SCOR Working Group Proposal

Title:

Impact of biotoxins on marine apex predators in Upwelling Systems

Acronym: ToxMAP

Summary/Abstract

Unusual vertebrate mortality events may result from changes to the marine environment and those associated with harmful algae blooms (HABs) are increasing. Algal blooms can be exacerbated by human activity such as eutrophication and rising water temperatures linked to climate change. The ecosystem and public health threats posed by HABs are well recognised in some parts of the globe. For example, within the California upwelling system along the west coast of North America, the diatom genus *Pseudo-nitzschia* is known to impact fish, bird and marine mammal health, and result in shellfish fishery closures to protect human health. Although HABs are documented in eastern boundary upwelling systems in the Southern Hemisphere (e.g. Benguela, Humboldt), trophic effects in these systems are largely undocumented. In recent years there have been several unexplained mortality events affecting marine life across the Benguela Upwelling Ecosystem indicative of toxicosis via the biotoxin domoic acid (DA). Initial testing has identified DA in seals from South Africa (Gridley, unpublished data). This working group will establish connections between expert biotoxin scientists from regions where the effects are well understood, and those in the Southern Hemisphere where the impacts of biotoxin exposure are relatively unknown and only recently emerging. Expected outputs include knowledge transfer, training in optimal sampling and detection methodology, and collaboration on globally relevant scientific research articles. We will generate preparedness for toxic events affecting wildlife, aquaculture and fisheries in light of the climatic changes which are predicted to increase HAB occurrence and frequency across the globe.

Scientific Background and Rationale

Phytoplankton are microscopic algae that form the fundamental building blocks of marine food webs. However, when algae bloom at high concentrations, they can become deadly, generating poisons known as biotoxins that can have disastrous consequences to both marine life and human food security. The impacts of harmful algae blooms (HABs), principally from the genus *Pseudo-nitzschia* are considered by Trainer et al., (2010) to be especially problematic in upwelling systems and are best understood in the California Current System. Several species of planktonic diatomaceous algae, mainly in the genus *Pseudo-nitzschia* produce domoic acid (DA), a potent excitatory neurotoxin (Bates et al. 2018; Pitcher and Pillar 2010). The trophic transfer of DA up the food chain via filter feeders such as molluscs, crustaceans and planktivorous fish can lead to both acute and chronic toxicity in marine birds and marine mammals such as cormorants, sea lions, fur seals, otters and cetaceans, often leading to mass mortality events (Bargu et al. 2011; McGabe et al. 2016, Zabaglo et al. 2016, Work et al., 1993). In humans, ingestion of DA via food chain accumulation causes amnesic shellfish poisoning (ASP), which was first observed in Canada in 1987. This toxicity event alone, linked to the consumption of cultivated blue mussel (*Mytilus edulus*) affected 153 people, causing four deaths and significant long-term neurological issues in other victims.

Biochemically, DA is a glutamate agonist which targets the AMPA and kainite receptors in the body particularly affecting the brain. In laboratory trials with mammals, the responses to domoic acid are similar across taxa following a typical sequence of events: hyperactivity and sedation (with gagging and vomiting), akinesia, rigidity, stereotypic behaviour, lost of postural control, convulsions and death (reviewed in Tasker 2021). In Californian sea lions (*Zalophus californianus*) for example, chronic effects observed include atrophy of the hippocampus resulting in complete disorientation (Berman et al. 2002; Qiu et al. 2006). A range of symptoms including ataxia, head weaving, muscle tremors, seizures, and death occurred as a direct result of DA toxicosis (Gulland et al. 2002). Additionally, Brodie et al. (2006) observed stillbirths, abortions and neonatal death in pregnant females. In humans with ASP, adverse health outcomes include gastrointestinal distress, confusion, disorientation, seizures, short-term memory loss, and, in rare cases, death (Jefferey et al. 2004; Levebre and Robertson 2004). Consequently, the presence of DA threatens the prosperity of fisheries and aquaculture with economic losses and negative socioeconomic impacts among communities that are dependent on these industries and resources as well as the disruption of cultural and social practices and tourism (Hoagland et al. 2002; Dyson and Huppert 2010).

