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## Report of the Workshop on Harmful Algal Blooms (HABs) and Associated Toxins, 7-8 May 2017, Bled, Slovenia

International Whaling Commission



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### Report of the Workshop on Harmful Algal Blooms (HABs) and Associated Toxins

Members: Rowles (Co-chair), Hall (Co-chair), Baker, Brownell, Cipriano, Glibert, Gulland, Kirkpatrick, Paerl, Schwacke, Simeone, Stimmelmayr, Suydam, Trainer, Van Dolah.

#### 1. CONVENERS' WELCOME AND INTRODUCTIONS

Rowles and Hall welcomed the group to Bled, Slovenia and the participants were introduced. The meeting was held at the Hotel Golf in Bled, Slovenia from 7-8 May 2017. A list of participants is given as Annex A.

#### 2. APPOINTMENT OF CHAIR AND RAPPORTEURS

Rowles (US) and Hall (UK) were appointed co-chairs of the meeting and Simeone was appointed rapporteur.

#### 3. REVIEW AND ADOPT AGENDA

The agenda was adopted by the workshop participants. The adopted Agenda is given as Annex B.

#### 4. INTRODUCTION AND BACKGROUND

#### 4.1 Harmful algal bloom dynamics and drivers

The majority of algae in marine and fresh waters are not only beneficial but also necessary to the functioning of aquatic ecosystems. They form the base of the food webs and it is this microscopic life that all aquatic life ultimately depends upon for food. Yet, a comparatively small subset of the total known microscopic algal species may produce toxins that directly or indirectly interfere with the growth or survival of other organisms, or may alter ecosystem functioning through their large accumulation, as blooms. These algae are known as harmful algae, and their associated proliferation events are referred to as harmful algal blooms (HABs). HABs are found in all parts of the world, in all types of waters and they



Fig. 1. Harmful algal blooms (HABs) are affected by multiple factors, both natural and anthropogenic. HABs, in turn, have direct (including through *in utero* exposures) and indirect effects on cetaceans.

are expanding both in global distribution and in their harmful impacts. Not all harmful algae cause large blooms; some can pose an ecosystem threat even when their relative abundance in the overall algal assemblage remains low compared to other algae. The HAB problem is significant. It poses a major threat to public health, ecosystem function, fisheries sustainability and health, and increasingly may be a threat to cetacean health (Fig. 1).

There are many species of harmful algae, each of which may have different ecological drivers and ecological impacts, resulting in different HAB species being predominant in different parts of the world or at various times. Although some of the factors contributing to the global expansion are natural, such as biological species dispersal, many others are considered to be driven by ever-increasing human population growth and associated urbanization, agricultural and industrial development (Paerl 1988; Nixon 1995; Boesch *et al.*, 2001, Anderson *et al.*, 2002, Glibert *et al.*, 2005, 2014, Heisler *et al.*, 2008). Human activities have altered the nutrient regimes of coastal waters tremendously, primarily as a

result of increased applications of chemical fertilizers and generation of wastewater which ultimately enter into the aquatic environment through direct runoff, groundwater or atmospheric deposition (Glibert *et al.*, 2014). Increased nutrient inputs to enclosed and nearshore ecosystems have resulted in widespread coastal eutrophication throughout Europe, U.S and Asia (Paerl 1988; 1997). These nutrients provide the fuel on which these HABs may grow. There are many reports of increases in HABs associated with eutrophication or nutrient loading (e.g., Anderson *et al.*, 2002, Heisler *et al.*, 2008), but the complexity of the relationship is far from understood. It is clear that nutrient inputs yield changes in algal biomass (Ryther and Dunstan 1971), but the impact of these changes on biodiversity are far more complicated. The success of HABs lie at the intersection of the physiological adaptations of the harmful algal species and/or strain (population), the environmental conditions, and interactions with co-occurring organisms (both biogeochemically and trophodynamically) that alter abiotic conditions and/or aggregate or disperse cells (or can alter abiotic conditions in a favourable or unfavourable manner), in turn promoting or inhibiting their growth (Glibert and Burford 2017).

In addition to increases in nutrient loading, changes in aquaculture practices, overfishing, ballast water discharge, alteration of coastal circulation due to the construction of harbours and confinement areas for aquaculture, and global climate change may all be important drivers of the global increase in HABs. Aquaculture is rapidly increasing globally, while fish supply from capture fisheries has been relatively stable (FAO 2013). Hence, increases in fish production have come, and will increasingly come, from aquaculture systems. The contribution of aquaculture to global fish supply increased from 4% in 1970 to 25% in 2000 and to 40% in 2010. Over 70% of this production is in developing countries, mostly in Asia (FAO 2013) and this industrial expansion will continue to contribute to the persistent occurrence of HABs, through nutrient enrichment, habitat alteration and microbial contamination associated with aquaculture operations.

#### 4.2 Global distribution of HABs

It is now well recognised that globally harmful algal blooms are increasing, occurring more often, in new and different places, often lasting longer, and with a range of toxicities. The increase in the occurrence of many of these blooms is often related to an increase in nutrient pollution (Paerl 1988; Glibert *et al.*, 2005, 2014, Glibert and Burford 2017), however many of them can occur in pristine areas with little to no influence from anthropogenic nutrient inputs (e.g. Trainer *et al.*, 2002). Nutrient loads are changing regionally, in both proportion, and in the dominant form of the nutrient. The fact that nutrient loads have generally increased is, in itself, insufficient for the promotion of HABs. This workshop reviewed the global trends in plant nutrients entering coastal and inland waters, and then identified some of the physiological adaptations of the different HAB species that make them particularly successful under nutrient-enriched regimes. Using empirical data and modelling examples, these trends were described, using examples from around the world (McCabe *et al.*, 2016).

#### 4.3 Major HABs and their toxins of concern for cetaceans

A diversity of algal toxins with documented impacts on marine mammals are produced by dinoflagellates, diatoms and cyanobacteria (Landsberg *et al.*, 2005). Bloom-forming marine dinoflagellates are prolific producers of polyketide neurotoxins including the alkaloid, saxitoxin, and ladder polyethers, brevetoxin, ciguatoxin, and palytoxin, as well as a diversity of polyketides that cause gastrointestinal or dermal symptoms. Diatoms of the genus *Pseudo-nitzschia* produce the tricarboxylic amino acid neurotoxin domoic acid. Cyanobacterial toxins such as the cyclic peptide, microcystin, generally associated with freshwater systems, can also occur in estuarine or coastal marine waters. The different HAB groups and species have different growth dynamics and factors which drive their life cycles and toxin production, and therefore how they affect marine mammals (through the food chain or via direct contact, inhalation/aspiration, or incidental ingestion) will vary (see Appendix 1 and Table 1).

