

**Report of the Pollution 2025
- Cumulative Effects -
Multiple Stressors IWC
Intersessional Workshop**

Virtual Meeting, 1-4 November 2021

Pollution 2025 – Cumulative Effects – Multiple Stressors IWC Intersessional Workshop¹

Online, 1-4 November 2021 (19:30-23:30 UK time)

1. INTRODUCTORY ITEMS

1.1 Welcoming remarks

Holm welcomed participants to the workshop, acknowledging that they were calling in from various time zones. She looked forward to a lively and wide-ranging discussion on how the impacts of the cumulative effects of multiple stressors on cetacean populations could be investigated and potentially progressed with the support of the Scientific Committee's Sub-Committee on Environmental Concerns. Holm acknowledged that whilst it was very unlikely that the workshop would end with a 'blueprint' to solve all the questions on multiple stressors and cumulative effects, it should progress our understanding of this topic. This topic is very important to the IWC, due to it's the previous concerns voiced over the effect of environmental change on cetaceans, as noted in IWC Resolutions 1998-5, 2000-7 and 2009-1. The importance also follows on from former IWC workshops; for example, on 'Habitat Degradation' in 2004 (IWC, 2006) and on 'Assessing the Cumulative Effects of Multiple Stressors on Cetaceans at the Individual and Population Level' in 2018 (IWC, 2019).

1.2 Introduction of participants

A list of participants is provided as Annex A.

1.3 Election of Chair

Holm was elected chair of the workshop.

1.4 Appointment of Rapporteurs

Plön, Hosen, Mayaud, Allen and Gross were appointed as rapporteurs.

1.5 Adoption of Agenda

The draft agenda was adopted (see Annex B).

1.6 Available documents

Documents for this intersessional workshop were made available on the SharePoint site, under each associated agenda item. A list is provided as Annex C.

2. BACKGROUND AND GOALS OF POLLUTION 2025

Holm opened the workshop by drawing attention to the report of the earlier IWC workshop on the same topic and summarising its recommendations (IWC, 2019).

- Improve knowledge and enable quantitative assessments on the data available for cumulative effects.
- Develop a widely applicable approach for providing precautionary advice for populations in which cumulative effects are of concern.
- Establish cumulative effects as a standing item on the E agenda.
- Encourage additional efforts to identify the relevant threats in Important Marine Mammal Areas (IMMAs) in order to assist with the management of cumulative effects.
- Endorse the results of the IUCN/ACCOBAMS workshop entitled 'Towards understanding the overlap of selected threats and Important Marine Mammal Areas (IMMAs) across the Mediterranean Sea'.
- Encourage that such an effort – aimed at overlaying different sources of threat and pressure on existing Important Marine Mammal Areas (IMMAs) – be continued and carried out in more detail in the other marine regions where IMMAs have already been identified.
- Offer its assistance in such assessments.

The Scientific Committee at SC/68C **agreed** on the work plan of Pollution 2025, with a particular focus on the topic of 'cumulative effects' and 'multiple stressors/factors'. To further this issue, this intersessional workshop was suggested by the sub-committee, with the main objectives as follows.

- Discuss new results in this field.

¹Presented to the Scientific Committee as SC/68D/Rep/02.

- Discuss and evaluate new methods, techniques, and methodological approaches available for assessing the effects of stressors on cetaceans, both at the individual and at the population level. The primary focus is on cumulative effects and multiple stressors, one being chemical pollution, and to discuss how to evaluate the potential of such new methods in addressing these.
- Decide which study designs could be appropriate to address this topic.
- Evaluate cumulative effects with respect to their effects at the population level, taking into consideration the potential of various models, both individual models as well as population-based models.
- Discuss case studies on specific species and populations.
- Develop the criteria required for robust case studies.
- Discuss emerging issues of concern, including both emerging pollutants and other issues, such as direct and indirect effects of climate change.
- Discuss and suggest mitigation strategies.
- Recommend a strategy to progress this work and communicate the potential negative impact of multiple stressors on cetaceans to a wider audience.

3. FRAMEWORK FOR INTEGRATING DATA OF DIFFERENT SOURCES AND TYPES

3.1 Presentation of framework

Lori Schwacke, NMMF, Johns Island, USA

Schwacke provided a summary and background of the different types of conceptual frameworks that currently exist for integrating data of different sources and types and detailed two conceptual frameworks. She first described the Population Consequences of Disturbance (PCoD) framework, which was developed with the aim to consider how behavioural changes and/or physiological responses to disturbance ultimately may impact vital rates and dynamics of marine mammal populations (Pirota *et al.*, 2018)2018; and the Adverse Outcome Pathways (AOP) framework, which has generally been applied for assessment of chemical stressors. It describes the activity of a chemical from an initial molecular interaction through intermediate key events at increasing levels of biological organisation, ultimately connecting to an organism level outcome or a population level outcome (Ankley *et al.*, 2010). Whilst the AOP framework is well suited to integrate information from *in vitro* studies, as well as outputs from emerging methodologies (e.g. omics *approaches or high throughput screening*), AOPs to date have been described in qualitative terms rather than modeling quantitative relationships among organisational levels (Sewell *et al.*, 2018). In that respect, Schwacke discussed and used several studies that followed the PCoD framework to provide relevant examples of how to integrate data from varying levels of biological organisation to quantitatively assess population consequences of chemical pollutants for cetaceans.

Examples discussed included: (1) using an Effects of Pollutants on Cetacean Populations (SPoC) model to infer population consequences of PCB exposure on a number of species (bottlenose dolphin, humpback whale and killer whale), where different data types were integrated through physiological/ cellular responses through to health assessments and vital rates (Hall *et al.*, 2018); (2) population consequences of the *Deepwater Horizon* oil spill for Gulf of Mexico cetaceans, where the model integrated age-, sex- and exposure classes as well as included formal expert elicitation to better describe distributions of input parameters (Schwacke *et al.*, 2017); and (3) Veterinary Expert System for Outcome Prediction (VESOP) framework to estimate population survival rates from health data collected from a sample of individual animals (Schwacke *et al.*, 2019). In this case study, using such a VESOP framework has identified several key health parameters that predict survival (e.g. individuals diagnosed with inflammation and anaemia). Through exploring these various single stressor studies, it was demonstrated that a firm understanding of processes is needed before trying to develop a model to integrate data, and that often many contextual variables, such as sex, age, and reproductive status, complicate exposure characterisations. In addition, it was noted that a lack of baseline data is a large source of uncertainty, particularly for information regarding adult survival rates. The transitions involving the health of an individual can be particularly difficult to measure, and so studies often rely on data with high uncertainty, including information based on extrapolation from other species, however, it was then demonstrated through example 2, that expert elicitation may help in some of these circumstances to synthesise diverse information and sparse data. In addition, significant advances have been made through: (1) long-term monitoring studies; and (2) collection of standardised and consistent health measures that can be pooled across study sites covering a gradient of stressor exposure.

Integrating data across organisational levels is even more important when considering the combined effects of multiple stressors as different stressors with potential commonality in their effect pathways may combine and interact at various levels. An extension to the PCoD framework, the Population Consequences of Multiple Stressors (PCoMS), was proposed by an US National Academies Panel (National Academies of Sciences Engineering and Medicine, 2017). In addition to the multiple exposures to stressors included in the PCoD model, the PCoMS model also includes multiple doses specific to an individual. In this way, one can investigate how different individual doses may interact to cause different effects on physiological and behavioural responses in a particular individual. While research to implement initial PCoMS models is

just beginning, it may provide a useful conceptual framework to organise and understand the underlying processes for combined and potentially interacting effects of multiple stressors. Schwacke concluded by stating the need to monitor vulnerable populations now, before stressors are introduced, to better input parameters in such a framework, with priority given to monitor the most sensitive parameters, e.g. adult survival rates in long-lived species, such as cetaceans. The need for collaboration to build sufficiently robust datasets was also stressed.

