusual mortality events as well as on timely relaying information on their occurrence to the Member States and to the suggested CEUM Sub-Committee. The easy and open communication between OSCBs will help determine when a die-off is underway, ensure a timely and adequate intervention and, ultimately, uncover the cause of the die-off and explore environmental factors that may have enhanced its severity. Minimal personal of an OSCB should be one scientist, preferably a marine mammal research veterinarian with good knowledge in the biology of cetaceans and of the different factors involved in cetacean strandings.

2.1.1. Administrative support team

At least one person should be in charge of the administration of the OSCB. His/her responsibilities should include:

- Coordination with local authorities;
- Coordination with the Naval Forces and Oil and Gas Companies;
- Contact with the authorities that will deliver CITES permits;
- Contact with the airlines that will transport the samples: ask for their specific requirements for the packaging and dispatch of biological materials;
- Communication with media and public;
- Development of education activities and material;
- Management of volunteers;
- Building of a website;
- Finance management.

2.1.2. Scientists

A biologist and a veterinarian, both ideally with experience with cetaceans, should be appointed by the OSCB. Their responsibility should include the following items:

- Develop a stranding network that can react quickly to cetacean mortality events;
- Develop protocols for attending strandings and for the collection of tissues for chemical, acoustic and biological pollution;
- Prepare the material necessary for attending a die-off (everything should be ready and at hand for instant leave);
- Provide field staff and build capacity;
- Recruit and manage volunteers;
- Timely intervention and incident control coordination: an educated decision on response level (equipment and personnel);
- Coordination with other similar networks within and outside the Member States;
- Adequate decision regarding the fate of live-stranded cetaceans (release, rehabilitation, euthanasia);
- Collection of biological data and pictures;
- Necropsy of dead cetaceans;
- Collection of samples;
- Contact with laboratories that will process the samples;
- Contact with research centres that could provide free CT examination;
- Prepare a protocol for packing and dispatching biological material;
- Send the samples;
- Carcass disposal in agreement with national regulation.

2.1.3. Volunteers

Volunteers should be recruited to help with strandings. They may have distinct backgrounds and personalities and should be given tasks according to their respective skills.

2.2. Memoranda of understanding with collaborators

Memoranda of understanding should be established with the Naval Forces, Oil and Gas companies as well as with universities, research/medical institutes and laboratories willing to help at the occasion of an outbreak of mortality. Laboratories (toxicology, microbiology and acoustic research) should be asked to send specific protocols for sampling, preserving and sending the samples. Ideally they should provide the vials, fluids and other material required for sampling. Otherwise they should specify the material needed for sampling and the firm where to buy it.

3. OUTLINE OF A PROGRAMME TO BUILD CAPACITY

Capacity building is a prerequisite to explore factors involved in a die-off. It should concern the staff of the OSCB, volunteers, coastguards and navy officials, fishermen and the general public (please see § 1.2.3.). The following programme outlines the steps that may be taken to realize this target.

- Organization of annual, national workshops on cetacean outbreaks of mortality for the staff of the OSCBs. National and international experts in the fields of toxicology, acoustic contamination and microbiology should ideally be invited to participate;
- Organization of training courses on cetacean strandings, on acoustic, chemical and biological contamination and sample collection for the staff of the nascent OSCBs. These training courses may take place at the OSCB, CEUM facilities or at the laboratory of a national stranding network;
- Organization of national meetings with other relevant bodies related to strandings (universities, coastguards, oceanaria, naval forces, fishermen, etc) and presentation of documents on cetacean mortality events;
- Acquire capacity building material (books, papers, reports, CDs, DVDs, protocols) from other stranding networks, universities, research groups, NGOs and scientists;
- Development of a library dedicated to marine mammal strandings, acoustic, biological and chemical contamination and epidemics;
- Communication with other OSCBs;
- Preparation of leaflets on the biology of cetaceans and the reasons of cetacean mortality events targeting the general public;
- Preparation of children booklets and posters on whales and dolphins and stranding events.

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Emergency task force:
Guidelines for a coordinated cetacean stranding response during mortality events caused by infectious agents and harmful algal blooms[7]

1. GUIDELINES CONCERNING BEST PRACTICES AND PROCEDURES FOR ADDRESSING CETACEAN STRANDING DURING EPIDEMICS CAUSED BY INFECTIOUS AGENTS AND HARMFUL ALGAL BLOOMS

1.1 Introduction on main marine mammal die-offs

1.1.1 Morbilliviruses

1.1.1.1 Morbillivirus epidemics in pinnipeds

1.1.1.2 Morbillivirus epidemics in cetaceans

1.1.2 Herpesviruses

1.1.3 Brucella spp.

1.1.4 Leptospira spp.

1.1.5 Toxoplasmosis

1.1.6 Harmful Algal Blooms

1.2 Things to do in preparation for an epidemic

1.2.1 Technical and administrative infrastructure needed in each Member State to best address emergencies caused by cetacean epidemics

1.2.2 Equipment list

1.2.2.1 Crowd control, public relations

1.2.2.2 Recording material

1.2.2.3 Animal relief

1.2.2.4 Emergency Medical Supplies

1.2.2.5 Euthanasia

1.2.2.6 Necropsy

1.2.2.7 Specific sampling

1.2.2.8 Personal

1.2.2.9 Large equipment

1.2.2.10 Dispatch

1.2.2.11 Minimal equipment

1.2.3 Capacity Building

1.2.3.1 Scientists

1.2.3.2 Volunteers

1.2.3.3 Local government officials

1.2.3.4 Public

1.3 Actions to take during an epidemic event

1.3.1 Protocols for intervention on site

1.3.1.1 Live cetaceans stranded on the beach

1.3.1.2 Dead whales and dolphins

1.3.2 Protocols for collection, transportation and storage of specimens and sample

1.3.2.1 Protocols for sample collection

1.3.2.1.1 Basic Data Protocol

1.3.2.1.2 Specific sample collection

1.3.2.1.2.1 High priority samples

1.3.2.1.2.2 Intermediate priority samples

1.3.2.2 Protocol for transportation and storage

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[7] Document prepared by Dr Marie-Françoise Van Bressem, Cetacean Conservation Medicine Group, CMED/CEPEC, Cra 74, 139-33, Bogota, Colombia
E-mail: mfb.cmed@gmail.com

1. GUIDELINES CONCERNING BEST PRACTICE AND PROCEDURES FOR ADDRESSING CETACEAN MORTALITY EVENTS CAUSED BY EPIDEMICS

1.1. Introduction on main marine mammal die-offs

Marine mammal epidemics have occurred in pinnipeds and cetaceans worldwide and are the subject of continued scientific research. Repeated outbreaks may have long-term effects on the affected populations (Van Bressem *et al*., 1999, 2009; Lonergan and Harwood, 2003; Härkönen *et al*., 2006). Among the micro-parasites causing marine mammal mass-mortalities, morbilliviruses appear by far to be the more lethal and widely distributed of all (e.g. Kennedy, 1998; Duignan *et al*., 1995a,b; Van Bressem *et al*., 2001a, 2009). Herpesviruses, the bacteria *Brucella* spp. and *Lepstospira* spp. as well as the protozoan *Toxoplasma gondii* have also triggered severe diseases and mortalities in a number of cetacean and pinniped species (Gulland *et al*., 1996; Foster *et al*., 2002; Dubey *et al*., 2003; Smolarek Benson *et al*., 2006). Harmful algal blooms (HBAs) are increasingly recognized as a cause of die-offs in marine animals (Flewelling *et al*., 2005). Below I summarize information on these infectious diseases and intoxications.