#### 4.3.1 Cyanobacterial HABs

Cyanobacteria are the Earth's oldest oxygenic phototrophs and they have had major impacts on shaping its biosphere. Their long evolutionary history (~ 3.5 billion years) has enabled them to adapt to geochemical and climatic changes as well as to recent anthropogenic modifications of aquatic environments, including nutrient over-enrichment (eutrophication), warming, altered precipitation patterns, water diversions, withdrawal and salinisation. Eutrophication has promoted a worldwide proliferation of cyanoHABs that is harmful to ecological and animal (including mammalian) health by outcompeting beneficial phytoplankton, depleting oxygen upon bloom senescence, and producing a variety of toxic secondary metabolites (e.g., cyanotoxins) (Otten and Paerl 2015). While CyanoHABs are most profound and deleterious in eutrophic freshwater environments, they can also proliferate in brackish estuarine and full-salinity marine ecosystem, especially those that are impacted by excessive nutrient enrichment. Evidence is mounting that cyanotoxins originating from freshwater and estuarine blooms make their way into estuarine and coastal food webs via filter-feeding bivalves and zooplankton, which upon consumption by higher ranked animals including mammals (e.g., sea otters), can lead to adverse health effects and fatalities (Miller *et al.*, 2010). Some cyanotoxins can contaminate freshwater drinking water supplies as well as waters in bays, sounds, and estuaries, leading to a variety of adverse acute and chronic health effects (e.g., neurological, hepato-digestive, dermatitis) when contaminated water is contacted or ingested (Carmichael 2001).

#### 4.3.2 Coastal and Oceanic HABs

The majority of eukaryotic marine HABs are the dinoflagellates that produce a diverse group of toxins, and include several genera that are represented in Appendix 1. The dinoflagellates, their toxins, and syndromes (illnesses) caused in humans and marine mammals include *Alexandrium* (saxitoxins that cause paralytic shellfish poisoning), *Karenia* (brevetoxins that cause neurotoxic shellfish poisoning); *Dinophysis* (okadaic acid and the dinophysistoxins that cause diarrhetic shellfish poisoning); *Gambierdiscus* (ciguatoxin that causes ciguatera fish poisoning). For *Alexandrium*, a cyst or "seed-like" resting stage has been identified (see description below). For *Karenia* and *Dinophysis*, no cyst stage has been identified. The benthic HABs, including *Gambierdiscus*, most typically are found associated with reefs and other hard surfaces. They can be bioaccumulated over many years by benthic feeders including reef fish. The dinoflagellate *Ostreopsis* cf *ovata*, has recently been identified as a producer of palytoxin (Table 1).

The only diatom known to produce a biotoxin is *Pseudo-nitzschia*, a chain-forming diatom. This cell is non-motile but typically is concentrated in near-surface oceanic waters. However, when single *Pseudo-nitzschia* cells or chains run out of nutrients, they become less buoyant and "rain' out of the surface waters into the benthos as marine snow. These cells can survive and retain toxin for many years where they cause benthic feeders to accumulate domoic acid. For example, sea otters have been known to become ill after ingesting benthic sand crabs in Monterey Bay, California, and the surrounding region.

Although is likely that the majority of HABs initiate in the nearshore coastal region, some HABs such as *Pseudo-nitzschia*, can initiate in offshore oceanic "hotspot" sites, such as the Juan de Fuca eddy and Heceta Bank (Hickey *et al.*, 2013). These ocean features have retentive circulation that allows natural sources of nutrients to be concentrated at these sites. These highly productive regions support robust food webs, therefore the impacts of HABs originating from these oceanic hotspots on migrating marine mammals should be considered.

#### 4.4 Factors affecting the spread of HABs and their toxins

How environmental factors impact toxin production is the subject of ongoing research, but nutrient [nitrogen (N), phosphorus (P)] supply rates, light, temperature, oxidative stressors, interactions with other biota (bacteria, viruses and animal grazers), and most likely, the combined effects of these factors are all involved (Paerl and Otten 2013; Glibert and Burford, 2017). Accordingly, strategies aimed at controlling and mitigating harmful blooms have focused on manipulating these dynamic factors, with a focus, to date, on the freshwater HABs. The applicability and feasibility of various controls and management approaches was discussed for aquatic ecosystems (Paerl *et al.*, 2016), including those utilised for fisheries, recreation, drinking water supplies (in the case of cyanobacterial HABs or "cyanoHABs") and that serve to support the health of coastal, riverine, bay, sound, and estuarine cetaceans. Strategies based on physical, chemical, and biological manipulations of specific factors show promise, in particular for the cyanoHABs, however, a key underlying approach that should be considered in almost all instances is nutrient (both N and P) input reductions, which have been shown to effectively reduce CyanoHAB biomass, at least in inland waters, and therefore limit health risks (Paerl *et al.*, 2016).

HABs can be harmful in several fundamental ways. The HAB problem and its impacts are as diverse as are the causes and underlying ecological factors leading to blooms. HABs may be caused by the explosive growth of a single species that rapidly dominates the water column, but may also be the result of highly toxic cells that do not necessarily accumulate in high numbers. They may cause direct lethal or sublethal effects to all age classes as well as foetuses due to the action of the toxin(s). Some of the algal toxins are among the most potent known, but there is a wide range of potency depending on which algae predominates and the toxin it may synthesise, or the degree of toxicity of that particular algal bloom. These toxins affect cetaceans either directly (inhalation, dermal, aspiration, or through incidentally ingested toxic waters) or because they are transferred through the food chain. Summary figures illustrating the behaviour and food chain transfer for each of the major HAB species and their toxins relevant to cetaceans are given in Appendix 1. A survival and growth strategy that is important to many HAB species is a complex life cycle, or a life cycle involving resting or benthic stages, such as spores or cysts. These life cycle stages provide a recurrent seed source or inoculum for planktonic populations and this characteristic may be a critical factor in determining not only the geographic distribution of species, but also how it may proliferate when future conditions become favourable.

Understanding the interactions between the environmental factors favouring HABs, when, why and which toxins may be produced and how they may affect cetacean health is difficult. Not only is there wide diversity in HAB species and toxin type, rate of production, extent to which it is labile or stable in the environment and how it is transferred through the ecosystem, but there is also large disparity in availability of data about these HABs, the environment, and their toxins upon which relationships can be investigated. Some regions have excellent coverage of HAB abundance but little data on toxicity, or in other cases toxins may be detected in animals but the source of the HAB may be difficult to unravel due to spatial or temporal offsets. More often sampling of all of the relevant parameters, both with respect to the HABs and the cetaceans, has not been made at the appropriate temporal or spatial scale or with the right methodologies to directly assign cause and effect relationships.

A wide array of initiatives, programmes and expertise in the HAB and phytoplankton community is available to cetacean biologists and the IWC regarding the exposure to, risks of, and the impact of HABs to cetaceans. These are summarised in Figure 2.