The focus of this working group will be on increasing preparedness for biotoxin impacts on the west coast of Africa where the Benguela Upwelling Ecosystem generates high levels of productivity and nutrient densities. Like other eastern boundary upwelling systems it is susceptible to HABs (Pitcher et al. 2010, 2020). Species of *Pseudo-nitzschia* including those known to produce DA such as *P. australis* have been identified several times in the Southern (South Africa) and Northern (Namibia) Benguela regions (Gai et al. 2018; Louw et al. 2018; Pitcher et al. 2014). On the east coast of Africa, within a Western boundary upwelling recent monitoring of phytoplankton and domoic acid levels identified *Pseudo-nitzchia* and peaks in DA during the austral winter (Kelchner et al., 2021).

In recent years there have been several unexplained mortality events affecting marine life across the Benguela Upwelling Ecosystem ranging from fish and crustacean species to marine mammals. High incidences of premature abortion have been documented at fur seal colonies throughout the Benguela and there have been increasing reports of lethargic, emaciated, disorientated and aggressive animals. Observations of stereotypic behaviours indicative of DA toxicosis have been recorded as well as seizing animals. Seal mortality along the west coast was particularly high in 2020 and 2021 when seal colonies in both Namibia and South Africa experienced elevated mortality across age ranges, with thousands of carcasses reported by members of the public and cleared from beaches by local municipalities. This continued into 2022, with crustaceans, fish and marine mammal species affected at multiple sites spanning the Benguela ecosystem. Preliminary testing has identified domoic acid in samples from necropsied seals and crustaceans, and further testing is underway (Gridley unpublished data).

Over the same time frame, avian influenza has killed tens of thousands of sea birds in both South Africa and Namibia, wiping out over 20% of the endangered Cape cormorant (*Phalacrocorax capensis*) population of South Africa in less than a year. Other critically endangered African sea bird species such as African penguin (*Spheniscus demersus*), bank cormorant (*Phalacrocorax neglectus*) and Cape gannet (*Morus capensis*) have also succumbed, with unsustainable losses. The lethal effects of domoic acid on Brant's cormorants (*Phalacrocorax penicillatus*) and brown pelicans (*Pelecanus occidentalis*) in the California Current have been documented, and in the latter many signs of neurotoxicity were identified (Work et al., 1993). It is therefore conceivable that biotoxin exposure is impacting birdlife in the Benguela as well - a possibility which warrants investigation as marine bird species in the region are facing extinction, notably the African penguin and bank cormorant. Indeed, seabirds in the Southern and Northern Benguela have recently been observed showing signs of exposure to toxic algal blooms (seizures, head tremors etc.) which could not always be directly linked to a known cause of death. A lack of capacity and resources meant these were not investigated more thoroughly for possibly biotoxins. As many marine top predator populations are already threatened, they are particularly susceptible to catastrophic events (such as the avian flu induced mass mortality recently seen in African penguin and cape cormorant), so understanding the effects of DA in these systems is critical. Therefore, sampling protocols and training are urgently needed to understand whether such deaths are indeed related to biotoxin exposure, while an improved understanding of the vectors of biotoxin accumulation and the clinical signs in avian species will assist monitoring efforts. In endangered species, such as the African penguin, this could also facilitate rehabilitation of affected animals and even more importantly – the response to unusual mortality events because the response to a transmissible disease like avian flu and DA exposure would likely be completely different.

Around Southern Africa, there has been no investigation into the impact of DA on marine top predators due to lack of funding, capacity, and research interest. Now is the time to focus scientific effort on the role of biotoxin exposure in UME, including an understanding of the trophic pathways and bioaccumulation of domoic acid. Understanding the lethal and sub-lethal effects of this biotoxin on marine apex predators is much needed and the working group will facilitate transfer of knowledge from similar species and systems. For example California sea lion and the brown fur seal (*Arctocephalus pusillus*, known as the Australian fur seal in Australia and as the Cape fur seal in southern Africa) belong to the same family, Otariidae, and thus the physiological and health effects of DA toxicity are expected to be the same as glutamate receptors in the brain are the same across these species.