1.1.1. Morbilliviruses

The genus *Morbillivirus* belongs to the Family *Paramyxoviridae* and includes measles virus (MV) in humans and other primates, canine and phocine distemper viruses (CDV and PDV) in carnivores, cetacean morbillivirus (including the strains porpoise, dolphin and pilot whale morbilliviruses) in cetaceans, rinderpest (RPV) and peste des petits ruminants (PPRV) viruses in artiodactyls. Morbilliviruses are pleiomorphic, enveloped virions about 150 nm in diameter with a single-stranded RNA of negative sense polarity (Fenner *et al*., 1993). They require large populations of individuals (e.g. 300,000 for measles virus in humans) to be maintained endemically and induce serious, often lethal, systemic diseases in their hosts (Black, 1991). Transmission probably occurs through the inhalation of aerosolised virus, shed by infected individuals.

Since the late 1980s, at least three different morbillivirus species have caused outbreaks of lethal disease in pinnipeds and cetaceans. The existence of immunologically-naïve marine mammal communities and the introduction of morbilliviruses from other aquatic or terrestrial mammals where these viruses are endemic may be the decisive factors involved in triggering an epidemic. Factors influencing contact rates between individuals are very important in determining the spread of the disease (Harris *et al*., 2008). Biological and environmental factors such as inbreeding, high contaminant loads and limited prey availability may synergistically interact to increase the severity of the disease (Van Bressem *et al*., 2009).

1.1.1.1 Morbillivirus epidemics in pinnipeds

Phocine distemper virus (PDV) caused mass mortalities in harbour seals (*Phoca vitulina*) from Northern Europe in 1988 and 2002 (Osterhaus and Vedder, 1988; Jensen *et al*., 2002). On both occasions the epidemics started in central Kattegat (Denmark) and subsequently spread to other colonies around the northern European coast. More than 23,000 seals (an estimated 60% of the population) died in 1988 and 30,000 (approximately 47% of the population) in 2002 (Hammond *et al*., 2005; Härkönen *et al*., 2006). Clinical signs observed in seals were those typical of canine distemper and included respiratory, digestive and nervous problems and abortions. Histological findings included interstitial and purulent pneumonia and generalised lympho-depletion (Kennedy *et al*., 1989). Arctic seals may be the reservoir of the virus. Harp (*Phoca groenlandica*) and grey (*Halichoerus grypus*) seals may be the vectors (Härkönen *et al*., 2006).

An outbreak of CDV caused the death of 5,000-10,000 Baikal seals (*Phoca sibirica*) in 1987-1988 (Grachev *et al*., 1989; Mamaev *et al*., 1996). Clinical signs were similar to those of canine distemper in dogs (Grachev *et al*., 1989). It is likely that this epizootic resulted from contact with CDV infected terrestrial carnivores (Mamaev *et al*., 1996).
Several thousands of Caspian seals (*Phoca caspica*) died in Azerbaijan on the western shore of the Caspian Sea in 1997. A strain of CDV, distinct from the one found in Baikal seals and other field CDVs, was detected by polymerase chain reaction (PCR) in the brain of an adult female suggesting that this virus could have caused the epidemic (Forsyth *et al*., 1998). A confirmed CDV outbreak occurred in this species in the spring of 2000, killing more than 10,000 animals. Broncho-interstitial pneumonia and lymphocytic necrosis and depletion were common findings. Terrestrial, sympatric carnivores may be a reservoir for CDV (Kuiken *et al*., 2006).

Morbilliviruses were isolated from Mediterranean monk seals (*Monachus monachus*) during an outbreak of mortality in 1997 (Osterhaus *et al*., 1997) thought to have primarily been caused by HABs (Hernandez *et al.*, 1998; Harwood, 1998).

1.1.1.2. Morbillivirus epidemics in cetaceans

Concurrently with the first PDV outbreak in harbour seals, porpoise morbillivirus (PMV) caused mortalities in harbour porpoises (*Phocoena phocoena*) from European waters in 1988-1990 (Kennedy *et al.*, 1988, 1992a; Visser *et al*., 1993). A dolphin morbillivirus (DMV) ravaged the Mediterranean striped dolphin population (*Stenella coeruleoalba*) in 1990-1992 and again in 2007-2008 (Domingo *et al.*, 1990; Van Bressem *et al.*, 1993; Fernandez *et al.*, 2008; Raga *et al*., 2008). DMV-affected dolphins were first detected in the vicinity of Valencia, Spain, at the beginning of July 1990. The epidemic subsequently expanded to the western and eastern Mediterranean and vanished in the spring of 1992 after reaching the coasts of Greece (Bompar *et al.*, 1991; Bortolotto *et al.*, 1992; Aguilar and Raga, 1993; Van Bressem *et al.*, 1993; Cebrian, 1995). Although no precise mortality rates could be estimated for this die-off, it is likely that thousands of animals perished (Aguilar and Raga, 1993). As a relative measure of the impact, the mean school size in the epidemic core regions significantly decreased to less than 30% of the pre-outbreak number (Forcada *et al*., 1994). Serological data indicated that the virus did not persist endemically in striped dolphins and that this population was losing its immunity to DMV and was at risk from new virus introductions (Van Bressem *et al*., 2001a). Pilot whales (*Globicephala* sp.) as well as other gregarious cetacean species were suggested as reservoir and vector of the virus (Duignan *et al*., 1995b; Van Bressem *et al*., 1998, 2001a). Between October 2006 and April 2007, at least 27 long-finned pilot whales (*Globicephala melas*) stranded along the southern Spanish Mediterranean coast and the Balearic Islands (Fernández *et al*., 2008). In early July 2007 dead or moribund *S. coeruleoalba* and *G. melas* were found in the Gulf of Valencia (Raga *et al*., 2008). Morbillivirus lesions and antigen were observed in stranded pilot whales and striped dolphins. A DMV strain closely related to the virus isolated during the 1990-1992 epidemic was detected in several stranded odontocetes by PCR (Fernández *et al*., 2008, Raga *et al*., 2008). In summer-autumn 2007, over 200 *S. coeruleoalba* were found dead along the coasts of Spain. Juveniles were more frequently affected than adults, likely because older dolphins were still protected by the immunity developed during the 1990-1992 epidemic (Raga *et al*., 2008). The virus apparently reached the French Mediterranean coast in August 2007 and Italy's Ligurian Sea coast in August-November 2007 (Garibaldi *et al*., 2008). It could still be detected by PCR in dolphins stranded along the Mediterranean coast of France in May 2008 (Dhermain *et al*., unpublished observations). As both DMV epidemics started close to, or in the Gibraltar Strait and, as DMV was circulating in the North Sea in January 2007 (Wohlsein *et al*., 2007), it was suggested that DMV-infected pilot whales entered the Strait of Gibraltar and transmitted the infection to striped dolphins (Van Bressem *et al*., 2009)

In the Northwest Atlantic, PMV and DMV infections killed about 27% of the inshore population of common bottlenose dolphins (*Tursiops truncatus*) along the Atlantic coast of the US, from New Jersey to Florida in 1987-1988 (Krafft *et al*., 1995, Taubenberger *et al.*, 1996, McLellan *et al*., 2002). In 1993-1994, PMV hit coastal bottlenose dolphins along the Gulf of Mexico coasts of Florida, Alabama, Mississippi and Texas (Lipscomb *et al*., 1996). Pilot whales (*Globicephala* sp.) and offshore bottlenose dolphins may have been a source of infection for the coastal dolphins (Duignan *et al.*, 1996). Broncho-interstitial pneumonia, non-suppurative encephalitis and lymphoid depletion were commonly seen in the affected porpoises and dolphins (Kennedy *et al*., 1991, 1992a; Domingo *et al*., 1992; Lipscomb *et al.*, 1994).