#### 4.4.1 Mechanisms underlying toxin production

The regulation of toxin production by HABs is complex because blooms often consist of multiple algal species, with temporal and spatial variation in species dominance that may be driven by changes in nutrient conditions or water mass. The presence of toxic or non-toxic haplotypes within a given species may undergo succession over the course of a bloom (Erdner *et al.*, 2011; Beversdorf *et al.*, 2016). Different algal species may display constitutive or inducible toxin expression, and a complex, dynamic bacterial community that occurs within algal blooms may influence toxin production (Kodama *et al.*, 2007;Van Dolah *et al.*, 2009; Anderson *et al.*, 2012). In spite of these complexities, progress has been made in understanding the regulation of toxin production in HABs. Many cyanobacteria genomes have now been sequenced, revealing toxin biosynthetic gene clusters that may lend themselves to understanding and monitoring bloom toxicity. For example, the regulation of the microcystin gene cluster by the central nitrogen regulator (ntcA) is now understood to link microcystin biosynthesis to high intracellular carbon (C)/nitrogen (N) ratios (Beversdorf *et al.*, 2015). In contrast to cyanobacteria, toxin biosynthesis in many dinoflagellates appears to be more or less constitutive, but different bloom phases may be dominated by clonal haplotypes that may differ in innate toxin levels. Although numerous polyketide synthases have now been identified in brevetoxin and ciguatoxin producing dinoflagellates, their regulation is



Fig. 2. IWC and contracting governments might interact with the international GlobalHAB Programme (formerly GEOHAB). GlobalHAB/ GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms) cooperates and coordinates with other relevant national, regional and international programmes. SCOR=Scientific Committee on Oceanic Research; IOC IPHAB= Intergovernmental Oceanographic Commission Intergovernmental panel on HABS; ICES= International Council for the Exploration of the SEA; PICES= North pacific Marine Science Organization; IOCCG= International Ocean-Colour Coordinates Council; IMBER= Integrated Marine Biogeochemisty and Ecosystem Research.

largely unexplored, due in part to their enormous genomes that to date have prevented genome sequencing (Kellman *et al.*, 2010; Kohli *et al.*, 2017). In blooms of the diatom *Pseudo-nitzschia*, domoic acid toxicity can be quite variable spatially and temporally, in part because of the presence both toxic and non-toxic *Pseudo-nitzschia* species, but possibly also because of co-occurring bacteria that may contribute to domoic acid biosynthesis. Production of domoic acid correlates with a decrease in diversity of the bloom-associated bacterial community, which may indicate that DA selects for specific bacteria or that selected bacteria contribute to toxicity (Sison-Mangus *et al.*, 2014). Current research efforts are directed at unravelling the pathway of DA biosynthesis and the possible role of bacteria. Other factors have been also demonstrated to play a role in DA biosynthesis, such as iron, copper, or silica limitation, and oxidative stress (Lelong *et al.*, 2012), illustrating that multiple, likely interactive, triggers for DA production exist.

#### 4.4.2 Potential issues of toxin exposure for cetacean mortality and morbidity

The HAB toxins of concern for cetaceans, together with the major phytoplankton groups that produce them were reviewed by the workshop. The most important routes of exposure, health effects and the reported evidence that indicates exposure or mortality in marine mammals are given in Table 1. Table 1. Summary of HAB toxins of concern for cetaceans, routes of exposure, mechanisms of action, signs and symptoms based on knowledge in other mammals, exposure or mortality evidence and regions where interactions between the various HABs and cetaceans are likely to occur.

Toxin	Causative Functional	Route of	Mechanism of	Target	Likely Signs and Symptoms	Evidence of	Evidence of	Habitat	Region
	Genera	Exposure	Action		in Marine Maninais	cetaceans	cetaceans		
Brevetoxins <sup>1</sup>	Dinoflagellates Karenia brevis; Karenia spp.	Aerosol; oral: shellfish, finfish	Neurotoxic; sodium channel activator	Brain; neuromuscular junctions; pulmonary system; immune system	Listlessness, difficulty righting, acute mortality of robust, healthy individuals	Yes	Yes	Coastal, oceanic	K. brevis prevalent in Gulf of Mexico; other Karenia spp. present elsewhere in temperate, eutrophic waters
Ciguatoxins <sup>1</sup>	Dinoflagellates Gambierdicsus spp.	Oral: reef fish	Neurotoxic; sodium channel activator	Brain; neuromuscular junctions; heart	Acute: nausea, vomiting, dysthermia, Chronic: fatigue, possibly inappetence	No	No	Coral reef	Patchy global distribution in tropics
Domoic acid <sup>2</sup>	Diatoms Pseudo- nitzschia spp.	Oral: shellfish, herbivorous fish; benthic invertebrates	Neurotoxin; glutamate receptor agonist	Brain, heart	Seizure, intense scratching, head weaving; presence in odd locations, cardiomyopathy, abortion, eosinophilia	Yes	Yes	Coastal oceanic; prevalent in upwelling regions	Broad distribution globally; upwelling coasts
Saxitoxins <sup>2</sup>	Dinoflagellates and cyanobacteria Alexandrium spp.; Gonyaulax spp; Gymnodinium spp.	Oral: shellfish; finfish	Neurotoxic; Sodium channel blocker	Brain; neuromuscular junctions	Acute mortality	Yes	Yes	Coastal, oceanic	Global
Diarrhetic shellfish toxins <sup>1</sup>	Dinoflagellates Dinophysis, Prorocentrum spp.	shellfish	Phosphatase inhibitor	GI tract	Unknown in marine mammals; GI illness in humans	Yes	No	Coastal, oceanic	Global
Microcystins and other cyclic peptides; <i>Cylindrospermopsin</i> Nodularin <sup>2</sup>	Cyanobacteria Microcystis spp. Nodularia spp. Cylindrospermopsis Dolichospermum spp. Aphanizomenon spp.	Water; benthic invertebrates	Phosphatase inhibitor	Liver	Icterus of oral mucous membranes; elevated serum bilirubin; hepatic necrosis	No	No	Usually freshwater, with coastal impacts, in nutrient enriched waters	Global in eutrophic lakes; potential for estuarine and coastal waters; in runoff entering estuarine/coastal waters
Anatoxins	Cyanobacteria Nodularia spp. Cylindrospermopsis Dolichospermum spp. Aphanizomenon spp. Planktothrix spp. Lyngbya spp.	Water; benthic invertebrates	Neurotoxic; Nicotinic acetylcholine receptor agonist	Brain Peripheral nerve function	Unknown in marine mammals	No	No	Usually freshwater, estuarine and coastal waters, in nutrient enriched waters	Global in eutrophic lakes; potential for estuarine and coastal waters; ; in runoff entering estuarine/coastal waters
Palytoxins <sup>1</sup>	Dinoflagellates Ostreopsis cf ovata	Aerosol; benthic invertebrates	Neurotoxic; Na:K pump	Brain; skin	Unknown in marine mammals; neurological symptoms and dermatitis in humans	No	No	Benthic community in rocky substrates	Reefs; emerging issue in Mediterranean
Lyngbya toxins <sup>2</sup>	Cyanobacteria Lyngbyatoxin A Debromoaplysia toxin	Benthic forage	Irritant	Skin	Skin lesions	No	No	Benthic overgrowth of reefs, mud & sand flats, seagrass beds	Temperate to tropics

 $^{1}$  = toxin is lipophilic,  $^{2}$  = toxin is hydrophilic

#### 4.4.3 Implications of climate change for HABs and their toxins

Climate determines environmental factors regulating algal growth, including water temperature, water transport circulation patterns (advection, upwelling and stratification), in turn affecting mixing and stabilisation and thus exposure of photosynthetic organisms to illuminated and nutrient rich layers of the water column. In addition, human activities have altered coastal environments affecting nutrients and circulation patterns. Thus, collectively these climate and anthropogenic changes have affected the composition of the algal community and the trophic structure and function of marine ecosystems at global, regional and local scales. Once a bloom is initiated, physical processes controlling bloom transport are of paramount importance. Coastal currents driven by wind, river inflow, buoyancy or other factors can transport blooms hundreds or even thousands of kilometres along the coast, often from one management area to another. Winds, tides, currents, fronts and other environmental features can create discrete patches or streaks of cells at all scales. The retentive nature of some semi-enclosed coastal systems, such as estuaries, sounds and fjords, can produce long residence times leading to prolonged periods suitable for cells to thrive. Substantial advances have been made over the past decade in unravelling all of these interactions but there is still much that is not understood (Wells *et al.*, 2015).