The Workshop thanked Schwacke for presenting a range of case studies where multiple data sources were integrated to connect stressors to consequences for the population. The Workshop noted the potentially powerful tool the VESOP model offers for those who study and use biomarkers of chemical effects to evaluate the health of populations. In discussion, Schwacke confirmed that some of the markers identified in the VESOP example could potentially be useful to evaluate health across different species. However, given the uncertainty in reference ranges for many biomarkers in other species, the effectiveness of VESOP model approaches may be limited under such circumstances and should be used with caution. As income breeders bottlenose dolphins do not rely on energy reserves, which may explain the observed decoupling between body condition and 1-2 year mortality occurrence.

Therefore, some markers may be extrapolated, but not all. The Workshop also noted the importance of incorporating the level of uncertainty derived from the different steps in each section of the frameworks into the estimation of population consequences, so that managers can consider both variability and uncertainty and can therefore incorporate precautionary management if needed. To tackle this problem, Schwacke reaffirmed the implementation of expert elicitation workshops to improve parameters where uncertainty remains high.

Attention: SC/E, R

*The Workshop identified several models with the potential to integrate data on combined effects of multiple stressors. Long-term monitoring studies, to provide base line data and investigate temporal variations, are vital to these models and the Workshop **recommended** that researchers/Scientific Committee:*

- (1) *monitor vulnerable populations as much as is practical BEFORE potential stressors occur or as soon as possible after stressors become a factor;*
- (2) *archive samples collected opportunistically from potentially vulnerable populations for future assessment;*
- (3) *monitor the most sensitive sub-lethal parameters (e.g. body condition and reproduction); and*
- (4) *build comparable datasets across sites or even species, noting potential ecological fallacy and/or interspecies differences.*

Attention: SC/E, R

*The Workshop **noted** the importance of incorporating the level of uncertainty derived from the different steps in each section of the conceptual framework (National Academies of Sciences Engineering and Medicine, 2017) into the estimation of population consequences, so that managers can consider both variability and uncertainty and can therefore incorporate precautionary management if needed. Interim but formal statistical methods such as expert elicitation can help to fill gaps where current information is lacking.*

4. NEW METHODS/METHODOLOGICAL APPROACHES FOR ASSESSING CUMULATIVE EFFECTS

4.1 Markers of toxicological pathways

Christina Fossi, University of Siena, Italy

Fossi presented case studies and methods available for the assessment of cumulative effects in the Mediterranean Sea, which was identified as a biodiversity hotspot, but also an area impacted by multiple direct and indirect stressors, including ship strikes, POPs, emerging pathogens, climate change, food depletion and marine litter. Any one factor alone can impact cetaceans differently, decreasing survival, reproductive success or recruitment. However, cumulative, and interactive impacts can be far greater than any one factor alone, potentially affecting population stability of cetaceans in the Mediterranean Sea.

Three case studies were presented to illustrate the effects of multiple stressors. A study on striped dolphins as sentinels of marine health in the Mediterranean Sea showed that this species is most impacted by multiple contaminant loads, and genetic variability was linked to resilience (Fossi *et al.*, 2013). A study comparing fin whale exposure to ingestion of microplastics, POPs and emerging contaminants in the Mediterranean Sea and the Sea of Cortez showed a significant difference between the two sites, with a much higher microplastic load in the Mediterranean Sea (Fossi *et al.*, 2016). A model confirmed an overlap of fin whale feeding areas with microplastic hot spots in the Mediterranean Sea, which has a microplastic load that is nearly as high as in the North Pacific Gyre (Fossi *et al.*, 2016). Metabolomic approaches, which determine individual pathways impacted by microplastic exposure, were successfully applied in both fin whales

and striped dolphins (unpublished data). A third case study of Cuvier's beaked whale population in the Pelagos Sanctuary, Mediterranean Sea, investigated correlations between demographic data and ecotoxicological responses (Baini *et al.*, 2020). There was a clear trend of increasing POP levels with age and more than 80% of the population exceeded the generally-accepted threshold for impacts on longevity or reproductive output (Jepson *et al.*, 2016), while 50% exceeded the high threshold. It is believed that the massive ingestion of marine litter led to cumulative effects of contaminants in this population (Baini *et al.*, 2020).

A need to develop new tools to quantitatively monitor and study highly synergistic effects of independent stressor combinations was highlighted. New 'omics' developments and epigenetics can contribute to the field of cumulative contaminant effects. It was proposed that baleen whale species, specifically fin whales, could be potential indicator species of microplastics and marine litter in the environment (Fossi *et al.*, 2018). The value of an interface between science and policy to consider multiple sources of morbidity and mortality when developing conservation and management plans was emphasised.

The Workshop thanked Fossi for the detailed presentation of the use of sensitive biomarkers in the study of cumulative effects. In discussion, it was recommended that the identification of hot spot areas like the Mediterranean Sea can be useful in impact assessments of threatened species. Monitoring of cetacean microplastic ingestion was also recommended to define categories of marine litter and to suggest mitigation strategies for identified litter. The Workshop acknowledged that the 2019 IWC workshop 'Marine Debris: The Way Forward' (IWC, 2021) had already recommended the formation of an international treaty for marine debris mitigation, which is currently being realised through the United Nations. The IWC Marine Debris workshop suggested that to avoid duplication of resources, the current workshop would support the previous IWC recommendation on developing an international treaty, and **agreed** that the current workshop could provide new elements for consideration in the mitigation of marine debris to the UN initiative.

Attention: S

The workshop **reiterates** previous recommendations (SC19195) made by the 2019 IWC Marine Debris workshop on the development of an international treaty for the management and mitigation of marine debris and **recommends**: (a) the report of this Pollution 2025 workshop be shared with the UN initiative (UNEA); and (b) where possible workshop participants continue to provide input in the form of new research/elements to the initiative regarding mitigation of marine debris.

Attention: SC/E

Recalling **recommendation** SC19193 the Workshop reiterated the need to identify potential indicator species in each sea (e.g., fin whale in the Mediterranean) for microplastics and marine litter in the environment.

4.2 Toxic effects of POPs on marine mammals

Kelly Robinson, University of St. Andrews, UK

Robinson introduced a method which allows researchers to use tissue culture approaches (specifically adipose tissue culture) to study multi-level POP effects on marine mammals in remote field locations. Adipose tissue is important, especially in marine mammals, as it provides essential resources involved in foraging ability, accumulation of contaminant loads, and affects both body condition and thermoregulatory ability. Adipose tissue culture techniques are incredibly powerful tools to investigate how the adipose tissue functions, especially in relation to different hormone levels, life history stages and pollutant exposures. Understanding how tissues function is essential to gaining a better idea of how stressors are affecting these tissues, which can then be used to inform what is occurring at the individual and population levels. Due to obvious constraints e.g. ethical constraints, logistical constraints, low sample sizes/technical repeats, limited experimental conditions, limited survival of cells, etc., such techniques are rarely used in the field. It was therefore desirable to develop a tissue culture approach to study live blubber tissue in wild marine mammals in a remote laboratory environment. Knowledge on tissue function has been achieved in a number of marine mammal species, but usually on skin fibroblast cells (Burkard *et al.*, 2015), and adipose tissue has remained a challenge. This study now demonstrates that a tissue culture protocol can be successfully adapted for use in field conditions (Robinson *et al.*, 2018).

Importantly, there are multiple opportunities for contamination in the field (during sample collection, processing for culture and active culture in the media). Typically, contamination is only detectable in the field with visual changes in culture media which can take several days. Robinson explained that while only 5% of their samples were contaminated, they wanted to develop a way to detect contamination in real time while in a remote location. They discovered that a PreSens (Precision Sensing GmbH) planar optode system to detect oxygen changes, typically used in the food industry, worked well to detect contamination in cell culture media in real time. Such oxygen detecting optodes not only allow detection of possible contamination, but can also be used to collect physiological data by looking at, inter alia, oxygen use rates from the blubber tissue in culture. Establishing such a robust protocol for conducting tissue culture in remote

settings enabled data collection and experiments to be conducted over multiple seasons. Using this in-field tissue culture approach, it was possible to conduct *in vitro* experiments on blubber tissue from biopsies of wild seals to explore the effects of bioaccumulated POPs and acute POP exposures on adipose tissue function. POP burdens are high enough in seal pups to alter adipose function early in life (Robinson *et al.*, 2018). By analysing data from *in vitro* experiments alongside *in vivo* measurements, it was also demonstrated that POPs may disproportionately affect smaller individuals (individuals at the lower end of the mass ranges seen in seal pups) and could continue to have population-level impacts even when levels are relatively low compared to historical values (Bennett *et al.*, 2021).