Finally, an uncharacterised morbillivirus was implicated in the die-off of short-beaked common dolphins (*Delphinus delphis ponticus*) in the Black Sea in 1994 (Birkun *et al*., 1999). Morbillivirus neutralizing antibodies were also detected in the sera of 53% of 73 harbour porpoises collected along the coast of the Black Sea in 1997-1999 (Müller *et al*., 2002).

1.1.2. Herpesviruses

Herpesviruses antigenically and genetically related to members of the Alphaherpesvirinae subfamily (Family Herpesviridae, order Herpesvirales) were detected in a harbour porpoise stranded along the west coast of Sweden in 1988, in two bottlenose dolphins beached in South Carolina and Delaware (US) in 1995-1999 and in one bottlenose dolphin stranded in Tenerife, Canary Islands, in 2001 (Kennedy *et al*., 1992b; Blanchard *et al*., 2001; Esperon *et al*., 2008). Gross and histological findings included encephalitis and necrotizing lesions in multiple organ systems as well as skin lesions (Kennedy *et al*., 1992b; Blanchard *et al*., 2001; Esperon *et al*., 2008). Sequencing data suggest that these viruses are cetacean-specific and have coevolved with their cetacean hosts (Smolarek-Benson *et al*., 2006). The

virus detected in the dolphin stranded in South Carolina had nucleotide and amino acid identities of 98.9% and 96.9%, respectively, with herpesviruses identified in skin lesions from two other Atlantic bottlenose dolphins, suggesting that similar viruses may be responsible for both cutaneous and systemic infections in this species (Smolarek-Benson *et al*., 2006). Herpesviruses have regularly been detected in skin lesions from porpoises, dolphins and belugas (Martineau *et al*., 1988; Barr *et al*., 1989; Van Bressem *et al*., 1994; Smolarek-Benson *et al*., 2006). They are possibly endemic in several cetacean species and populations (Mikaelian *et al*., 1999). After infection herpesviruses become latent and are excreted periodically or continuously during the host's entire lifetime (Roizman *et al*., 1995)

1.1.3. Brucella spp.

Brucellosis is a globally distributed, zoonotic, bacterial disease of mammals that is pathogenic for the reticulo-endothelial, reproductive, musculoskeletal and cutaneous systems and which may cause generalized infection with septicaemia in humans (Corbel, 1997). The causative agents are Gram-negative bacteria of the genus *Brucella* including *B. abortus* in cattle, sheep, goats and pigs, *B. melitensis* in goats, sheep and cattle, *B. canis* in dogs, *B. suis* in pigs, *B. ovis* in sheep and *B. neotomae* in the desert wood rat (*Neotoma lepida*). In the 1990s, previously unknown strains of *Brucella* were detected by serology, histology and direct isolation in free-ranging pinnipeds and cetaceans from the Americas, Europe, the Antarctic and western North Pacific as well as in captive bottlenose dolphins (Ewalt *et al*., 1994; Tryland *et al*., 1999; Van Bressem *et al*., 2001b; Foster *et al*., 2002; Ohishi *et al*., 2004). Disorders associated with brucellosis in cetaceans include placentitis, abortion, lung infection, orchitis and non-suppurative meningoencephalitis (Miller *et al*., 1999; Gonzalez *et al*., 2002; Ohishi *et al*., 2004). To date there are four known cases of humans infected with *Brucella* spp. from marine mammals, three naturally acquired and one of laboratory origin (Brew *et al*., 1999, Sohn *et al*., 2003, McDonald *et al*., 2006) indicating the zoonotic potential of marine brucellae.

On the basis of biological and molecular characteristics, Foster *et al*. (2007) proposed two *Brucella* species in marine mammals, *Brucella ceti* and *B. pinnipedialis* with, respectively, cetaceans and seals as preferred hosts. Groussaud *et al.* (2007) further suggested that brucellae isolated from cetaceans constitute two species with different preferred hosts, i.e. *B. phocoenae* in porpoises and *B. delphini* in dolphins.

1.1.4. Leptospirosis

Leptospirosis is a zoonotic bacterial disease of global distribution that affects many species of domestic and wild animals including pinnipeds and is considered as a re-emerging disease. It is caused by *Leptospira spp.* a flexible, spiral-shaped, Gram-negative spirochete (Family Leptospiraceae) with internal flagella. *Leptospira interrogans* is found in California sea lions (*Zalophus californianus*) while *Leptospira kirschneri* is specific of elephant seals (*Mirounga angustirostris*) (Cameron *et al*., 2008). Leptospirosis in pinnipeds typically presents as an interstitial nephritis with clinical signs of impaired renal function, including dehydratation, vomiting and depression (Cameron *et al*., 2008). Infective leptospires are shed in urine. *L. interrogans*, serovar Pomona caused several severe outbreaks of renal disease in sea lions resulting in the stranding and subsequent death of hundreds of individuals along the coast of California (Vedros *et al*., 1971; Dierauf *et al.*, 1985; Gulland *et al*., 1996). The epidemic occurrences are cyclical in nature, with a distinct 3- to 4-year periodicity separated by endemic maintenance of the disease (Lloyd-Smith *et al*., 2007). Close proximity to dog parks and high dog park density are significantly associated with leptospirosis in sea lions (Norman *et al*., 2008). So far reports of this disease in free-ranging marine mammals have been limited to North America but similar outbreaks could theoretically occur in marine mammals anywhere in the world where leptospirosis is present in sympatric domestic and wild mammals. An outbreak has occurred among pinnipeds kept in captivity in the Netherlands (Kik *et al*., 2006).

1.1.5. Toxoplasmosis

Toxoplasmosis is caused by *Toxoplasma gondii,* an obligate intracellular protozoan parasite, and occurs worldwide in human and other warm-blooded animals including cetaceans (Dubey *et al*., 2003). Wild and domestic felids are the

only animals known to serve as definitive hosts but many mammals can be intermediate hosts (Miller *et al*., 2008). Infection occurs through the ingestion of contaminated food or water, or transplacentally. Free-ranging dolphins with toxoplasmosis have been reported in Europe (including the Mediterranean Sea), the Americas and the Caribbean. They presented lymphadenitis, necrotizing adenitis, myocarditis, acute interstitial pneumonia, non-suppurative encephalitis and systemic disease (Dubey *et al*., 2003; Di Guardo *et al*., 2009). Transplacental foetal infection was reported in two dolphins (reviewed in Dubey *et al*., 2003). Toxoplasmosis in cetaceans was often, though not always, associated with immunosuppression following a morbillivirus infection and/or high concentrations of environmental contaminants including PCBs (Di Guardo *et al*., 1995, 2009; Mikaelian *et al*., 2000). Feline faecal contamination flowing from land to sea through surface run-off is a likely source of infection (Conrad *et al*., 2005, Miller *et al*., 2008). The possible reactivation of latent *T. gondii* infection during morbillivirus outbreaks may synergistically increase the severity and death rate of this viral disease (Van Bressem *et al*., 2009).