Cyanobacteria exhibit optimal growth rates and bloom potentials at relatively high water temperatures; hence global warming plays a key interactive role in the expansion and persistence of cyanobacterial blooms (Paerl and Otten 2013). Additional manifestations of climatic change, including increased vertical stratification, decreased salinity, and intensification of storms and droughts, play synergistic roles in promoting bloom frequency, intensity, geographic distribution and duration. Because we have no immediate control on climatic changes, nutrient input reductions are the key management strategy for mitigating CyanoHABs (Paerl and Otten 2016). Furthermore, rising temperatures cause shifts in critical nutrient thresholds at which cyanobacterial blooms can develop; thus, nutrient reductions for CyanoHAB control may need to be more aggressively pursued as they respond to climatic changes taking place worldwide (Paerl *et al.*, 2016).

Eukaryotic marine HABs, including *Pseudo-nitzschia*, recently have been associated with anomalously warm water ocean conditions. An analysis of historical records of shellfish closures due to elevated domoic acid, have shown that the longest closures due to the most intense blooms occur during or immediately following warm water years, including El Niño events and the anomalously warm "Blob" of 2015 that resulted in a coastwide closure of shellfish harvest along the Pacific coast of North America (McCabe *et al.*, 2016; McKibben *et al.*, 2017). In addition, other climactic factors, such as the reduction of ocean pH known colloquially as "ocean acidification", will result in a higher domoic acid quota per cell for *Pseudo-nitzschia australis* blooms (Sun *et al.*, 2011; Wingert 2017).

#### 4.5 Conclusions and recommendations

Following the presentations there was a discussion about mapping and whether a HAB 'hotspot' map would be useful for predicting impacts of HABs on cetaceans, and if so, what should be included. However, it was not clear what spatial or temporal scales to use, what oceanographic, nutrient or HAB occurrence information should be included, nor what quality or type of cetacean data should be overlain on such a map. It was concluded that there were many resources on HAB distribution available online, and thus what would be more useful would be a list of contacts in the HAB community, by country or region, that could be contacted by cetacean scientists and managers who might require advice and guidance during response to an unusual event in cetaceans that they may suspect is associated with exposure to HAB toxins as a causative agent or to evaluate the risks to some populations from HAB impacts.

The workshop concluded that we need to better understand the risks of HABs for cetaceans through a variety of studies including studies of the contribution of HAB toxins to marine mammal mortality and morbidity. Therefore, data from HAB monitoring, marine mammal strandings and toxin analysis in tissues and environmental samples should be integrated at an appropriate spatial and temporal scale, depending on the particular questions to be addressed. Assistance in this endeavor could be facilitated by:

- 1. Informing marine mammal scientists of HAB databases by country and region. This will enable them to see realtime data and annual summaries of HAB observations and will allow them to collaborate more closely with HAB scientists who are leading the monitoring programs. Examples of these programs include the Harmful Algal Event Database (HAE-DAT, an annual summary database led by researchers in many countries, <u>www.haedat.iode.org</u>), the SoundToxins database (<u>www.soundtoxins.org</u> that includes real-time HAB data from WA State and Alaska with the State of Oregon to join soon). A "traffic-light pattern" alerts managers to the realtime threat of HAB toxins in shellfish.
- 2. To work with these networks and others to collect routine water samples and prey at sites appropriate for marine mammals, rather than relying on the shellfish monitoring sites that have been set up for human health protection.
- 3. To partner with One Health initiatives, such as the database maintained by the CDC (https://www.cdc.gov/onehealth/) which strives to include both human and animal HAB associated illness data.
- 4. Include marine mammal scientists in HAB Bulletin (early warning) reporting systems that are developing in the US, Europe and other countries.
- 5. Work toward developing integrated programs that integrate monitoring of plankton to use of satellites for bloom detection. This could include data from animal-borne conductivity, temperature and depth sensors that are now

being deployed on a variety of marine mammals, including cetaceans such as beluga whales (*Delphinapterus leucas*) (Lydersen *et al.*, 2002).

The workshop **recommended** cetacean biologists should link with GlobalHAB, ICES, PICES, SCOR and other HAB groups (Figure 2). This could be done, for example, through the ICES Working Group community. ICES has a Marine Mammal Ecology Working Group and a Working Group on Harmful Algal Bloom Dynamics and PICES has a section on HABs. GlobalHAB (formerly GEOHAB) is an international programme focusing on HAB population dynamics, ecology, as well as development of predictive models and improved understanding of toxins and their effects (<u>www.geohab.info</u>). The workshop **recommended** that more communication and active information exchange could be facilitated through these groups and their respective agendas.

The workshop noted the rapid global expansion of aquaculture systems that enrich nutrients into these environments that trigger phytoplankton blooms, including HABs that can alter coastal habitats. The workshop therefore **recommended** that countries using open aquaculture and pond systems near coastal areas consider the ecosystem changes from these industries that might negatively impact cetacean health through enhancement of HABs. The workshop suggested that development and operation of this industry follow best management practices to reduce impacts of the activities on the local and regional cetacean environment.