The Workshop thanked Robinson for this interesting, exciting and impressive technique, which the Workshop acknowledged would be beneficial for cetacean fieldwork. Robinson strongly believes this method could be applied to cetacean species and various fieldwork locations with a few adaptations. In discussion, Robinson offered to discuss with any workshop participants how this method might be progressed or adapted for cetaceans. The possibility of taking tissue samples from individuals that had recently died was also discussed, and by using the oxygen sensing optode, this would give a good indication whether tissues are useable, although this is an area which needs further development. It was emphasised that there is a possibility for more work and research to be conducted on longevity of live samples, beyond the 24 hours achieved in this study. As the process involves generating trauma to the blubber tissue, however, the life span of the tissue is reduced compared to other tissue culture techniques. Workshop participants acknowledged that cell culture in cetaceans is often not as successful as pinnipeds. The Workshop **agreed** that this approach shows strong promise.

4.3 Biologging, telemetry

Kagari Aoki, University of Tokyo, Japan

Aoki offered updates on the use of aerial photogrammetry and tag-derived tissue density for the assessment of body condition of humpback whales (Aoki *et al.*, 2021). Tag-derived tissue density estimates for body condition determination is based on the concept that fatter animals are more buoyant than thinner animals, and hence sink more slowly (Biuw *et al.*, 2003). This method was initially applied to determine body density of elephant seals during drift dives (Aoki *et al.*, 2011), but this technique was also used successfully to determine body condition of humpback whales during gliding, which is observed in most cetacean species. Humpback whales that had a larger length-standardised surface area (calculated through the analysis of overhead images collected by UAVs) also had lower estimated tissue body density, which is indicative of a greater lipid store and hence better body condition. A Bayesian hierarchical model showed that the underlying tissue density decreased during the feeding season, adding confidence to the effectiveness of estimating body condition using this method. It was highlighted that future monitoring of energy stores at sea through on-board processing of dive data during longer term tag deployments can be used to assess where and how energy stores are gained or lost, and should be implemented. This will provide insights to assess trends in cetacean body condition in response to human activity and climate change, which can be used as an indicator for population and ecosystem health.

The Workshop thanked Aoki for the presentation and in discussion, highlighted the presentations recommendation that assessing body condition can be a tool to document climate change impacts.

4.4 Use of drones for cetacean research to estimate body condition and health

Fredrik Christiansen, Aarhus University, DK

Christiansen provided an overview of the advances made in the use of Unmanned Aerial Vehicle (UAV) photogrammetry to study the physiology and bioenergetics of free-living cetaceans. The steps required when conducting UAV photogrammetry were: (1) image quality control; (2) identifying the metabolically active areas of the species; and (3) calculating body condition from the residual of the relationship between body volume (or any other metric relating to the energy reserves of the animal) and body length. Changes to the body condition of whales during migration, pregnancy and lactation can be quantified to inform the energetic cost of different stages in the reproductive cycle of whales. Several case studies were used to illustrate how measures of body condition can evaluate multiple anthropogenic stressors on individuals and populations. A study of southern right whales (*Eubalaena australis*, SRW) in South Australia showed that female body volume declined proportionally to the growth in body volume of their dependent calf, indicating energetic transfer (investment) between the mother and calf. A subsequent cross-population comparison of three healthy SRW populations with the declining North Atlantic right whale (*Eubalaena glacialis*, NARW) population showed that NARW were in comparatively poorer condition and that the NARW somatic growth rate had decreased over time. A study of survival in gray whales (*Eschrichtius robustus*) during a recent unusual mortality event (UME) in 2019-20, showed that all age classes, except calves, declined in body condition during the UME. The study further showed that emaciated stranded animals had a body condition of approximately -45% (45% lower body volume than the population average), which could be the lower threshold for survival for this species. A limitation of the method is that once an animal reaches a very poor condition it is not possible to visually detect a further decline in body condition (a further reduction in lipid or protein concentrations in various tissues). A third case study on Western Australian humpback whales showed a decrease in juvenile and adult body condition from the start to the end of the migration period (June-October) using absolute changes in body condition.

The Workshop thanked Christiansen for his presentation. In discussion, it was suggested that this method should be applicable across species regardless of size, because it is standardised for body length, although it may be difficult for species, such as dolphins, that do not linger (and hence provide opportunity to measure stretched-out body length) at the surface. Long-term impacts on body condition due to chronic stressors, such as climate change, may best be studied through inter-population comparisons across several years (to increase sample size). As the technology develops, cheaper UAV systems with good cameras have the potential to make the method more accessible. Several limitations were acknowledged, including: (1) the lack of long-term data due to the novelty of the technique; (2) fat reserves that cannot be seen in dorsal view with the drone; and (3) visibility limits due to turbidity in the water. Potential ways of addressing these limitations included reiterating the requirement for high quality images in which the animal is straight and completely visible at the surface, as well as capturing video footage from which images can be selected. It was acknowledged that lipid and protein content in different tissues cannot yet be measured with this method, but that it is a likely next step.

4.5 New 'omics approaches to investigate cetacean health and responses to multiple stressors

Jo Kershaw, Plymouth, UK

Recent developments in high-throughput nanotechnologies and bioinformatics have enabled the examination of biological systems in increasing detail, which have allowed a more mechanistic understanding of complex cellular functions, cellular networks and signalling, organ homeostasis and whole organism physiological functioning. Collectively, these have been referred to as 'omics technologies', and, overall, the application of these new technologies and techniques aims to understand a complex biological system, considered as a whole. To date, the genomes of only nine marine mammal species have been fully sequenced and annotated. Kershaw described three main themes where these technologies are particularly important and being used in current research: to investigate overall individual health, to understand and measure stress responses (both acute and chronic stress) and how to investigate physiological changes associated with contaminant exposure. 'Omics' approaches to address such themes are being used to develop biomarkers that can then be applied to marine mammal populations.

After a brief description of the different levels of organisation (e.g. genomics: universal detection of genes; transcriptomics: the totality of the mRNA transcript; proteomics: the study of the entire set of peptides and proteins; and metabolomics: the study of all the intermediate products of metabolism), Kershaw described some of the challenges associated with 'omics approaches that are common across other cetacean research studies. For example, challenges include sample collection (blood, biopsies and breath), as well as the requirement for good baseline information to allow for interpretation of samples to assess between individual variation, as well as temporal changes within individuals. Examples of different 'omics approaches using different case studies were provided.

Kershaw presented the paper of Trego *et al.* (2019) who used transcriptomics to investigate overall individual health of bottlenose dolphins in the Southern California Bight using biopsied skin samples. This study is a good example of the importance of the ecological context when trying to characterise transcriptome variability between individuals, and demonstrates the use of transcriptomics as a tool to understand how physiological responses shift with changing environmental conditions or demographic states. Deyarmin *et al.* (2020) used northern elephant seals as a model species to investigate the downstream effects of both single and repeated stress responses on blubber tissue homeostasis. This experimental study administered adrenocorticotrophic hormone (ACTH) to stimulate the production of glucocorticoids by the adrenal glands, i.e. essentially stimulate a stress response in order to identify proteins that were expressed under various stress scenarios. The study found that distinguishing between single and repeated responses to stress was captured in blubber protein profiles. The changes in expressed proteins (e.g. heat shock proteins, metabolic enzymes, and metabolite transporters) suggest that repeated stress may affect blubber tissue proteostasis, mitochondrial activity, adipogenesis and metabolism in marine mammals. Hence, this study is one of the first to demonstrate that proteomics can be used as a novel method to identify chronically stressed animals, through differentially expressed proteins in adipose tissue. Simond *et al.* (2020) aimed to investigate the potential mechanisms of toxicity as a result of exposure to a wide range of organohalogen contaminants through metabolic profiling of the endangered beluga whale population in the St. Lawrence Estuary, Canada. A targeted metabolomic approach was used to characterise profiles of amino acids, biogenic amines and fatty acids in the skin of male belugas, and their relationships with blubber concentrations of major organohalogens were examined while accounting for differences in habitat use in the estuary. Although the mechanisms of toxicity of these organohalogens cannot be identified using the correlative approach used here, these results suggest that SCCP exposure in particular could alter lipid metabolism. These results highlight the important of considering ecological factors, like diet and habitat use, in metabolomic studies.