1.1.6. Harmful algal blooms

HBAs are proliferations of microscopic algae that harm the environment by producing toxins that accumulate in shellfish or fish, or through the accumulation of biomass that in turn affects co-occurring organisms and alters food webs in negative ways (HARRNESS, 2005). They occur worldwide and have apparently increased in global distribution, intensity and occurrence over the past few decades (Fire *et al*., 2008). Approximately 20 of the more than 1,000 known dinoflagellate species produce toxins that may cause mortality in fish, birds and mammals (Steidinger and Baden, 1984). Domoic acid (DA) is a potent marine neurotoxin produced by diatom species of the genus *Pseudo-nitzchia*. Brevetoxins are powerful natural neurotoxins emitted by *Karenia brevis* and related species of dinoflagellates. Saxitoxin is generated by the dinoflagellates *Alexandrium tamarense* and *A. catenella*. Human intoxication is characterized by acute gastrointestinal illness with neurological symptoms that, in some cases, may lead to death. Brevetoxins, DA and saxitoxins have been implicated in the die-offs of birds and marine mammals, worldwide (Gilmartin *et al.*, 1980; Geraci *et al.*, 1989; Bossart *et al.*, 1998). Paralytic phycotoxins may have played a role in the mortalities observed in 1997 in the Western Sahara population of Mediterranean monk seal (*Monachus monachus*) (Hernandez *et al.*, 1998; Harwood, 1998). DA caused the deaths of hundreds of California sea lions along the central coast of California in 1998 (Scholin *et al*., 2000) and was associated with an unusual marine mammal mortality event along the southern California coastline in 2002 (Torres de la Riva *et al*., 2009). Brevetoxins caused the death of more than 100 coastal bottlenose dolphins along the coast of Florida in March –April 2004 (Flewelling *et al*., 2005). Primary prey items of Sarasota Bay bottlenose dolphins with elevated levels of brevetoxins are vectors for their predators during the *K. brevis* blooms (Fire *et al*., 2008).

1.2. Things to do in preparation for an epidemic

Marine mammal strandings attract a lot of public attention. Epidemics may cause the beaching of several dolphins over weeks along thousands of kilometres across borders. The degree of response of each country will depend on the existence of active stranding networks and marine mammal research groups as well as on its economic and logistic possibilities. Some countries may be able to provide most of the scientific, technical and administrative infrastructure needed to face a massive stranding while others may only offer a more reduced support or none at all. Collaboration between Member States will be a plus to effectively attend these events. The foundation of an expert Sub-Committee on Cetacean Unusual Mortalities (CEUM) within the ACCOBAMS Scientific Committee would optimise the answer to die-offs in the Agreement Zone. The CEUM Sub-Committee should ideally have the equipment described in 1.2.2.

The following guidelines are designed for an optimal response to an epidemic. Nevertheless, much can be done with a more reduced infrastructure and equipment (please see 1.2.2.11).

1.2.1. Technical and administrative infrastructure needed in each Member State to best address emergencies caused by cetacean epidemics

All Member States should at least have an on-scene coordinator body (OSCB) that would contact the CEUM Sub-Committee and any other relevant institution in the case of a suspected mass-mortality, send data to the Mediterranean Database of Cetacean Strandings (MEDACES- http://medaces.uv.es/home_eng.htm), deal with the public and media, ensure that the proper samples are taken, be responsible to obtain all necessary permits and deal with the carcasses. The OSCB should ideally depend on an existing stranding network, a natural science museum, a university or a ministry (Agriculture, Environment, Fisheries). It should collaborate with existing national entities

related to marine mammal stranding such as active stranding networks and marine mammal research groups, wildlife conservation and rescue centres, aquaria and oceanaria, coastguards, park officials, navy and local authorities.

The OSCB basic technical and administrative infrastructure should include:

- A stranding hotline telephone, dedicated to record any stranding occurring along the coast and operating 24 hours, seven days a week;
- A computer with internet access;
- A printer;
- Portable telephones;
- A GPS to register stranding locations;
- Digital cameras;
- DVD reader;
- A specialized marine mammal library;
- A website describing the activities of the OSCB as well as the names of the persons in charge and to be contacted in the event of an epidemic;
- A database on cetacean mortality events
- Educative material;
- A centrifuge to spin blood samples;
- A large fridge to keep samples at 4°C;
- A –80°C freezer to store samples for longer periods of time.

1.2.2. Equipment list

The following is an optimal equipment checklist to face stranding of live and dead animals (Geraci and Lounsbury 2005; Raverty and Gaydos, 2007). However, much can still be done with less material and infrastructure (§ 1.2.2.11.).

1.2.2.1. Crowd control, public relations

- Plastic tape and pylons to cordon off necropsy site;
- Signs: WARNING—PUBLIC HEALTH HAZARD—DO NOT ENTER;
- Educative material on stranding and epidemics as well as on the stranding network;

1.2.2.2. Recording material

- Waterproof pencils;
- Metal clipboards, waterproof labels;
- Data forms, necropsy and collection protocol forms;
- Camera and film, extra batteries, video camera with additional memory cards;
- Tape measure (metric), at least 20 meters long (plastic and metallic);
- Hoist/crane, scales to record organ weights (0,1-10kg);

1.2.2.3. Animal relief

- Zinc oxide;
- Blankets and towels;
- Shovel (to dig pits for fins and tail);
- Ice packs (to keep the extremities cool);
- Tarpaulins;
- Foam mattresses;
- Water sprayers
- Inflatable rescue pontoon system
  http://www.jwautomarine.co.uk/images/SlideSh/show024/default.htm
  http://www.jwautomarine.co.uk/pr_sb.htm;
- Thermal space blankets (for warming or cooling);

1.2.2.4. Emergency medical supplies

- I.V. Fluids and infusion sets (droppers, 10& 60 drops/min.);
- Basic diagnostic set (stethoscope, thermometers);
- Stimulants;
- Tranquillizers;
- Adrenalin;
- Steroids.

1.2.2.5. Euthanasia[8]

- Needles for large animals;
- Sedative: midazolam (0.02 mg/kg);
- Barbiturate: Large Animal Immobilon (Etorphine) administered intramuscularly is recommended (see footnote 1);

1.2.2.6. Necropsy

- Rope, at least 20 meters, blankets, stretchers to move carcasses, if necessary;
- Standard necropsy instruments. Multiple scalpel handles, scalpel blades, scissors, forceps and knives;
- Knife sharpener, if possible in secure pack;
- Flensing knives and hooks with appropriate sharpening tools, chain saw, axe, or reciprocating saw to cut through the cranium, chest or vertebrae;
- Hammers, chisels and handsaws;
- Retractors of various sizes and shapes. Self-retaining retractors with one or two movable arms mounted on a slide bar are most useful;
- Sterile instruments for culture collection;
- Whirlpacks;
- Jars, vials;
- Buckets;
- Flashlights with extra batteries and light bulbs;
- Containers (from vials to garbage cans) for sample collection, including ice chest, dry ice and if possible liquid nitrogen;
- Gas generator and flood lights with extra bulbs and gasoline;
- Lights;
- Portable or electric circular saw;
- Accessible water supply with hose;
- Buckets;
- Garbage bags, dish soap, paper towels for clean-up.

1.2.2.7. Specific sampling (histology, microbiology, HBAs)

- 10% neutral buffered formalin;
- 4% buffered glutaraldehyde;
- 20% diethyl sulfoxide (DMSO) saturated saline solution for genetic analysis, in vials;
- Isopropanol alcohol, for contaminant sampling;
- Needles and syringes;
- Heparinized syringes;
- Culture vials for virology and bacteriology;
- Transport medium for bacteriology and virology;
- RNA later (Ambion; http://www.ambion.com/techlib/resources/RNAlater/index.html)
- Sterile swabs;
- Sterile urine cups;
- Glass slides;
- Serum tubes for blood and urine collection and gas burner to sear organ surfaces and sterilize scalpel blades;

[8] Legislation regarding euthanasia and the use of euthanizing agents may vary between countries. Local laws should be checked before deciding which agent is to be used. The OSCB should obtain an authorization from the local authorities to perform euthanasia on cetaceans before life-strandings occur.