#### 5. HEALTH IMPACTS OF HAB TOXINS ON CETACEANS

#### 5.1 Review of health effects of toxins on marine mammals

Harmful algal blooms are increasing globally, and toxins they produce have been conclusively associated with large-scale mortality events in marine mammals (Bossart et al., 1998; Scholin et al., 2000). Effects of toxins on cetaceans are less well-understood, due to more limited opportunities to examine live and freshly dead animals to identify lesions and detect associated toxin. The mere presence of a biotoxin in marine mammal tissue or fluids does not necessarily allow attribution of biotoxin exposure as the sole cause of death; in many instances it is possible that multiple stressors may have contributed to mortality. The first biotoxin suspected to impact marine mammals was ciguatoxin, which was detected in liver samples by mouse bioassay from two Hawaiian monk seals (Neomonachus schausinlandii) sampled during a dieoff of 50 seals on Laysan Island in 1978 (Gilmartin et al., 1980). Since then ciguatoxin has been detected in blood of 19% of 55 live seals using the Neuro 2A cytotoxicity assay, raising concerns about the potential of sub-clinical impacts (Bottein Dechraoui et al., 2011). In 1987, the deaths of 14 humpback whales (Megaptera novaeangliae) off Cape Cod, Massachusetts, U. S., was attributed to saxitoxin, as this toxin was detected in stomach contents of two whales and in mackerel from the area (Geraci et al., 1989). In the same year, mass mortality of bottlenose dolphins along the eastern seaboard of the U.S. was associated with brevetoxin exposure, although later analyses revealed cetacean morbillivirus in these animals. Since then, brevetoxin has been associated with multiple mortality events of bottlenose dolphins in the Gulf of Mexico and along the east coast of the U.S., with epidemics of morbillivirus occurring concurrently in some years (Flewelling et al., 2005; Twiner et al., 2012; Fire et al., 2015). Brevetoxin exposure can alter in vitro activity of some lymphocyte populations, suggesting that interactive effects of biotoxin exposure and viral infection likely occur (Gebhard et al., 2015; Walsh et al., 2015). Saxitoxin also alters in vitro proliferation of harbour seal (Phoca vitulina) T lymphocytes and their susceptibility to morbillivirus infection (Bogomolni et al., 2016). Saxitoxin was associated with a multispecies die off (invertebrates, fish, seabirds, harbour and grey seals, harbour porpoise, beluga and fin whales) in the Gulf of St Lawrence in 2008 (Starr et al., 2017), and with death of Mediterranean monk seals in 1997 (Reyero et al., 1999). It was also detected in gastrointestinal contents from two of over 300 sei whales stranded in the Golfo de Penas in southern Chile in 2015 (Haussermann et al., 2017). However, in these mortality events (Mediterranean monk seal, sei whale, Gulf of St Lawrence cetaceans) no characteristic lesions were identified. Saxitoxin has also been reported in faeces of Northern right whales, raising concerns about potential impacts on reproduction (Doucette et al., 2012). The most detailed information on health effects in marine mammals associated with biotoxin exposure comes from examination of California sea lions (Zalophus californianus) stranding during Pseudo-nitzschtia blooms. High levels of domoic acid in body fluids have been associated with mortality, neuronal necrosis in the hippocampus and cardiomyopathy. More chronic and latent effects observed in sea lions include epilepsy due to hippocampal atrophy, impaired spatial navigation, memory loss, reproductive failure and circulatory eosinophilia (Brodie et al., 2006; Goldstein et al., 2008; Thomas et al., 2010; Cook et al., 2015). Cetaceans have stranded along the California coast during these blooms, but even though some have high levels of toxin in gastrointestional contents or urine, typical pathological lesions associated with domoic acid toxicity have not been described (de la Riva et al., 2009; Fire et al., 2010). This may be due to the acute neurotoxic effects and rapid death that may be occurring in these animals combined with decomposition state of many stranded cetaceans. A biotoxin recently reported to have caused hepatic necrosis and death in Monterey Bay, California, sea otters is microcystin associated with run off of freshwater cyanobacteria blooms (Miller et al., 2010).

#### 5.2 Learning from the effects on human health and linkages to cetacean health

In both humans and marine mammals, the major routes of exposure to HABs are via ingestion, inhalation, and dermal contact<sup>1</sup>. Understanding the way that human HAB associated illnesses present and are investigated, described below,

<sup>&</sup>lt;sup>1</sup>Two basic definitions when discussing public health are: (a) Epidemiology, the application of the scientific method to study the occurrence or risk of adverse health outcomes in populations. Surveillance, the processes of collecting, analysing, and disseminating data on specific health effects.

provides insights into approaches for cetacean HAB associated illnesses or mortality investigations. The first, ingestion, is the route of exposure in humans most often caused by the consumption of contaminated shellfish (primarily for PSP, NSP, DSP, ASP, AZP<sup>2</sup>) although some poisonings occur through the consumption of finfish (CFP<sup>2</sup>, and perhaps brevetoxin) (Berdalet et al., 2015). However, the only biomarker of exposure is through the recovery of the partially consumed food, which is infrequently recovered by physicians or emergency room attendants. The food poisonings have different rates of onsets, different symptoms, and different resonance time in the body depending on the associated toxin. All of the toxins are tasteless and odourless and resistant to both heat and cold. The second route of exposure is through inhalation (primarily for Karenia red tides, cyanobacteria, and Ostreopsis) and can have upper respiratory effects such as nasal congestion, rhinorrhea, and cough. Karenia brevis aerosols have an impact on the lower airways of people with asthma and can cause decreased pulmonary function and increased symptoms for 3-5 days after a 1 hour exposure. Hospital admissions for both respiratory illness and gastrointestinal illness have been documented during a Karenia bloom. Given the unique anatomical and physiological adaptations in the respiratory tract of cetaceans, the inhalation or aspiration of toxin at the air/sea interface is of high concern. The toxin load may be much higher than any air measurements taken on land. Dermal exposure is the third route of exposure and although there have been anecdotal reports of swelling of the mucous membranes and skin rashes, little epidemiology has been conducted to date in humans and no studies in cetaceans.

Many of the compounding issues when investigating a human exposure to a HAB are also true for marine mammal investigations. Often, little is known about the duration and dose of the exposure, the toxicity of the bloom, overall health prior to the exposure, and exposures to other possible contaminants concurrently (for example, in the case of people with asthma, exposure to pollen, dust and other allergens).

The traditional tool for bloom detection and monitoring has been microscopic enumeration from water grab samples. This requires a skilled microscopist and reporting can be delayed by several days. Remote sensing can also be a useful tool in bloom detection and tracking assuming that the bloom is at the surface and is dense enough to be detected. Given the critical need for an operational and near real time reporting system for beaches and human health, newer methods that are not designed for regulatory purposes but for bloom tracking and intensity assessment are currently in use in some areas (Mote's Beach Conditions Reporting System and HABscope). The workshop encourages the HAB monitoring community to continue to develop monitoring methods that provide the spatial and temporal coverage needed to adequately understand human and animal exposures.



Figure 3. Summary of factors affecting harmful algal bloom (HAB) presence, HAB toxins and impacts on cetaceans.

<sup>&</sup>lt;sup>2</sup> PSP = Paralytic Shellfish Poisoning, NSP = Neurotoxic Shellfish Poisoning, DSP = Diarrheic Shellfish Poisoning, ASP = Amnesic Shellfish Poisoning, AZP = Azaspiracid Shellfish Poisoning, CFP = Ciguatera Shellfish Poisoning.

#### 5.3 Investigative approaches

Linking HABs and their toxins to cetacean impacts is difficult because of the multiple species (HABs and cetaceans) involved, varying oceanographic conditions and organismal biology, and data availability and data quality at all levels (summarised in Figure 3).

#### 5.3.1 Review of HAB detection methods

To determine the geographic distribution of offshore phytoplankton blooms, satellite chlorophyll imagery (e.g. MODIS) is useful. However, because all phytoplankton cells contain chlorophyll, including chlorophyll a and accessory pigments that have optimal wavelengths for light harvesting, satellite images are generally not useful for distinguishing phytoplankton of different genus or species. However, algorithms have been developed to detect certain phytoplankton that sometimes form visible blooms, such as *Karenia brevis* off the Florida coast and *Pseudo-nitzschia* off the California coast. It is necessary to ground truth these satellite images by collecting field samples to ensure the accuracy of satellite detection. The detection of a genus or species by satellite and algorithm in one geographical region does not ensure that the algorithm developed for this area will work well in another part of the world. In addition, satellite imagery cannot be accessed on cloudy days and is not accurate close to shore where turbidity impacts sensor accuracy.