In discussion, the future of these 'omics approaches was considered, highlighting that as an increasing number of the health and stress biomarkers are identified, it would be advantageous to apply different biomarkers to at-risk populations and those that face multiple stressors. Combining the study of multiple genes, proteins and metabolites can produce a multiomics approach that provides a more holistic understanding of individual health. In line with that, the examination of

multiple tissues or body fluids will provide a better understanding of the whole organism's physiology and the consequences of changing environmental conditions. Further work into 'metagenomic' studies of marine mammals could be focused on the viral and microbial communities in these species, which will be valuable in identifying emerging diseases of potential concern.

The Workshop thanked Kershaw for a comprehensive overview of these new methods and the possibilities they open up for health assessment of cetaceans. It was acknowledged that one of the barriers facing the 'omics' field is the cost of sample analysis. However, cost is decreasing and thus 'omics' approaches will likely see more use in wildlife research. It was noted that moving from the 'shotgun' approach of looking at everything towards a more targeted approach, such as focusing on specific, validated biomarkers indicative of immune system function, chronic stress responses, or contaminant exposure, for example, will bring down cost as well. Concerns were raised regarding the storage of samples for 'omics' work; as such samples are very sensitive to degradation. It was explained that mRNA is more unstable than proteins and transcriptomics work therefore requires placing of samples as quickly as possible into temperature-stable storage media such as RNA-later. The need for studies to distinguish between pre- and post-sampling changes in gene expression, especially for samples collected from dead animals, was highlighted. Storing the tissues appropriately and as quickly as possible is critical to preserve that snapshot in time.

Attention: SC

The Scientific Committee is **encouraged** to continue investigating new 'omics' approaches as a means of screening for new biomarkers.

4.6 Sentinel parameters

Susan Bengtson Nash, AUS

An overview of the humpback whale sentinel program, which provides a reliable and consistent monitoring opportunity of the Antarctic sea-ice ecosystem through studying biopsied tissue samples of free-roaming humpback whales was presented (Bengtson Nash *et al.*, 2018). Various sentinel parameters (adiposity, diet and fecundity) have been identified and developed for humpback whales over the years, and seem to follow trends of Antarctic environmental variability. Some of the ecological tracer techniques to measure these sentinel parameters were described, e.g. the Adipocyte Index, which measures the change in volume of adipocyte cells, giving an indication of the individual's lipid reserves and thus body condition (Castrillon *et al.*, 2017), as well as the use of lipophilic POP burdens measured in adult male outer blubber layers. A few months of seasonal fasting resulted in an increase in chemical burdens in outer blubber layers of humpback whales - up to a 500-fold increase Bengtson Nash *et al.* (2013). As the outer blubber layer supports a number of vital functions (e.g. thermoregulation and buoyancy), this layer represents an increasing proportion of the individuals' remaining lipid stores during fasting, and so accumulates greater pollutant loads. Therefore, measuring POP burdens in the outer blubber layer of adult males can provide an indication of adiposity (Bengtson Nash *et al.*, 2018). Diet is measured through traditional carbon and nitrogen Bulk Stable Isotope analysis and fatty acid analysis, whilst fecundity rates are measured through migratory sex ratio, as well as steroid hormone analysis for pregnancy. The need to include disease and toxicity as sentinel parameters was highlighted, and the usefulness of including steroid hormones (e.g. cortisol) to measure stress, as well as working towards 'omics' approaches to contribute new biomarkers of toxicity and disease.

The longest record of annual measurements (13 years) is for the E1 migrating stock (east Australian migrating humpback whale population) and has captured two extreme climatic events in Antarctica: the La Niña event of 2010/11, and most recently, the anomalous climatic events of 2017. In these years, whales migrated in poorer body condition, fewer females participated in the migration, and higher calf mortalities were recorded. As such, this long-term monitoring and timeline of humpback whales provides confidence that, through standardised, consecutive, reliable and annual monitoring, it is possible to capture environmental trends in Antarctica's sea ice ecosystem. Through international collaboration, the sentinel program is able to capture samples of humpback whales from the Brazilian (A), western (D) and eastern (E1) Australian, New Caledonian (E2) and Colombian (G) breeding grounds, allowing for circumpolar surveillance of the Antarctic sea-ice ecosystem. The sentinel program offers a good approach to investigate cumulative stress through environmental monitoring by interrogating humpback whales for every signal that can be accessed and analysed through their blubber tissues.

The Workshop thanked Bengtson Nash for an excellent overview of the sentinel program and how the developing situation of climate change can both exacerbate known stress factors and lead to new ones, creating additional burdens to humpback whales. The limitations of using lipophilic POP burdens as markers of adiposity in delphinid species that may feed on multiple prey sources was discussed. As prey source affects POP accumulation and contamination, feeding on multiple prey sources could lead to regional variability in POP burdens. Concomitantly, the humpback whale, which feeds on one prey item and in one location, provides greater confidence that the variability in POPs is due to a change in diet or body condition. Nevertheless, if there is substantial variation in POPs within a population over time, body condition is the

first factor that should be investigated. The Workshop also noted alternatives to measuring body condition, such as visual health assessments, which may not be as accurate as other techniques, including the adipocyte index. It was noted that further work is planned to compare the adiposity index with other body condition matrices, such as photogrammetry. It was also noted that it was as yet unknown whether the adipocyte index can work for species other than humpback whales. In addition, the adipocyte index only captures the outer blubber layer, an important functional layer (e.g. used in buoyancy and thermal regulation), and so animals are thought to be in really bad condition if they need to utilise this layer, which could be a limitation of the technique.

Attention: S

The workshop **requests** the Secretariat to communicate with the Stockholm and Minamata Conventions to consider the explicit inclusion of cetacean blubber (of model species) as a core media for long-term (and retrospective) monitoring of chemicals of Arctic/Antarctic concern (CEACs) to demonstrate biomagnification potential and hereby facilitate expedited chemical regulation.

4.7 Epigenetic approaches

Ashley Barratclough, National Marine Mammal Foundation, San Diego, USA

Barratclough introduced the concept of epigenetics to enable DNA methylation analysis of remotely collected skin samples to provide a chronological age estimate of cetaceans. Age is important to improve our understanding of population dynamics, for example to know how many animals are contributing to reproduction. In addition, age can provide an indication of the health status by investigating whether an individual is undergoing age acceleration, i.e. where biological age (defined as the functional capability of an organ) is higher than chronological age (defined as time since birth). As different species age at different rates, it is important to create a species-specific age clock. Therefore, Barratclough described their first phase of this study, which was to establish the chronological aging clock of bottlenose dolphins. By using samples from 34 bottlenose dolphins of known-age ranges (from 8 months-58 years) from the U.S. Navy Marine Mammal Program, Barratclough described how she and her co-authors created the first multi-tissue bottlenose aging clock (Barratclough *et al.*, 2021). Barratclough and her co-authors were able to examine the changes in DNA over a defined time frame (at least 5 years apart) of the same individuals and identified methylated CpG sites (cytosine-guanine nucleotides) associated with biological aging. An elastic net regression analysis and identification of 195 CpG sites strongly correlated with chronological age ($R^2=0.95$), thus showing the ability to predict the dolphins age within two and a half years of the actual age. This diagnostic tool is currently being applied to wild dolphins from different geographical locations to assess the effects of environmental stressors on biological aging. Identifying biological aging sites that could be associated with specific pathologies or environmental stressors can provide a good indication of the health status of that individual. By comparing the complete medical, nutritional, and environmental history of each dolphin from the Navy population, it is possible to identify biological age changes for each individual and relate this as a proxy for health status. Comparison of both chronological and biological age allows determination of the presence or absence of 'age acceleration'. Are environmental factors driving age acceleration? Are there differences in age acceleration between geographic location based on variation in environments? Being able to use epigenetics to quantify health status as well as just the age demographic within the population could be pivotal to our understanding of the overall health of such populations. Comparing the chronological ageing clock obtained from the Navy dolphins with wild dolphin populations will allow assessment of the presence or absence of age acceleration. Toxins and poor nutrition are known to affect the human genome; PCBs and reduced food availability could affect cetaceans similarly. Further work addressing these issues is currently being undertaken by Barratclough and colleagues.