- Culture vials for bacteriological and virological analysis;
- Aluminum foil and plastic bags for freezing tissues;
- Coolers for samples refrigeration;
- Plankton net.

1.2.2.8. Personal

- Protective clothing for staff and volunteers (hats, boots, protective wear, wet and dry suits);
- Coveralls, aprons, gloves, caps, disposable masks, protective eye and head gear;
- Hand soap and towels;
- Disinfectant;
- First aid kit.

1.2.2.9. Large equipment

- All terrain vehicle with trailer;
- A boat to reach floating dead cetaceans;
- 30m^2 walk-in fridge;
- A wet laboratory to carry out the necropsies.

1.2.2.10. Dispatch

- CITES permits;
- Contact airlines that may dispatch the samples and ask where to buy IATA-approved containers. They will be required to send samples by airplanes.

1.2.2.11. Minimal equipment

The following minimal equipment also permits to alleviate the suffering of a stranded live dolphin and take valuable biological and microbiological samples from freshly dead dolphins:

- Recording material;
- Camera;
- Mobile phone;
- Buckets;
- Blankets;
- Water sprayer;
- Zinc oxide, shovels;
- Gloves, plastic boots and masks;
- Wide plastic sheets;
- Butcher knives;
- Butcher saws;
- Scalpel and scalpel blades;
- Vials and jars;
- Ropes.

1.2.3. Capacity building

Different levels should be considered for capacity building according to the persons concerned i.e. scientists of the OSCB, volunteers and public.

1.2.3.1 Scientists

Scientists of the OSCB with no previous knowledge of marine mammal die-offs should receive specific training to attend live animals, do necropsy, take samples, manage the public and dispose of the carcasses. It would be recommendable that the proposed CEUM Sub-Committee and/or Member States with a large experience in cetacean stranding arrange training courses for scientists of the nascent OSCBs with less practice. Training in rescue

techniques and stranding are also offered by several NGOs and marine mammal centres in Spain, Italy, the UK and other European countries. Scientists may start to build a specialized marine mammal library including valuable books such as 'Marine Mammal Ashore, a Field Guide for Strandings' (Geraci and Lounsbury, 2005) and 'Stranded Cetaceans: Guidelines for Veterinary Surgeons', RSPCA (1997). Free scientific papers on infectious diseases and marine mammal mortalities available on the World Wide Web and specifically at pubmed (http://www.ncbi.nlm.nih.gov/pubmed/) should be downloaded and printed. International workshops on cetacean epidemics should be planned within the Member States.

1.2.3.2. Volunteers

Volunteers should be given a formation allowing them to efficiently help during outbreaks of mortality. Workshops on the general biology of dolphins and whales, the reasons why they strand and the pathogenic agents they may harbour, should be organized. Volunteers should in particular be informed of the potential health risks stranded marine mammals represent. Each volunteer should be given a role according to his/her personal skills. Stranding simulations with inflatable plastic whales may be a good idea to give participants a feel how a real event might evolve.

1.2.3.3. Local government officials

Leaflets describing the basic biology of cetaceans and explaining stranding events and epidemics, and how to react to them, should be written, printed and distributed to local government officials. These leaflets should provide the hotline for strandings as well as the names of the people in charge. Members of the OSCB may arrange talks on marine mammal epidemics for government officials and distribute educational material at this occasion.

1.2.3.4. Public

Booklets for children addressing the basic biology of cetaceans and the possible reasons for their die-offs should be written, printed and distributed to kindergartens and local schools. Posters on the same topics and including the health risk posed by marine mammal strandings should be designed and distributed in schools, libraries, museums, tourism information centres, national parks, universities, etc. National or local companies and businesses may be keen to offer support for printing this material. A website or a newsletter detailing the activities of the OCSB would be useful to help the general public to understand its activities.

1.3 Actions to take during an epidemic event

Several situations may occur during an epidemic:

- Single stranded dolphins may be found dead or agonizing on different beaches
- Several dead dolphins stranded on the shore
- Dead and live cetaceans stranded simultaneously on a beach

In all cases, excellent coordination between the OSCB staff, the proposed CEUM Sub-Committee and other organizations specialised in these events will be the key for a successful answer. The protocols given below are broadly based on Geraci and Lounsbury (2005) and the Irish Whale and Dolphin Group (2007) (http://www.iwdg.ie/content.asp?id=31). The second edition of 'Marine Mammal Ashore: A Field Guide for Strandings' provides extensive information on how to deal with stranded, live or dead dolphins and whales and one or more copies should be in the library of all bodies involved with cetacean strandings. It would be wise to carry one copy to the field.

1.3.1. Protocols for intervention on site

1.3.1.1. Live cetaceans stranded on the beach

The event should be evaluated and attempts made to determine the species and appraise the length of the specimens. The number of stranded dolphins of each species should be estimated. Live animals should be stabilized to ensure that they can breathe and will not overheat or become too stressed:

- Support the animal in an upright position if possible, digging trenches under the pectoral fins;
- Keep the animal moist by covering it with wet blankets or towels, sprayed or doused with a constant supply of water;
- Protect damaged skin with zinc oxide;
- Do not cover or obstruct the blowhole and make every effort to keep sand and water away from the blowhole;
- In sunny weather try to provide shade for the animal by erecting a tarpaulin above it;
- In very cold or windy weather, try to erect a windbreak around the animal;
- If the animals are in the surf zone, move them into deeper waters or shift them so they are perpendicular to the water's edge, with the head facing land;
- Caution: care should be taken around the tail fluke as a thrashing cetacean can maim or kill. Also minimize contact with the animal (use gloves and mask if contact is necessary) and avoid inhaling the animal expired air;
- All noise, contact and disturbance around the animal must be kept to a minimum. Erect a rope barrier to cordon off the area (apart from essential personnel caring for the animal) and ask the local authorities to assist with crowd control at the scene;
- When available, a coastguard or beach-master should be appointed to liaise with media and control onlookers, and to ensure that the veterinary and rescue teams can get on with the job, without unnecessary interference;
- Contact all people and organizations that have shown interest in helping rescue live stranded cetaceans;
- Evaluate the health of the animal according to the following parameters:
    - presence of obvious injuries;
    - entangled nets or ropes around flukes, fins and beak;
    - breathing pattern:
      small cetaceans (eg. porpoise or common dolphin): Normal breathing rate = 2-5 breaths/min;
      medium-sized cetaceans (eg. pilot whale): Normal breathing rate = 1 breath/min;
      large Cetaceans (eg. sperm whale): Normal breathing rate = up to 1breath per 20mins;
    - skin integrity;
    - nutritional status;
    - heart rate (from 30 to 100 beats/ minute in bottlenose dolphin) using a stethoscope for small dolphins and a hand firmly placed under the axillary region for larger cetaceans;
    - behavioural criteria: alert (responsive to environment stimuli: palpebral reflex), weakly responsive (responsive only after much stimulation), non-responsive (not responsive to noise or touch);
    - presence of blood in the mouth or blowhole (critically poor health);
    - core body temperature: normal range 36.5 to 37°C. Critical hypothermia: below 35.6°C; critical hyperthermia above 40°C;
- When the animal seems healthy, attempts should be made to re-float it and guide it to deeper waters by lifting with a tarpaulin or a stretcher, by dragging with slings or using a rescue pontoon system. This should only be attempted when a sufficient number of experienced people are available (e.g. 6 for a medium-sized bottlenose dolphin). Re-floats should be attempted on rising tides. Once the animal is towed back to the sea, it should be supported, with its blowhole kept above the surface. Acclimation is complete when the whale is able to surface on its own to breathe. This may take several hours and, in cold water, a relief team should be available. A mother and calf should be acclimated together. If several cetaceans beached together they should be released together. All supporting devices should be easy to remove;
- Under no circumstances should attempts be made to re-float calves that are likely not weaned;
- When the animal is unfit for immediate release the other options should be considered i.e. rehabilitation or euthanasia. Rehabilitation will only be possible when a facility exists in the country and is reachable by road in no more than two hours;
- If the animal cannot be rescued, humane killing should be considered. Euthanasia is an option for odontocetes and small whales and should be done through the administration of 'Large Animal Imobilon' (see footnote 1), possibly after sedation. Larger whales should be allowed to die naturally.