#### 5.3.2 Review of phytoplankton and toxin exposure detection methods

Detection methods for HAB toxins include ELISA, receptor binding assay and liquid chromatography mass spectrometry. The limitations of each assay should be understood and proper controls must be used prior to their use. For example, ELISAs can be subject to matrix effects (false positives due to compounds in particular tissues or fluids) and must be used at proper dilutions in order to eliminate the possibility of false positive readings. In addition, ELISA results do not always correlate with total toxicity but correlate with affinity of an antibody to a particular toxin structure. For example, a saxitoxin ELISA will not recognize all toxin isoforms equally (e.g. STX, neosaxitoxin, gonyautoxins). In contrast, because domoic acid is the major isomer produced by the various species of *Pseudo-nitzschia*, the domoic acid ELISA assay is effective in estimating total toxicity. Other methods for toxin testing are the receptor binding assay (most typically used for saxitoxin analysis) that measures total toxicity and is based upon the recognition of the toxin by a purified receptor, usually from rat or pig brain. The liquid chromatography mass spectrometry method measures individual toxins with high molecular specificity and detection sensitivity. The choice of method(s) to be used depends upon equipment available, needs for screening large numbers of samples, and the need for absolute quantification or estimation of toxins in a particular sample.

Cell-based detection of organisms is most typically done using light microscopy. The disadvantage of light microscope detection is that often organisms can be detected only to the genus level. Identification to the species level often requires molecular probes or higher magnification to see fine organismal structure, through the use of scanning electron microscopy. Often detection to the genus level using light microscopy is used in conjunction with toxin detection. For example, the genus *Pseudo-nitzschia* has >32 species, each with different cellular toxin levels, therefore toxicity cannot be predicted merely by cell observation. Light microscopy can be used to determine whether a threshold abundance of cells is reached, at which time toxin testing is performed to confirm whether or not domoic acid is present.

An example of how the current detection methods that are being used for surveillance was given in a study to document the exposure of a range of cetaceans inhabiting Scottish waters to the neurotoxin, domoic acid (DA) (Hall *et al.*, 2017). Overall, approximately 40% of the individuals screened (n=158) had detectable DA in their urine or faeces. This included 12 different species, such as harbour porpoise, long finned pilot whale, minke whale, white-beaked dolphin and sei whale. DA levels were generally low (median  $\sim 2$  ng/g or ng/ml) but concentrations in excreta were difficult to interpret as time since exposure was not known. One harbour porpoise had a very high level in its urine ( $\sim 2500$  ng/ml) suggesting in at least one case exposure was at acutely toxic levels. However, these results were from stranded animals that, at post mortem examination, died of various causes and were not associated with any obvious signs of neurotoxicity, suggesting the overall exposure is likely to be low level but possibly chronic. Pseudo-nitzschia spp. diatoms are now highly prevalent in Scotland throughout the year and may produce large toxic blooms during the summer months. The consequences of prolonged DA ingestion for the health of individuals and cetacean populations in this region remains unknown.

#### 5.3.3 Use of 'omics approaches for health, physiology and biomarker identification in cetaceans

'Omics, or systems biology approaches, have evolved over the past two decades into powerful tools to aid in understanding how biological networks respond to perturbations (Veldhoen *et al.*, 2012). Genomics, transcriptomics, proteomics and metabolomics have been successfully applied to gain insight into physiological processes in marine mammals, such as diving physiology and fasting, and responses to contaminant exposure. The advancement in instrumentation (sequencing, mass spectrometry), bioinformatic algorithms (for analysing genomic and proteomic data), and public databases of DNA sequences, peptide profiles, and metabolites, continues at a rapid pace that will make 'omics approaches increasingly tractable for non-model organisms such as marine mammals. Transcriptomic and proteomic profiles in blood, now routinely used in human medicine to identify prognoses of cancer, heart disease, and other diseases and exposures, make blood a compelling tissue to monitor in accessible marine mammals for exposure and disease status (Morey *et al.*, 2016). However, blood sampling in free ranging cetaceans is problematic, making blubber, skin, and breath the subjects of current research. The use of 'omics to investigate biotoxin exposures is limited to date, but these methods hold promise for the development of biomarkers that can inform us of the role of biotoxins in unexplained mortality

events or the extent of chronic exposures within marine mammal populations. Both blood transcriptomes and serum proteomes can identify domoic acid poisoning in California sea lions (Mancia *et al.*, 2012, Neely *et al.*, 2012); however, neither approach has yet been developed into robust tests that can be applied in clinical or field settings. Transcript profiles in blubber and skin biopsies have been interrogated with some success to identify indicators of contaminant exposure (Van Dolah *et al.*, 2015), but require further development and validation, and have not yet been used to investigate biotoxin related events. Metabolomic profiling of volatiles in exhaled breath from bottlenose dolphins shows promising results that this method can identify metabolic responses indicative of oil exposure (Pasamontes *et al.*, 2017); however, sampling techniques for large free ranging cetaceans will require additional development. All 'omics approaches need the establishment of "healthy" profiles and insight into natural variation that allows the identification of alterations indicative of adverse health. In human medicine, databases of 100's to 1000's of profiles are used. The investment in such baseline information in marine mammals is needed. Publicly available databases are already in place that can accommodate data sharing.

#### 5.3.4 Strategies to investigate die-offs potentially attributable to HABs

When faced with an unusual cetacean event, it is often unclear what the causative agent(s) might be and in some cases the scientists dealing with the event may suspect that HABs and their toxins may be involved. An unusual event may raise concern because it is higher than expected, or may be different in some way but it is important to have baseline data for comparison.

The major steps in an investigation strategy are given in Table 2. In addition to this strategy, there are other resources available to help guide an investigation such as the Canadian Cooperative Wildlife Health Centre: wildlife disease investigation manual (2007).

Stage	Description	Comments		
Strandings information	Collect basic (level A) data, remembering modifications may be needed if strandings are live	Species, sex, decomposition code, age class structure and/or size		
	External and internal exam to rule out trauma, obvious infectious disease and potentially determine lesions (including histopathology)	Photographs		
	Nutritional condition			
Sample collection (animal)	Fluids	Urine (preferred), faeces, aqueous humour, cerebrospinal fluid, milk, amniotic/allantoic fluid		
	Tissues for analyses	liver, kidney, foetus		
	Presence of GI contents and status of GI tract	GI contents for prey ID – take multiple replicate samples for confirmatory testing		
Location	GPS location of carcasses or live stranded animals			
	Geography – site characteristics (tide line, position, etc.)			
Timing/seasonality, duration of event	Environmental information such as wind, currents, weather, visual bloom, other species involved, anthropogenic activities (Navy, fishing fleet, oil spill, contaminant spill, fish farms etc.)	Including personal accounts/observations of the scene and any observed human symptoms		
Sample collection (environment)	Water samples, sediment			
	Prey sampling – whole, and ideally at the same time as water sampling			
Environmental sample shipment, analysis and storage (dark, cold options)	Archive for future testing where appropriate			
Remote sensing data (noting limitations of satellite imagery), algal identification, or toxin levels	Contact GlobalHAB for expertise to guide local knowledge, laboratories	A local laboratory may not have capacity for toxin identification – can get connected to ICES/PICES working group or GlobalHAB to identify contacts		
Synthesize findings into a report	Even negative findings are important to report	Potentially convene an interdisciplinary team in- person meeting to put all data together and develop report		
Post-event monitoring plan	Temporal trophic-level effect			

 Table 2

 Main stages in an investigation of die-offs potentially related to HABs and their toxins.