The Workshop thanked Barratclough for sharing these initial results and discussing the next phases and future work in the field of epigenetics. In discussion, it was acknowledged that this technology is still in its early stages, and that ideally there needs to be a larger sample size, noting this could be achieved relatively quickly through collaborations with other long-term studies, if appropriately preserved tissue samples are available. The major limiting factor is the cost of using this technology due to its novelty, however, as it becomes more readily available, these studies can be conducted on a larger scale. Concern were raised regarding the state of preservation of a sample, which could restrict the method utilised in this study. Collaboration with NOAA is working towards addressing this question, but skin samples successfully used in current studies have been stored in a freezer at minus 80°C for 20 years. It is therefore believed this technique can be used with archived tissue samples. Further concerns were noted with regards to age estimation in cetaceans that have very low metabolic rates, such as baleen whales. Although this may affect the precision of age estimation, Barratclough believes that in animals with low metabolic rates there are more opportunities to learn as methylation changes occur over longer periods; this may not be picked up in animals with faster metabolism.

Attention: SC, Sec, IWC database group, Industry, Research Community

The workshop **recommended** that an international database be established as well as sample archives, to analyse samples with new techniques.

Furthermore, the workshop **requests** the Secretariat to seek formalised arrangements with the global network of Environmental Specimen Banks (ESBs) to ensure the routine collection and archiving of cetacean tissues according to standardised protocols. ESB are the time capsules of the environment and allow for retrospective analysis as new information is gained. They also limit loss of valuable archived materials through e.g. researcher movement, freezer failures, unsuitable collection or storage etc.

5. CASE STUDIES

5.1 Deepwater Horizon oil spill

Teri Rowles, NOAA Fisheries, and Cynthia Smith, National Marine Mammal Foundation

The *Deepwater Horizon* oil spill exposed marine mammals to impacts through multiple significant routes, including inhalation, aspiration and ingestion. This susceptibility is largely due to their living at the air-surface interface; evaporated oil compounds created toxic fumes at the sea surface, and droplet size can be decreased by dispersants and potentially become more toxic. A case study of the Barataria Bay bottlenose dolphin population, in which injury was assessed between 2010 and 2015 during an unusual mortality event following the spill, was presented (Smith *et al.*, 2017). Poor health was indicated in 'oiled' individuals found within the spill footprint. There were significantly higher instances of lung disease, adrenal cortical thinning, and perinatal loss for oiled vs non-oiled individuals. Lung disease is likely to impact both individual and population health. Cortical thinning is of particular concern due to its importance in adrenal health, which is needed to adapt to environmental changes and for parturition. Perinatal loss, severe fetal broncho-pneumonia, and high perinatal stranding rates, along with high rates of maternal illness and placental dysfunction, overall indicate reproductive failure within the population. The long-term population trajectory will rely on metrics, such as maximum proportional decrease, years to recovery and lost cetacean years. The scientific community needs to learn from each spill in every country, as well as improve measurement of exposure, assessment, quantification and integration of live and dead animal data. To do this, there have been attempts to use expert elicitation to fill data gaps and improvements have been made to the understanding of aspiration/inhalation risks. Rowles and Smith emphasised that the Gulf of Mexico is a high-risk area due to continued oil and gas development. Overall, the *Deepwater Horizon* oil spill may have significant and long-lasting impacts on individuals, populations and habitats.

The Workshop thanked Rowles and Smith for their update and acknowledged that it is difficult to identify which components or types of oil are most responsible. Areas that need more investigation are: (1) the pathway of volatile organic compounds in droplets, specifically dosage; and (2) the mechanism of injury with adrenal cortical thinning. Comparative studies with human responders were suggested, specifically the Coast Guard response to *Deepwater Horizon* and previous work on 9/11 responders. These may provide a path forward in terms of long-term health and possible treatments. Suggestions for mitigation pathways included identifying means of effectively cleaning the surface water during spills without exacerbating conditions for cetaceans, as current dispersants can break down the oil in a way that is potentially more harmful.

Attention: I, SC, S, CG,G

The Workshop **agreed** that current dispersants can break down oil in a way that is potentially harmful to cetaceans (see also previous recommendation SC1608). It therefore **encouraged** the oil spill response community, National Governments, maritime industry, oil and gas extraction industry to:

- (a) consider the impacts on the physiology and health of cetaceans and other marine life of their surface oil response activities; and
- (b) identify means of effectively cleaning the surface water during spills without exacerbating conditions for cetaceans (and other marine species).

Attention: SC

Given the threat to cetaceans from oil spills the Scientific Committee is **encouraged** to: (a) study effects of multiple stressors; (b) identify areas of high risk for spills and high risk for cetaceans by establishing heat map and early warning systems; (c) follow existing guidelines and principles (e.g. NOAA guideline on oil spill response; and (d) on assessing exposure and impacts of oils spills on MarMam) (e.g. IPIECA/IOPG key principles for the protection, care and rehabilitation of oiled wildlife; wildlife response preparedness, etc.).

Attention: CG, I

The workshop recalled recommendation SC1609 calling on Contracting Governments and industry to share information on exposure of, and impacts to, cetaceans; increase efforts on prevention of spills; and focus research to improve tools to detect exposure and evaluate impacts of oil spills on cetaceans. Building on this, the Workshop **urged** industry to establish precautionary measures to prevent oil spills., e.g. by properly maintaining their current equipment and facilities, and increase efforts to contain oil in case of oil spills rather than the use of dispersants.

Attention: S, I, CG, SC, C, R

The workshop **requests** the:

- (a) IWC Secretariat: (i) circulate the report of this workshop to appropriate entities to communicate the workshop discussions; and (ii) draw the attention of the Commissioners to the report of the workshop and, in particular, the finding that oil exposure can lead to significant adverse acute and chronic pathologies (i.e. respiratory diseases, immunological disorders, endocrine disruption, reproductive disorders) threatening cetacean life with population effects; and
- (b) Commissioners bring this information to the attention of their relevant national authorities.

5.2 Arctic monitoring, real time emerging cumulative issues

Raphaela Stimmelmayer North Slope Borough, Alaska, and Gay Sheffield, University of Alaska Marine Advisory Program

Stimmelmayer presented a characterisation of multiple external stressors on Arctic marine mammal species through harvest monitoring and ongoing health assessments of landed Bering-Chukchi-Beaufort (BCB) bowhead whales. Overall, this has provided solid baseline data on population status and general health status, which are considered to be positive. Ongoing risk surveillance of bowhead whale habitat and increasing understanding about direct and indirect climate change impacts on the Arctic-subarctic marine ecosystem indicates an increasing complexity of environmental, ecological and anthropogenic stressors. For example, ongoing environmental and ecological changes are numerous, ranging from dynamic northward distribution shifts of Bering Sea Pacific cod and pollock; extended residence time and further northward movement of subarctic baleen whales; changes in timing of bowhead whale spring and fall migration, as well as novel overwintering in the Beaufort Sea; increasing killer whale-baleen whale predation; multi-year sea surface temperature departures from normal; continued decline in sea ice coverage; increasing presence of harmful algal toxins; ongoing unusual mortality events in gray whales, seals and seabirds.