1.3.1.2. Dead whales and dolphins

- Autopsy on the beach is a valid option when strandings occur in remote areas, away from public presence, do not threaten human health and weather conditions are favourable. It is recommendable for large dolphins and

whales or when no transport is available. If feasible, the animals should be placed on a wide plastic sheet before the autopsy is undertaken. Freshly dead dolphins should be given priority. When the day is hot, attempt to collect the basic information and then quickly open the specimen and collect samples for virology, bacteriology, parasitology and HBA research.

- When feasible, dolphins and porpoises should be transported to an appropriate facility for complete necropsy. All endeavours should be made to retrieve the animal in as short a time as possible to avoid deterioration of the body before analysis. While awaiting necropsy, specimens should be kept in a cold room.

- In all cases, photographic documentation is strongly recommended.

1.3.2. Protocols for collection, transportation and storage of specimens and samples

1.3.2.1. Protocols for sample collection

Prior to sample collection, some basic data should be collected in order to be able to know indispensable biological parameters. Recording the whale/dolphin condition is important to determine which samples should be given priority. Only the animals considered fresh or slightly decomposed are worth sampling for microbiology. All samples collected for microbiology should be taken as aseptically as possible. Ideally, the necropsy should be carried out by an experience scientist. Notes should be taken by an assistant.

After collection of the basic data, the body may be opened, preferably on a wide plastic sheet or on a necropsy table. All instruments necessary, collecting, bags, jars and vials with or without liquids should be at hand before making the first incision. An assistant should label the containers and take notes and pictures.

The protocols provided here below and the sample priority and field tissue checklist provided in the Annex will be useful to make sure that all the necessary samples are collected and preserved adequately.

1.3.2.1.1. Basic Data Protocol

- Investigator (name, telephone, affiliation, address, e-mail):

- Date:

- Location of stranding:

- Presence of other dead aquatic animals:
  - Species:
  - Number (estimation):
- Indication for an algal bloom: YES/NO

- Field number:

- Species[9]:

- Sex[10]:

- Standard body length[11]:

- Condition:
  - alive
  - fresh

[9] Species identification should be done by qualified persons. Ideally a picture of each specimen with its field number should be taken.
[10] A picture of the genital region with field number will help to confirm the sex.
[11] Precise how it was taken (measurements should be parallel to the dolphin body, e.g. total length from snout to fluke notch).

- early decomposition
- advanced decomposition
- mummified

- Evidence for human interactions: YES/NO
  - Net marks
  - Knife cuts
  - Wounds caused by vessel strikes
  - Description-pictures

- Presence of skin lesions and wounds: YES/NO.
  - Description – pictures
  - Collect samples in formalin, DMSO and, if possible, freeze at –80°C

- Lactating: YES/NO

1.3.2.1.2. Specific sample collection [12]

1.3.2.1.2.1. High priority samples

Reproductive tract

Ovaries and testes should always be examined, weighed, photographed and collected in 10% formalin (4% end concentration) to assess sexual maturity. The presence/absence of corpora albicantia and a corpus luteum should be recorded. Uterus should be opened to check for a foetus. The latter should be measured, weighed and sexed and, if small, conserved in formalin. Presence of sperm in the epidydimis should be evaluated. A piece of at least 1x1x1 cm of both testes should be collected in formalin. The following questions may be answered in the field if time permits otherwise in the lab after addressing the mortality event.

- Ovaries:
  - presence of corpus albicans: NO, YES
  - presence of corpus luteum: YES, NO

- Foetus in uterus: YES, NO
  - sex
  - length
  - weight

- Testes: YES/NO
  - Right:
    presence of seminal fluid
    length
    weight

  - Left:
    presence of seminal fluid
    length
    weight

Virology and serology

- The following organs are targeted by morbilliviruses and herpesviruses and should be carefully examined for any changes and lesions. Use gloves, wash them frequently and change them between each specimen:

- Lungs
- Spleen
- Liver

[12] Basic and advanced data protocols are also available at the Medaces website: http://medaces.uv.es/home_eng.htm

- Lymph nodes
- Kidneys
- Brain[13]
- Thymus
- Heart
- Skin

- Document, describe and take pictures[14] of any change in organ gross morphology. Take pictures of skin lesions.

- Ten grams or 2x2x2cm of each organ should be conserved on ice and then frozen at –80°C for virus isolation. Each sample should be carefully labelled. When no freezer or liquid nitrogen is available, cut tissue samples to ≤ 0.5 cm in any single dimension and preserve in 'RNA later' (Ambion) for PCR studies. Once submerged in 'RNA later' samples may stay at room temperature for a week. If a longer delay is expected then freeze them at –20°C or –80°C after a night at room temperature (no more than 25°C).

- Preserve small samples of the previously mentioned organs in 10% formalin and 20% DMSO for histo-pathological and molecular studies.

- Extract 5-10 ml blood directly from the heart or major blood vessels after disinfecting the surface with alcohol and put on ice. You may attempt to centrifuge the blood and take the supernatant before freezing to avoid further hemolysis.

- Take some pleural, peritoneal and pericardial fluids, urine, fluid from vesicles in sterile tubes, keep on ice and store at –80°C.

<u>Bacteriology</u>

- Document, describe and take pictures of any change in organ gross morphology.

- Collect 5-10grs samples from the kidneys, testes, uterus, placenta and foetus (if available), mammary glands, spleen, eventual subcutaneous abscesses, keep on ice and refrigerate at –4°C or freeze at –80°C if long delays are unavoidable (> 24h) before further analysis. When no freezing facilities are available, smaller samples should be kept in DMSO.

- Preserve 1x1x1 cm samples of the same organs in formalin and DMSO.

- Take a blood sample from the heart and process as described above.

- Collect pleural and peritoneal fluids, urine and pus from abscesses and store half in aerobic containers and half in anaerobic containers. Keep on ice and then freeze at –80°C if a laboratory is not at hand.

- If feasible (a laboratory is ready to receive and analyse the samples in a short time) take swabs from the eyes, blowhole and throat and place them in an appropriate bacterial medium transport and refrigerate.

<u>Protozoans</u>

- Document, describe and take pictures of any changes in organ gross morphology.