#### 5.3.5 Chronic, acute and interactive effects

Currently, the best information detailing chronic, acute and interactive health effects of HAB toxins on marine mammals is for domoic acid. California sea lions show acute, chronic and latent effects (Goldstein *et al.*, 2008, Gulland *et al.*, 2012), but there is still a need to characterize developmental effects (Lefebvre *et al.*, 2017). Rodent studies show developmental effects of *in utero* exposure, suggestive of memory loss, demyelination, and ataxia (Doucette *et al.*, 2004). Toxicological effects are dependent upon the inter-uterine day of exposure. In humans, the regulatory levels of 20 ppm is probably insufficient to protect the developing foetus, with suggestions that perhaps pregnant women should be advised to avoid all potential DA ingestion. The effects of HABs and their toxins on the developing foetus in exposed cetaceans needs to be considered and further studies in this area would be encouraged.

Chronic and latent cases are frequently not spatially or temporally associated with HABs (Goldstein *et al.*, 2008) and are difficult to detect and investigate. Mixed exposures are beginning to be investigated (Fire *et al.*, 2011) as are issues of cumulative effects, particularly interactions between pesticides or contaminants and HAB toxins together which may result in synergistic effects (Tiedeken and Ramsdell 2010).

#### 5.3.6 From concentrations to impacts (including modelling)

The workshop reviewed a summary of cetacean mortality events in the U.S. that have been attributed to HAB toxins, focusing on the multiple bottlenose dolphin die-offs associated with exposure to brevetoxin in the northern Gulf of Mexico. The workshop also discussed the findings from live bottlenose dolphin health assessment studies, as well as findings from strandings of multiple cetacean species, in which urine/faeces samples were screened for algal toxins (primarily, brevetoxin and domoic acid). It was concluded that there is evidence that cetaceans along the majority of the U.S. coast have been exposed at some point in time to algal toxins. In some cases (e.g., bottlenose dolphins in Sarasota Bay and St. Joseph Bay, Florida), there is evidence for concurrent exposure to multiple toxins over several years. While the exposures in some cases have led to mortality events and/or sublethal effects, many of the measured toxin concentrations are fairly low and it is unclear whether or not the exposures have been sufficient to lead to adverse health effects. This suggests a need to develop approaches for conducting both individual- and population-level risk assessments. A number of examples of modelling approaches have been previously pursued to better understand pathways for HAB toxin exposure, and for characterizing potential risk of mortality and/or morbidity under various exposure scenarios. Examples included a bioenergetic model and a food web model to examine variation in HAB exposure related to lifestage (and associated energetic requirements), and in relation to prey composition and food web dynamics (Bejarano et al., 2007). An example of a spatially-explicit individual-based model to characterize population-level risk was also discussed.

#### 5.4 Conclusions and recommendations

One of the primary information needs for better understanding the health risks for cetaceans from algal toxins is an understanding of the dose-response relationships for both sublethal and lethal effects across the multiple toxin groups. While development of dose-response relationships may be unfeasible for any cetacean species, data could be synthesized from multiple sources including laboratory experiments of other species as well as measured concentrations from marine mammals with confirmed acute toxicosis (both cetaceans and pinnipeds), as well as control cases without evidence of HAB-related disease. The workshop **recommended** such datasets be identified or developed and synthesis approaches be pursued as a priority.

Other data gaps relate to exposure assessment and include prey preferences and/or composition and range of toxin concentration in important prey species. Cetaceans may be particularly vulnerable to aerosolized toxins at the water-air interface due to their unique respiratory physiology, and currently measurements are lacking for aerosolised toxins. The workshop **recommended** that toxins in prey species (not fillets) be included in surveillance and research studies, as well as toxins in tissues, as these samples may prove to be more valuable in determining exposure due to the very short half-life of many hydrophilic toxins in tissues and excreta.

Characterizing exposure is complex, and data needs will be case-dependent, depending on the algal toxin of concern, its receptor, the local food web, and potentially other environmental variables. Note that many of the observed sublethal effects (e.g., immune system perturbations) may interact with other stressors (such as exposure to intracellular pathogens, or chemical contaminants that may influence the toxin binding to common receptors) so interactive effects of algal toxins with other environmental stressors should be considered.

The workshop **recommended** sampling on a temporal and spatial scale that is relevant to both human health and coastal cetacean health.

The workshop **recommended** that the development of biomarkers of exposure and effects in relevant (and obtainable) tissues be pursued as a priority.

In relation to current surveillance approaches using the ELISA approach for DA and STX needs to be confirmed by mass spectroscopy and standardisation of methods are **recommended**. The workshop **recommends** that appropriate limits of detection and limits of quantification with appropriate uncertainty levels be developed for each approach being used.

#### 6. SUMMARY OF CONCLUSIONS

SC/67a/Rep09

The workshop concluded that the global distribution and increasing ubiquity of HABs and their toxins has resulted in an increasing risk of impacts on cetaceans, both at the individual health and population dynamics levels. We need to better understand the contribution of HAB toxins to marine mammal mortality and morbidity and that data from HAB monitoring, marine mammal strandings and toxin analysis in tissues and environmental samples should be integrated at an appropriate spatial and temporal scale, depending on the particular questions to be addressed. Assistance in this endeavor could be facilitated by:

- 1. Informing marine mammal scientists of HAB databases by country and region. This will enable them to see real-time data and annual summaries of HAB observations and will allow them to collaborate more closely with HAB scientists who are leading the monitoring programs. Examples of these programs include the Harmful Algal Event Database (HAE-DAT, an annual summary database led by researchers in many countries, <u>www.haedat.iode.org</u>), the SoundToxins database (<u>www.soundtoxins.org</u> that includes real-time HAB data from Washington State and Alaska with the State of Oregon to join soon). A "traffic-light pattern" alerts managers to the real-time threat of HAB toxins in shellfish.
- 2. To work with these networks and others to collect routine water samples at sites appropriate for marine mammals rather than relying on the shellfish monitoring sites that have been set up for human health protection.
- 3. To partner with One Health initiatives, such as the database maintained by the CDC (https://www.cdc.gov/onehealth/) which strives to include both human and animal HAB associated illness data.
- 4. Include marine mammal scientists in HAB Bulletin (early warning) reporting systems that are developing in the U.S., Europe and other countries.
- 5. Work toward developing integrated programs that integrate monitoring of plankton to use of satellites with appropriate algorithms for bloom detection. This could include data from animal-borne conductivity, temperature and depth sensors that are now being deployed on a variety of marine mammals.

It was concluded that there were many resources available online and that a list of contacts in the HAB community by country or region that could be contacted by cetacean biologists who might require input during an unusual event that they may suspect is associated with exposure to HAB toxins as a causative agent would be useful.

The workshop noted the rapid global expansion of aquaculture systems that enrich nutrients into these environments that can be a source of HABs themselves and can alter coastal habitats.