With respect to human impacts, since 2019/20 novel fishing for Pacific cod and pollock has successfully expanded northward in the Bering Strait and into the southern Chukchi sea. Within the same time frame, maritime traffic along the Northern Sea route has significantly increased and traffic is anticipated to become year-round by 2022. In 2020/21, several foreign marine debris events, likely associated with northern fishing and commerce vessel traffic, have occurred along the Alaskan coastline within the Bering Strait region. Taken together, this indicates that a real-time status change of the Arctic-subarctic marine habitat is occurring from a low-level stressor exposure regime to a novel habitat with potentially concurrent high-level stressors. Given the current evolving dynamic landscape of known and emerging stressors within core areas of bowhead whale habitat, there is much urgency for federal co-management partners and coastal communities to engage in targeted research. Real-time Arctic stressor identification and impact characterisation is needed to develop actionable transboundary mitigation strategies. It was suggested that the accumulated ecological and biological knowledge about the bowhead whale can provide valuable, relevant and transferable information to manage core large whale habitats in the Arctic-subarctic and devise realistic management strategies for a multi-user/multi-cetacean species Arctic seascape.

The Workshop thanked Stimmelmayer for the overview and **agreed** that mitigations should be a priority for this workshop. These included identifying the source of marine debris, so that responsible parties could be identified and the problem addressed legally. Furthermore the future use of polycyclic aromatic hydrocarbon (PAH) fingerprinting (provided the source oil is on file) was suggested, which would allow a determination of exposure and oil source in case of unknown oil spill exposure (e.g. 2006 oil exposure in bowhead whales as evidenced by retrospective PAH tissue screening). It was further acknowledged that any mitigations need to be in a bilateral or transboundary capacity, and that change is needed quickly. This was emphasised as the Arctic is interconnected with other habitats and other nations. It was suggested that lessons learned from other areas be applied to better protect the Arctic.

Attention: CG, I

The workshop **strongly encouraged** governments to implement preventive policies and producers responsibility, and reiterated recommendation CC1406 asking the IWC (and other IGOs) to encourage their member states to review national level implementation of MARPOL and other conventions relevant to prohibition of dumping of waste/marine debris etc.

Attention: S, SC/E, CG, I

The Workshop **requests** the Secretariat and members of the Sub-Committee on Environmental Concerns and the marine debris ICG to continue international engagement on debris tracing and prevention (e.g. via monitoring of cetacean microplastic ingestion, Extended Producer Responsibility polycyclic aromatic hydrocarbon (PAH) fingerprinting), to pursue solutions on marine debris and to suggest mitigation strategies.

Attention: S, SC/E, CG, I

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Attention: S

The workshop endorses the importance of precautionary areas in Bering sea and Arctic waters as outlined in the Polar Code of the IMO at its MSC99 meeting 2018 (<https://www.imo.org/en/MediaCentre/HotTopics/Pages/Polar-default.aspx> Ship routeing in the Arctic).

5.3 Pollutants in cetaceans in Spain

Graham Pierce, Instituto de Investigaciones Marinas (CSIC), Spain

Pierce summarised the progress of the Transfers of Anthropogenic and Natural Stressors Involving Trophic Interactions of Ocean Nekton (TRANSITION) project. The project's aim is to model the effects of PCB ingestion on cetaceans at individual and population levels, and consequences for the ecosystem, with a focus on Galicia in Northwest Spain. POPs data were collected in fish and three cetacean species (common dolphins, bottlenose dolphins, and harbor porpoise (Pierce *et al.*, 2008)). Levels were substantially higher in cetaceans compared to fish, with the highest concentrations in males. A case study of common dolphins showed a downward trend in concentrations over time. Additionally, concentrations in males showed a positive correlation with body length. Comparisons across France, Galicia, and the Netherlands showed that concentrations varied across countries, although overall these remain high across Europe. A Leslie-matrix simulation model has been developed using mortality, reproduction, and PCB bioaccumulation, although it is still being validated at a basic level. A GADGET ecosystem model has also been developed using diet and prey consumption (Saavedra, 2017). It was emphasised that these models are preliminary and do not currently look at mechanisms. There is a need for more data from foetuses, calves and pregnant/lactating females. Future work also aims to extend the models to include relevant pathogens.

The Workshop thanked Pierce for presenting these TRANSITION project updates. It was noted that the project is still at an early stage, as it began in 2019 and was interrupted by the COVID-19 pandemic. PCB concentrations are used as a proxy for total PCB burden, but this may be skewed for animals in poor condition, where concentrations will increase. Future directions for the project include incorporating the relationship between PCB burdens and diet, as all study animals are known individuals, so dietary information is available, and looking at contaminants for transition through the food chain.

5.4 Multispecies PFAS assessment

Karen Stockin, New Zealand

Stockin presented an initial review on the magnitude and impacts of Per- and Polyfluoroalkyl Substance (PFAS) occurrence in stranded individuals from 12 odontocete species in New Zealand waters, including coastal species, such as Hector's, Maui's and bottlenose dolphins, as well as offshore species, such as Gray's beaked and pygmy sperm whales. The highest PFAS levels were found in Southern Ocean species frequenting New Zealand waters. In addition, offshore species had higher PFAS levels than coastal species. These findings were contextualised with a focussed discussion of a recently published paper (Stockin *et al.*, 2021) on common dolphins. Most PFAS were below the level of reporting in common dolphins, but the authors found PCBs and DDT above threshold levels despite both toxins having been banned in New Zealand since 1996. Common dolphin PFAS profiles did not vary significantly by body condition, life history or location, but a positive correlation between PFAS and trace elements was observed. The predominant PFAS compounds found in common dolphins were long-chain PFAS, including Perfluorooctyl Sulfonate (PFOS). Stockin also noted that a decline in PCB levels has not yet occurred in orcas feeding on stingrays and marine mammals in New Zealand waters. Next steps in the project will include the examination of toxins in a large dataset of common dolphins and pilot whales, as well as investigating contaminant burdens in a nutritional context.

The Workshop thanked Stockin for presenting these initial findings of PFAS patterns and profiles in New Zealand cetaceans. In discussion, Stockin recommended that future research should focus on multiple sampling opportunities of

the same individual and species to inform mechanisms of PFAS exposure. Discussion focused on how baselines can be determined, considering that stranded cetaceans may not represent a healthy population. In New Zealand, not enough species have been examined for contaminant levels and large datasets for baseline considerations currently only exist for the endemic Hector's and Maui's dolphins. It was argued that live stranded cetaceans may be representative of a healthy population, and it was highlighted that non-healthy population members need to be examined more closely for baseline evaluations.

Attention: SC/E

The Workshop recommended that in order to inform mechanisms and trends in legacy chemicals and exposure and effects to emerging contaminants of concern, future research should focus on multiple sampling opportunities of the same individual and species.

5.5 Baltic Sea – an ecosystem with multiple stressors

Ursula Siebert, Veterinary School Hannover, Germany

Siebert provided an update on the monitoring of harbour porpoises in the Baltic Sea, an ecosystem affected by multiple anthropogenic activities, such as shipping, fisheries, offshore construction, marine litter, chemical pollution, military and seismic activities. Due to the multitude of stressors, cumulative assessments and interdisciplinary approaches are required to understand ecosystem processes and impacts on harbour porpoises, especially the endangered southern sub-population, estimated at 600 individuals. Long-term study results on chemical pollution through necropsy, histology, microbiology, virology, parasitology, age structure, reproduction biology and genetics revealed that harbour porpoises had lower PCB loads than other marine mammals in the Baltic Sea, but that their livers had higher pentabromodiphenyl ether (PentaBDE) levels due to cumulative effects of contaminants. The highest number of lesions was found in the respiratory tract, decreasing lung capacity and reducing the ability to compensate stress and feeding interruption. There were higher incidences of parasitic infections, pneumonia and chronic disease compared to Arctic marine mammal species. It was concluded that Baltic Sea harbour porpoises were more susceptible to infectious diseases, and impacts on the endocrine and immune system when compared to animals from the Arctic area where animals experience less human pressure (Siebert *et al.*, 2020). Another study showed that many harbour porpoises, especially females, have a decreased life expectancy, with many dying prior to reaching sexual maturity (Dietz *et al.*, 2021).

In discussion, it was noted that there is a need for more interdisciplinary research on Baltic Sea harbour porpoises at the national and international level, as well as for long-term datasets to investigate temporal variations. The Workshop also noted the need for a systematic health investigations and interpretations by trained experts. It was proposed that such needs could be addressed by securing funding for Baltic Sea projects for transnational (rather than national) areas inhabited by marine mammal species. Additionally, it was recommended that an international database as well as sample archives, to analyse samples with new techniques, be established. The need for cooperation with international partners was emphasised, using new investigation possibilities to establish sensitive indicators for cumulative effects and to formulate a definition for international threshold levels, securing good environmental practices. Suggestions for mitigation included reduction of chemical pollutants, marine litter, underwater noise and bycatch.