- Collect samples of the following organs, keep on ice, refrigerate at –4°C and send with cold pack to a specialized research institute if possible. Otherwise preserve small samples in 10% formalin and DMSO:
    - Brain
    - Heart
    - Skeletal muscles

[13] If the skull is to be preserved for a museum collection, separate the head from the body and introduce a small spoon into the foramen magnum to collect a piece of brain/cerebellum. An electric saw could be used to cut a sharp-edge window in the skull. The two pieces could be later glued together.

[14] Always place a piece of paper with specimen field number close to the lesion you wish to photograph, to be able to identify its origin when the event is over.

- Lymph nodes
- Spleen
- Thymus
- Lungs
- Foetus
- Placenta

- Take a blood sample from the heart and process as described above.

Biotoxins

- Collect 5 to 10ml of blood in a heparinized syringe, separate the serum and freeze for shipment. If not possible, keep he sample on cold packs and ship to the lab. As several toxins may cause marine mammal mortalities and concentrate in different organs, it is recommended to take a wide range of samples including:
  - 50 grs of liver, kidney, lung (cranial pole), stomach contents, faeces, brain as well as bile and at least 3ml of urine. These samples should be kept on ice until frozen at –20°C.
  - Samples of brain, lungs and upper respiratory tract should also be preserved in 10% formalin.

- Collect water samples, keep on ice until frozen

- Collect fish and plankton with a plankton net, keep on ice until frozen

- Record any other aquatic animal mortality occurring concurrently with the cetacean outbreak of mortality

1.3.2.1.2.2. Intermediate priority samples

- When possible document and describe any change in the gross morphology of all organs not mentioned in 1.3.2.1.2.1. The following should always be examined:
  - Adrenals
  - Tonsils
  - Stomach
  - Intestine
  - Pancreas
  - Bladder

- Collect samples and store according to the procedures described in 1.3.2.1.2.1. for virology and bacteriology.

- Check the mouth, tongue, teeth and/or baleen plates, document and take pictures of any abnormalities and collect samples for virology and bacteriology as described in 1.3.2.1.2.1.
  - Description

- Examine the genital slit, penis (whole) and vagina (whole) for the presence of warts or vesicles, describe and take samples for virology as described in 1.3.2.1.2.1.
  - Warts: YES/NO
    Describe and take pictures
  - Vesicles, ulcers: YES/NO
    Describe and take pictures

1.3.2.2. Protocol for transportation and storage

All fresh samples should be kept on ice or cold packs, away from the sun while waiting for further processing. Upon arrival in the laboratory, they should be frozen at –20°C or –80° C according to the above mentioned protocols. Storage should be organized in a way that samples are easily found when the freezer is full which may be quite a task! Records should be kept of any sample location. Contact the local CITES Management Authority (http://www.cites.org/common/directy/e_directy.html) to know the requirements to obtain permits to export cetacean samples.

1.3.3. Carcass disposal

Carcass disposal may depend on the laws of each Member State. In some countries local authorities are responsible for the disposal of dead cetaceans. When it is not the case the OSCB should develop plans in advance in accordance with national authorities. Their feasibility should be discussed with the bodies that should intervene to help with carcass disposal (coastguards, navy, landfill site owners). The costs of each plan should be established. Here are some recommendations extracted from Geraci and Lounsbury (2005) and a background document from South African National Parks (online http://www.sanparks.org/about/news/2006/july/whale.php).

1.3.3.1. Let it lie

In uninhabited areas the carcass may be left on the beach. Weather, tide and scavengers will do the work. Before leaving the carcass baleen or teeth should be extracted. Open the abdomen and thorax to prevent any bloater decomposing in the sun. Care should be taken with large whales.
Specimens that were euthanized represent a risk to scavengers and should be buried, taken to a sanitary landfill, composted or destroyed by incineration

1.3.3.2 Bury it

Burial of small cetaceans in a sandy beach may be relatively easy after cutting the carcasses. Burial of large cetaceans requires heavy equipment and experienced operators. Environmental damage and disturbance should be considered. The burial site should be above the water table to avoid contamination with body fluids. The hole should be deep so that the carcass is buried under at least one or two meters of earth.

1.3.3.3. Burn it

Burning the carcass reduces the mass and volume, allowing for whatever is left over to be cut up and removed either into the sea or to a landfill site. The burn will involve stacking a cremating pyre of wood around the whale and using solid accelerants in the slits of the blubber, burning it for a few days and then assessing the situation. Anti-oil pollution solvents may be used to mop up the resulting oil effluents.

1.3.3.4. Tow it out to sea

The carcass may be towed out to sea, providing it is released far enough offshore (about 80 km or more) so that currents and winds do not bring it back, it is clear of a shipping lane and has enough ballast to sink. The carcass should be cut opened to avoid bloating and favour sinking. Collaboration with scientists studying ‘whale falls’ (Hagg, 2005) would be beneficial. Before considering this option, contact the relevant authorities (navy, coastguards) and ask their permission and requirements to minimize problems with boat traffic.

1.3.3.5. Compost it

Carcasses up to 640 kg may be placed in a composting bin and covered with a ‘bulking agent’ such as sawdust or straw, high in carbon. As anaerobic microorganisms break down the carcass, fluids and odorous gases diffuse into the bulking material where they degrade to carbon dioxide and water. A properly functioning composting unit requires minimal maintenance, emits little odour, has no effects on groundwater, reaches internal temperatures high enough to kill pathogens and break down chemical euthanasia agents. Please see the website of the Minesota Department of Agriculture for more details www.mda.state.ms.us.

1.3.4. Communication management

At least one person of the OSCB should be in charge of communication management. His/her job would include calling the local authorities, giving the volunteers their tasks, write down the name, coordinates (telephone number, e-mail) and tasks of the participants, manage the public and contact other facilities that may help with the stranding event, animal rescue and carcass disposal.

1.4. Activities to implement after the epidemic is over

1.4.1. Debriefing meeting

Organize a debriefing meeting with all the people involved in the stranding and ask them their opinion on the event, the number of dolphins they counted and attended, the presence of other dead aquatic animals on the beach, if the response to the stranding was adequate in their opinion, what material was missing. Thank all volunteers for their help and distribute any new information material and stickers.

1.4.2. Preliminary report

Write an initial report as soon as possible. Points to summarize in the report should include the following (Geraci and Lounsbury, 2005):

- Date and location of the stranding, type of beach;
- Nature, timing, effectiveness of the initial response;
- Account of the scene as described by the team:
  - species involved and number of specimens per species,
  - pattern of stranding,
  - presence of other dead or sick aquatic animals,
  - cetacean condition,
  - indication for an epidemic,
  - environmental conditions.
- Necropsy findings;
- Specimens collected, place where they are stored, condition for storage;
- The actions taken and reason for decisions:
  - intended response plan,
  - impediments to implementation,
  - eventual action.
- Additional information:
  - photographs, maps, drawings,
  - reports from independent groups (police, coastguards, stranding networks, rehabilitation facility),
  - Things to be improved.

1.4.3. Media communication and alert

Write a brief note on the event for the media. Alert the media and public for the possibility of more cetacean strandings on every beach and encourage them to report.

1.4.4. Contacts

Contact the laboratories that will analyse the samples and coordinate for sample dispatch according to the airline procedures. Make sure that somebody will collect the samples at their arrival and that the person in charge is not on holidays at the time you send the samples. Keep telephone contact until you are assured that the samples arrived and were properly stored.

1.4.5 Follow-up

Ask for a follow-up of the analysis and prepare a manuscript on the findings together with all involved institutions.