Many of the compounding issues when investigating a human exposure to a HAB are also true for marine mammals. Often, little is known about the duration of the exposure, the toxicity of the bloom, overall health prior to the exposure, and exposures to other possible contaminants concurrently.

Linking HABs and their toxins to cetacean impacts is difficult because of the multiple species involved, varying oceanographic conditions and organismal biology, and data availability and data quality at all levels. In addition, the use of 'omics to investigate biotoxin exposures is limited to date, but these methods hold promise for the development of biomarkers that can inform us of the role of biotoxins in unexplained mortality events or the extent of chronic exposures within marine mammal populations. Whilst the acute, chronic and latent effects of DA on marine mammals is now well documented, the impact of chronic low-level exposure and the impact of other HAB toxins is not known. The effects of HABs and their toxins on the developing foetus in exposed cetaceans needs to be considered and further studies in this area would be encouraged.

#### 7. SUMMARY OF RECOMMENDATIONS

The workshop **recommended** cetacean biologists should link with GlobalHAB, ICES, PICES, SCOR and other HAB groups. This could be done through the working group community. For example, ICES has a Marine Mammal Ecology Working Group and a Working Group on Harmful Algal Bloom Dynamics and PICES has a Section on HABs. The workshop **recommended** that more communication and active information exchange could be facilitated through these groups and their respective agendas.

The workshop noted the rapid global expansion of aquaculture systems that enrich nutrients into these environments that can be a source of HABs themselves and can alter coastal habitats. The workshop therefore **recommended** that countries using open aquaculture and pond systems consider the ecosystem changes that could negatively impact cetacean health. The workshop suggested that countries ensure development of this industry is in line with best management practices.

The workshop **recommended** that more communication and active information exchange could be facilitated through these Intergovernmental ocean management groups and their respective agendas.

The workshop therefore **recommended** that countries using open aquaculture and pond systems consider the ecosystem changes that could negatively impact cetacean health. The workshop suggested that countries ensure development of this industry is in line with best management practices.

The workshop noted that although the climate cannot be changed in the short term, nutrient input can be controlled and it **recommended** that efforts to reduce the global use of nitrogen and phosphorus be supported.

While development of dose-response relationships may be infeasible for any cetacean species, data could be synthesized from multiple sources including laboratory experiments of other species as well as measured concentrations from marine mammals with confirmed acute toxicosis (both cetaceans and pinnipeds), as well as control cases without evidence of HAB-related disease. The workshop **recommended** such datasets be identified or developed and synthesis approaches be pursued as a priority.

The workshop **recommended** that toxins in prey species be included in surveillance and research studies as well as toxins in tissues as these samples may prove to be more valuable in determining exposure due to the very short half-life of many hydrophilic toxins in tissues and excreta.

The workshop **recommended** sampling on a temporal and spatial scale that is relevant to both human health and coastal cetacean health.

The workshop **recommended** that the development of biomarkers in relevant (and obtainable) tissues, both of exposure and of effects, be pursued as a priority.

In relation to current surveillance approaches using the ELISA approach for DA and STX needs to be confirmed by mass spectroscopy and standardisation of methods should be **recommended**. The workshop **recommends** that appropriate limits of detection and limits of quantification with appropriate uncertainty levels be developed for each approach being used.

#### 8. ADOPTION OF REPORT

The report was adopted at 15:00 on 9 May 2017.

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#### ANNEX A

#### LIST OF PARTICIPANTS

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#### ANNEX B

#### AGENDA

#### 1. INTRODUCTORY ITEMS

#### 2. APPOINTMENT OF CHAIR AND RAPPORTEURS

3. REVIEW AND ADOPT AGENDA

#### 4. INTRODUCTION AND BACKGROUND

- 4.1 Harmful algal bloom dynamics and drivers
- 4.2 Global distribution of HABS
- 4.3 Major HABs and their toxins of concern for cetaceans
- 4.3.1 Cyanobacterial HABs
- 4.3.2 Coastal and Oceanic HABs
- 4.4 Factors affecting the spread of HABs and their toxins
- 4.4.1 Mechanisms underlying toxin production
- 4.4.2 Potential issues of toxin exposure for cetacean mortality and morbidity
- 4.4.3 Implications of climate change for HABs and their toxins
- 4.5 Conclusions and recommendations

#### 5. HEALTH IMPACTS OF HAB TOXINS

- 5.1 Review of health effects of toxins on marine mammals
- 5.2 Learning from the effects on human health and linkages to cetacean health
- 5.3 Investigative approaches
- 5.3.1 Review of HAB detection methods
- 5.3.2 Review of phytoplankton and toxin exposure detection methods
- 5.3.3 Use of 'omics approaches for health, physiology and biomarker identification in cetaceans
- 5.3.4 Strategies to investigate die-offs potentially attributable to HABs
- 5.3.5 Chronic, acute and interactive effects
- 5.3.6 From concentrations to impacts (including modelling)
- 5.3.7 Conclusions and recommendations
- 6. SUMMARY OF CONCLUSIONS

#### 7. SUMMARY OF RECOMMENDATIONS

8. ADOPTION OF REPORT

#### Annex C

#### Harmful algae figures

1. The HAB taxa *Alexandrium* has a complex life cycle and population dynamics (upper left). Its most common toxin is saxitoxin which causes paralytic shellfish poisoning (lower left). It has been found to impact harbor seals and humpback whales (upper right) and to be distributed around the world (lower right). Global map from http://www.whoi.edu/redtide/

2. The HAB taxa *Karenia* has complex population dynamics and toxins that are carrier in aerosols and stable in the water column and which cause fish kills (upper left). Its most common toxin is brevetoxin which causes neurotoxic shellfish poisoning (lower left). It has been found to impact manatees and bottlenose dolphin (upper right) and to be most common in the Gulf of Mexico (lower right), although it can be found on other parts of the world (not shown on the map). Global map from http://www.whoi.edu/redtide/

3. The HAB taxa *Dinophysis* has a complex life cycle and population dynamics (upper left). Its most common toxin is okadaic acid which causes diarrheic shellfish poisoning (lower left). It has been found to impact harbor seals (upper right) and to be distributed around the world (lower right). Global map from http://www.whoi.edu/redtide/

4. The HAB taxa *Pseudo-nitzschia* forms large blooms (upper left). Its most common toxin is domoic acid which causes amnesic shellfish poisoning (lower left). It has been found to impact California sea lions and harbor porpoise (upper right) and to be distributed around the world (lower right). Global map from http://www.whoi.edu/redtide/

5. Cyanobacteria can form dense blooms in surface waters, as well in benthic systems depending on species (upper left). The toxin formed is dependent on species (lower left). It has been found to impact sea otters and manatees (upper right) and to be distributed around the world (lower right). Map shown is for cylindrospermopsin only and is from Kinnear (Mar Drugs 2010)

6. Benthic HABs form on coral reef and other rocky or solid benthic surfaces (upper left). There are multiple benthic HAB taxa. The toxin formed is dependent on species (lower left); the most common is ciguatoxin which causes ciguatera fish poisoning (lower left). It has been found to Hawaiian monk seals (upper right) and to be distributed around the world (lower right). Global map from <u>http://www.whoi.edu/redtide/</u>.