The Workshop thanked Siebert for her presentation, particularly for the detailed list of recommendations. In discussion, the need to define sensitive indicators that are likely to be specific to areas and populations was further highlighted. It was emphasised that lung health is an important health indicator for the porpoises of the North Atlantic, because the lung tells much about other chronic conditions. The health of the hearing system also needs to be investigated which is essential for the vital echo location. In addition, for those non-target analyses, the immune system has potential for measuring chronic stressors, and genetics investigations are very promising for understanding exposure to multiple stressors. A combination of relevant indicators has to be assessed for future monitoring of populations, e.g. health of the respiratory tract, life expectancy, immune competence.

Attention: SC/E, R, CG

The Workshop highlighted the need for:

- (1) *interdisciplinary research on Baltic Sea harbour porpoise populations at the national and international level (Baltic Range states, ASCOBANS, HELCOM);*
- (2) *the continuation of long-term datasets (and the creation of new datasets) to investigate temporal variations; and*
- (3) *systematic health investigations and interpretations by trained experts.*

The workshop suggested that funding for Baltic Sea projects should be secured for marine mammal species – which may be transboundary- rather than for national areas, which may only hold part of a species' population.

Attention: SC/E

To assess the impacts of cumulative stressors the workshop **proposed** that at least five different health indicators be monitored (lung and ear status are the best early indicators of overall animal health).

Attention: SC/E

The Workshop **recommended** the Scientific Committee, in cooperation with international partners, formulate a definition for international threshold levels for indicators and, using new investigation possibilities, establish sensitive indicators for cumulative effects.

6. MODELLING THE POPULATION CONSEQUENCES OF EXPOSURE TO MULTIPLE STRESSORS

6.1 PCOMS and other models

Enrico Pirotta, St. Andrews/CREEM, UK

Pirotta presented recent work on assessing, predicting and managing the combined effects of multiple stressors on individuals and populations using the population consequences of multiple stressors (PCoMS) conceptual model. This model captures and connects multiple scales, targets and organisational levels. Quantifying interactions between stressors is a cross-disciplinary goal, but the definition of interactions and methods to detect them are inconsistent within and between disciplines. The dose-response function of a stressor is generally non-linear, which implies that traditional factorial studies, analysed using linear models, are inappropriate to detect interactions between stressors. All existing approaches lie along a spectrum of increasing assumptions on the underlying mechanisms. This assumption spectrum can be used to reconcile cross-disciplinary methodological differences for the analysis of combined effects. Mechanistic assumptions will often be necessary, because we generally do not have sufficient data to rely on data-driven approaches. The choice of the analytical approach to use in each scenario should take into account the management goals and associated predictive needs. Stakeholders should find a shared framework for assessing combined effects of multiple stressors. Population Consequences of Disturbance (PCoD) models, extended to multiple stressors (PCoMS), offer a unifying framework to achieve this goal.

The Workshop thanked Pirotta for his presentation. In discussion, it was noted that current knowledge of many of the mechanistic pathways in the PCoMS framework (e.g. changes in immune status) is mainly theoretical. The bioenergetic pathway is very useful in many cases, because even stressors that are not operating intuitively along this pathway ultimately result in energetic costs to the individual animal. However, other currencies might be needed in some cases. For example, health assessments on bottlenose dolphins exposed to the *Deep Water Horizon* oil spill have shown that body condition is not a good metric for assessing the health status of these animals; their survival probability and reproductive output are better predicted using other health indicators such as adrenal function and lung status. This case study will thus explore other currencies to integrate combined effects.

6.2 News of SPOC and contamination mapping tool

Jo Kershaw, Plymouth, UK

Kershaw presented the use of two publicly accessible R Shiny online tools, hosted by the Sea Mammal Research Unit at the University of St Andrews, Scotland *The Contaminant Explorer*², is a global mapping tool, which can display published data on the concentration of POPs and mercury in cetacean tissues. Researchers can choose individual species and contaminant classes. By zooming in on a specific area, local studies are displayed for the chosen species and contaminant class.

The Effects of Pollutants on Cetacean Populations (SPoC) model³ can be used to explore the effects of pollutants on cetacean populations using an individual based model approach. Dose-response functions to simulate impacts on calf survival and calf immunity, population structure, population specific vital rates and number of simulations can be adjusted for a given species using a Leslie Matrix Model. Model outputs include simulated population trajectories, population growth rates and maternal contaminant loads. The model has been used to study the effects of PCB exposure on global orca populations (Desforges *et al.*, 2017; Murray *et al.*, 2021) and highly vulnerable Indo-Pacific humpback dolphins (Guo *et al.*, 2021).

The Workshop thanked Kershaw for her presentation and **agreed** that the Contaminant Explorer is a useful tool. It was recommended that the resource should be updated and funds may be sought within IWC to do so. It was highlighted that as open-source tools, the model and the map: (1) provide good opportunities for outreach; (2) are useful for graduate students; (3) are excellent risk communication tools; (4) are accessible to lay-people; and (5) are useful for research

²http://www.smru.st-andrews.ac.uk/IWC_Contaminant_Explorer/.

³http://www.smru.st-andrews.ac.uk/IWC_PCB_Cet_Pop_Model_v8/.

if population parameters are well understood for a given species. It was noted that the IWC Scientific Committee had already recommended the maintenance and updating of the mapping tool. The Workshop recommended that funding requirements should be assessed to facilitate the update of the tool moving forward.

Attention: SC, NI

The workshop **recommended** that Kershaw assess the funding requirements to facilitate the update and maintenance of the Contaminant Explorer tool and submit an application to the SC Research fund.

6.3 *In vitro* and *in silico* methods to investigate multiple stressors in marine mammals

Jean-Pierre Desforges, University of Winnipeg, Canada

Desforges presented novel *in vitro* and *in silico* methods for investigating the multitude of chemical, environmental and anthropogenic stressors on marine mammals. Single chemical testing provides a means of better understanding which pollutants should be focused on, given how many there are. However, individuals are exposed to hundreds of chemicals at a time, making single chemical testing somewhat irrelevant for wildlife risk assessment. To address this issue, concentrated blubber extracts were used to develop complex and realistic chemical cocktails based on killer whale and polar bear carcasses (Desforges *et al.*, 2017). Immunotoxicological results *in vitro* were extrapolated to *in vivo* impacts, and overall results suggested that toothed whales are the most at-risk marine mammal group for POP toxins. While this method is targeted and presents chemical mixtures for more realistic scenarios, the species and extraction methods are limiting and it cannot account for non-chemical stressors. *In silico* risk-quotient modelling was explored as a means of extrapolating individual-level chemical impacts in causative laboratory studies to population-level impacts in marine mammals (Dietz *et al.*, 2018). This model translates critical daily doses (CDD) taken during laboratory studies and converts them to critical body residues (CBR), which can be calculated for marine mammals. Results found that in all study polar bear populations across the Arctic, cumulative chemical risk was above the no effect threshold (risk-quotient >1) and that PCBs accounted for ~90% of all risk. This type of model is easy to disseminate and to identify cumulative impacts, but it relies on laboratory animals and is semi-qualitative in terms of risk of effects.

Finally, dynamic energy budget (DEB) modelling was presented as a means of incorporating how energetics is used for an agent-based model that captures species-specific life history, ecology and population dynamics. It allows for different combinations of stressors and simulation scenarios to be used, such as food limitation, chemical pollution and infectious disease. The drawback is that DEB Theory is complex and thus difficult to learn and disseminate, and also requires a substantial amount of data. However, it is a universal theory that can be applied to any species and integrates multiple stressors into a single currency and modeling framework. Thus it is proposed as the ultimate integrating tool that can be used to explore multiple stressor impacts in individuals and populations across spatio-temporal boundaries. Overall, it was concluded that a broad toolbox is needed to study chemical and other stressors, as well as integrative modelling to incorporate multiple stressors into a common research framework.