2. CONTINGENCY PLAN DRAFT

In the Mediterranean Sea, epidemics of morbillivirus have caused the death of thousands of striped dolphins in 1990-1992 and in 2007 as well as mortalities in long-finned pilot whales (Aguilar and Raga, 1990; Fernandez *et al*., 2008; Raga *et al*., 2008; Van Bressem *et al*., 2009). An uncharacterised morbillivirus was also detected in two short-beaked common dolphins stranded along the coast of Crimea in 1994 during an outbreak of mortality (Birkun *et al*., 1999). Herpesviruses, *Toxoplasma* spp. and *Brucella* spp. have been identified in odontocetes stranded along the coasts of Spain (Mediterranean Sea and Canary Islands) and Italy (Di Guardo *et al*., 1995, 2009; Van Bressem *et al*., 2001b; Esperon *et al*., 2008). Paralytic phycotoxins may have been responsible for the death of several

Mediterranean monk seals in the Mauritanian colony (Hernandez *et al.*, 1998, Harwood, 1998). Thus, Member States should be ready for the eventuality of cetacean mortalities in their waters due to viruses, bacteria, protozoans and HBAs. The development and strengthening of existing national and regional stranding networks will be key to properly address these mortalities. Importantly, data on strandings along the coasts of the Black and Mediterranean Sea as well as the contiguous Atlantic waters should be sent to MEDACES (http://medaces.uv.es/home_eng.htm) set-up in 2001 to co-ordinate all national and regional efforts for riparian countries. The establishment of a CEUM Sub-Committee within the ACCOBAMS Scientific Committee would further improve answer to strandings by facilitating coordination between Member States and helping with infrastructure and capacity building. The foundation of CEUM Working Group that would communicate by e-mail would facilitate information diffusion.

2.1 OSCB

An efficient contingency plan will be based on the foundation of a national OSCB that will be responsible for the activities and decisions related to unusual mortality event as well as on timely relaying information on their occurrence to the Member States and to the suggested CEUM Sub-Committee. The easy and open communication between OSCBs will help determine when a die-off is underway, ensure a timely and adequate intervention and, ultimately, uncover the cause of the die-off and explore environmental factors that may have enhanced its severity. Minimal personal of an OSCB should be one scientist, preferably a marine mammal research veterinarian with good knowledge in the biology of cetaceans.

2.2.1. Team

2.2.1.1. Administrative support team

At least one person should be in charge of the administration of the OSCB. His/her responsibilities should include:

- Coordination with local authorities;
- Communication with media and public;
- Development of education activities and material;
- Management of volunteers;
- Building of a website;
- Finance management.

2.2.1.2. Scientists

A biologist and a veterinarian, both ideally with experience with cetaceans, should be appointed by the OSCB. Their responsibility should include the following items:

- Develop a stranding network that can react quickly to cetacean mortality events;
- Develop protocols for attending strandings and for the collection of tissues for microbiology, parasitology and HBA testing;
- Prepare the material necessary for attending a die-off (everything should be ready and at hand for instant leave);
- Provide field staff and build capacity;
- Recruit and manage volunteers;
- Timely intervention and incident control coordination: an educated decision on response level (equipment and personnel);
- Coordination with other similar networks within and outside the Member States;
- Adequate decision regarding the fate of live-stranded cetaceans (release, rehabilitation, euthanasia);
- Collection of biological data and pictures;
- Necropsy of dead cetaceans;
- Collection of samples;
- Contact with laboratories that will process the samples;
- Contact with the authorities that will deliver CITES permits;
- Contact with the airlines that will transport the samples: ask for their specific requirements for the packaging and dispatch of biological materials;
- Prepare a protocol for packing and dispatching biological material;
- Send the samples;
- Carcass disposal in agreement with national regulation.

2.2.1.3. Volunteers

Volunteers should be recruited to help with strandings. They may have distinct backgrounds and personalities and should be given tasks according to their respective skills.

2.2 Memoranda of Understanding with Collaborators

Memoranda of understanding should be established with other institution and laboratories willing to help at the occasion of an outbreak of mortality. Laboratories (bacteriology, virology, parasitology, HBA research) should be asked to send specific protocols for sampling, preserving and sending the samples. Ideally they should provide the vials, fluids and other material required for sampling. Otherwise they should specify the material needed for sampling and the firm where to buy it.

2.3 Get ready to detect an epidemic and unusual mortality events

Regular visits to the beaches by scientists and volunteers of the OSCB should be organized, so that a baseline for a 'normal' stranding number may be established by species, geographic location, season of the year etc. All cetaceans that are fresh or moderately decomposed should be necropsied and samples sent for parasitological, bacteriological and virological to get an idea of the common macro- and micro-fauna in these populations. The OSCB should make sure that the media have the hotline phone number, distribute posters on epidemics in public places and regularly communicate with coast guards, fishermen associations and any person or organization susceptible to register unusual mortalities of marine mammals.

♦ Criteria pointing to the occurrence of an unusual mortality event[15] are:
- Marked increase in the magnitude or a marked change in the nature of morbidity, mortality or strandings when compared with prior records;
- A temporal change in morbidity, mortality or strandings is occurring;
- A spatial change in morbidity, mortality or strandings is occurring;
- The species, age, or sex composition of the affected animals is different than that of animals that are normally affected;
- Affected animals exhibit similar or unusual pathologic findings, behavior patterns, clinical signs, or general physical condition (e.g., blubber thickness);
- Morbidity is observed concurrent with or as part of an unexplained continual decline of a marine mammal population, stock, or species.

♦ The following criteria for defining an epidemic are:
- It is unexpected;
- It involves the stranding and death of unusual large number of cetaceans from one or several species;
- It may start in one country and progress to others;
- It may last for several months;
- It may recur;
- It demands an immediate response.

2.4. Get ready to attend an epidemic

When an epidemic is suspected, the OSCB should get in contact with national and international collaborators and the suggested CEUM Sub-Committee, and call its volunteers as soon as possible. Once ready, the OSCB scientists should go at once to the site of stranding taking all the necessary equipment already pre-packed. They should give

[15] Source: http://www.nmfs.noaa.gov/pr/health/mmume/criteria.htm

volunteers their tasks before attending the animals. The administrator should liaise with the local authorities, public and media.

2.5. Determine the end of the event

The end of the epidemic may be difficult to pinpoint but in the case of morbillivirus infection will likely be gradual. Collaboration between all Member States will be essential to estimate the end of the mortality event.

3. OUTLINE OF A PROGRAMME TO BUILD CAPACITY

Capacity building is a prerequisite to an efficient die-off response. It should concern the staff of the OSCB, volunteers, coastguards and navy officials, fishermen and the general public (please see § 1.2.3.). The following programme outlines the steps that may be taken to realize this target.

- Organization of annual workshops on cetacean epidemics and infectious diseases for the staff of the OSCBs. National and international experts of morbilliviruses, *Brucella* spp. and other bacteria as well as of HBAs should ideally be invited to participate;
- Organization of training courses on cetacean strandings, infectious agents and sample collection for the staff of the nascent OSCBs. These training courses may take place at the OSCB, CEUM facilities or at the laboratory of national and international stranding networks;
- Organization of national meetings with other relevant bodies related to strandings (universities, coastguards, oceanaria, etc) and presentation of documents on cetacean epidemics and diseases;
- Acquire capacity building material (books, papers, reports, CDs, DVDs, protocols) from other stranding networks, NGOs and scientists;
- Development of a library dedicated to marine mammal strandings and epidemics;
- Communication with other OSCBs;
- Preparation of leaflets on the biology of cetaceans and the reasons of strandings and mass die-offs targeting the general public;
- Preparation of children booklets and posters on whales and dolphins and stranding events.

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