The Workshop thanked Desforges for his presentation. In discussion, several cell types were suggested for *in vitro* impact analyses, such as brain tissue, to examine neurological function and adrenal glands to assess endocrine effects. It was noted that the inclusion of other stressors, such as shipping and noise, would be difficult to include *in vitro*. Exploration of a method that would include these stressors via a means of simulating these effects at a cell level was suggested. Concerns were raised over the inclusion of a mouse model, as this was not comparable to cetaceans for *in silico* modeling approaches like risk quotient analysis. It was noted that the inclusion of such models were justified as these are used in human toxicology studies, however, it was acknowledged that such data are unlikely to represent wildlife in terms of critical body residues. Exploring possible means of addressing this issue were suggested, such as scaling for body size.

Attention: SC/E,R

The workshop **recommended** further development of a broad toolbox (including *in vitro* and *in silico* methods, and other sensitive bioindicators) to study the effects of chemical and other stressors, as well as integrative modelling (such as dynamic energy budget) to incorporate multiple stressors into a common research framework.

Attention: SC/E

The workshop **agreed** that the use of cell and tissue culture and other *in vitro* approaches showed strong promise for allowing effect assessment and can be used to collect physiological data by looking at, *inter alia*, oxygen use rates from the blubber tissue in culture. The Workshop therefore **encouraged** the continued development and application of these (*in vitro* and *in silico*) techniques.

7. CONCLUSIONS AND OUTLOOK: EMERGING CONTAMINANTS, EMERGING ISSUES OF CONCERN DUE TO CLIMATE CHANGE

During the discussion on future work, it was highlighted that the workshop spent a considerable amount of time on methods and method improvements, but did not devote sufficient time for discussions of mitigation strategies, other than those recommended by Stimmelmayer and Siebert (see Item 5.1). The need for mitigation planning to prevent broad impacts due to future disasters, such as those documented after the *Deepwater Horizon* oil spill, was discussed. It was **agreed** that recommendations for the mitigation of individual pollutants are straightforward, but recommendations for cumulative stressors are more difficult and warrant further discussions. Whether the IWC has the remit to address mitigation measures specifically or should contribute to these matters at meetings of the United Nations or the Arctic Council was discussed, but no conclusions on which was most appropriate could be reached by this workshop. It was **agreed** that the IWC has been successful previously when recommending mitigation measures to different countries and that there are existing channels to move mitigation suggestions forward.

Attention: SC/E

Scientists across disciplines are **encouraged** to find a shared language and methodology to assess the combined effects of multiple stressors as it is a cross-disciplinary problem.

Attention: SC, R

The workshop encouraged the SC and wider Research Community to continue the investigation, and improve the understanding, of the cumulative impacts of human activity-induced stressors on marine ecosystems. These include, *inter alia*, climate change, ocean acidification, marine litter, noise, eutrophication, biomass overharvesting.

8. RECOMMENDATIONS

Attention: CG, S, SC/E, CC, I, R

The workshop emphasised the value of an interface between science and policy to consider multiple sources of morbidity and mortality when developing conservation and management plans and address the geopolitical importance.

The Workshop therefore **recommends**:

- (1) the Secretariat and the wider Commission continue communication and cooperation with range states and appropriate bodies e.g. NOAA, ASCOBANS; ASCOBAMS, etc. which develop management plans; and
- (2) improve, synthesise, and respond to emerging knowledge across all disciplines and sectors to include government, academic and industry information, and traditional and local knowledge.

Attention: S

The workshop **reiterates previous recommendations** for IWC to continue collaboration with the Arctic Council (SC1403), CCAMLR (CO1833) and other policy bodies to effectively communicate their expertise and the relevant parts of its annual SC report. The Secretariat is **requested** to establish observer status at these and other relevant organisations to expand the IWC's reach with policy makers.

Further, the Workshop **encourages** the IWC to work with these bodies and funding organisations (e.g. National Science Foundation (NSF)) in their aim to strengthen scientific cooperation and joint monitoring among the Arctic states, and with other states, organisations and stakeholders involved in Arctic research or traditional and local knowledge, with a focus on: (a) prioritising research issues, filling knowledge gaps, and developing mechanisms to share and exchange observational data; (b) real-time identification of arctic stressors and impact characterisation to develop actionable transboundary mitigation strategies; and (c) devise realistic management strategies for a multi-user/multi-cetacean species Arctic seascape.

Attention: S, SC

The Workshop **encourages** the Arctic Council and CCAMLR to consider the implementation of the great whales as sentinels of polar climate change and transboundary pollution.

Attention: SC

The workshop endorses the importance of precautionary areas in Bering sea and Arctic waters as outlined in the Polar Code of the IMO at its MSC99 meeting 2018: <https://www.wcdn.imo.org/localresources/en/MediaCentre/HotTopics/Documents/POLAR%20CODE%20TEXT%20AS%20ADOPTED.pdf> (accessed 15/03/2022).

9. CLOSING STATEMENT

Holm thanked all participants, Working Group leaders and rapporteurs. Staniland, on behalf of the IWC, thanked all participants for their contributions to the workshop. In particular, he thanked Holm for the organisation and smooth running of the workshop. The meeting closed at 23.20, Thursday 4 November 2021. The report was adopted by correspondence on 12 December 2021.

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Annex A

List of Participants

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Annex B

Agenda

1. Introductory items
 - 1.1 Welcoming remarks
 - 1.2 Introduction of participants
 - 1.3 Election of Chair
 - 1.4 Appointment of Rapporteurs
 - 1.5 Adoption of Agenda
 - 1.6 Available documents
 2. Background and Goals of Pollution 2025
 - 2.1 Pollution 2000 and 2020+ - achievements, open questions
 - 2.2 Pollution 2025 - goals and objectives
 3. Framework for integrating data of different sources and types
 - 3.1 Presentation of framework - Lori Schwacke
 4. New methods/methodological approaches for assessing cumulative effects
 - 4.1 Markers of toxicological pathways - Cristina Fossi, University of Siena, IT
 - 4.2 Toxic effects of POP's on marine mammals – Kelly Robinson, University of St. Andrews, UK
 - 4.3 Approaches on toxicological process and the respective pathways - Jean-Pierre Desforges, McGill University, CA
 - 4.4 Biologging, Telemetry - Kagari Aoki, University of Tokyo, JAP
 - 4.5 Use of drones for cetacean research to estimate body condition and health – Frederik Oscar Christiansen, Aarhus, DK
 - 4.6 Proteomics and other -omics biomarkers of use in cetacean physiology research to assess cumulative impacts – Jo Kershaw, Plymouth, UK
 - 4.7 Sentinel parameters – Susan Bengtson Nash, AUS
 5. Case studies
 - 5.1 Deepwater Horizon oil spill – Teri Rowles
 - 5.2 Arctic monitoring, real time emerging cumulative issues – Raphaela Stimmelmayer, USA
 - 5.3 Pollutants in cetaceans in Spain – Graham Pierce, ES
 - 5.4 Multispecies PFAS assessment – Karen Stockin, NZ
 - 5.5 Baltic Sea – an ecosystem with multiple stressors – Ursula Siebert, Veterinary School Hannover, GER
 6. Modelling the population consequences of exposure to multiple stressors
 - 6.1 PCOMS and other models - Enrico Pirotta, St. Andrews, CREEM, UK
 - 6.2 News of SPOC and contamination mapping tool, Jo Kershaw, Plymouth, UK
 7. Conclusions and outlook: Emerging contaminants, emerging issues of concern due to climate change
 8. Recommendations
 9. Other
 10. References
 11. Adoption of Report
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Annex C

Additional Literature⁴

A3.1 Additional Literature

- Alava, J.J. 2020. Modeling the bioaccumulation and biomagnification potential of microplastics in a cetacean foodweb of the Northeastern Pacific: A prospective tool to assess the risk exposure to plastic particles. *Front. Mar. Sci.* 7:566101. [Available at: <https://doi.org/10.3389/fmars.2020.566101>].
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A3.8 Outcomes and Recommendations

Previous recommendations are available from the IWC Database of Recommendations.