The proposed working group will collaborate to document historic unusual mortality events and using the information available (particularly observer reports, post-mortem examinations and remote sensing data to quantify ocean colour which is related to phytoplankton blooms etc.) to assess the likelihood that these were linked to HABs. We will then assess the risk of biotoxins on marine species. We will develop protocols for best practice when investigating HAB impacts and further develop protocols for the monitoring for biotoxin exposure in top predators. Training on data collection, analysis and interpretation of a multi-disciplinary group will facilitate preparedness for HABs and associated biotoxin accumulation in marine systems.

Terms of Reference

1. Data Mining and Review: Undertake a thorough review to understand trends in apex predator mortality with a focus on upwelling systems. Use available archived data to assess the likelihood of unusual mortality events links to HABs. Where possible, data mine the archived information from these events to generate timelines and hypotheses on causative factors of mortality.
2. Understanding Species at Risk: Conduct a risk assessment of the potential impact of HABs on top predator species, with a focus on apex predators (otters, seals, cetaceans) including critically threatened sea bird species (African penguin, Bank and Cape cormorants) which are already suffering from multiple impacts (low prey availability, habitat degradation, avian influenza). Elicit expert opinion from within the working group and their networks, to characterize likely or realized behavioural and pathological indicators of biotoxin exposure across species.
3. Vectors and Trophic Pathways: Use existing data and expert knowledge to understand vectors and bioaccumulation pathways considering spatial and temporal dynamics in food webs and species differences in susceptibility.
4. Training: Conduct training on best practice for routine monitoring and responses to unusual and mass mortality events which may indicate biotoxin exposure.
5. Future Proofing: Provide tools which enable appropriate monitoring and responses for sickness and mortality associated with biotoxicity. Grant writing and fundraising to ensure continuation of working group and outputs.

Working plan

The working group will bring together key players from multiple continents and countries to work collectively on a common theme: to understand the impact of biotoxins on marine apex predators in upwelling systems. The group of full and associated members has been selected to ensure geographic representation and inclusivity, with both local and global experts. The group includes early and mid career researchers who are developing to become leaders in their fields and will benefit immensely from the knowledge transfer gained by taking part in this group. The working group is motivated by a common goal to understand better biotoxin impacts within the Benguela Upwelling Ecosystem, and the WG will use this system as a model for other areas where information is lacking (for example other Eastern and Western upwelling regions such as Humboldt and Mozambique) where HABs and their impacts are also starting to be identified as upcoming threats to marine life (Kelchner et al., 2021).

ToR	Actions	Timelines
1) Data Mining and Review	Summarize stakeholders responsible for collating and disseminating relevant information on mortality events List authorities holding relevant information environmental data relevant to HAB generation and persistence and those with data on HAB occurrence Collaborate to detail relevant mortality events which may be HAB related and summarize the data collected during these events.	Year 1
2) Understanding Species at Risk:	Generate lists and maps of species at risk Understand the trophic relationships of at-risk species Identify specialists and stakeholders working with and responding to those species at risk	Year 1
3) Vectors and Trophic Pathways	Collate existing data and call on expert knowledge to understand the vectors and bioaccumulation pathways considering spatial and temporal dynamics in food webs and species differences in susceptibility.	Year 1
4) Training:	Focused training: a) Working group veterinary experts (Gulland and Gardner) will train the WG and invited participants in the recognition of clinical and gross pathological signs indicative of DA toxicosis. b) Optimal training in multi-species protocols to determine toxicity, considering differential or synergistic diagnoses (e.g. avian influenza, brucellosis, morbillivirus). c) Laboratory experts (Gulland, Hall, Lefebvre and Kershaw) will train WG and invited participants in the appropriate field and laboratory methods for analyzing samples for the presence of DA. d) In addition, training on rehabilitation of marine life exposed to biotoxin, with a focus on threatened and endangered species	Year 1 and Year 2

	(such as African Penguin and Cape clawless otter). Development of training materials: Diagnostic field and post-mortem protocols, laboratory protocols Larger scale training for routine testing and in preparation of UME: Foundational training to include post-mortem examination, methods to collect the most appropriate samples, sample storage. Training will facilitate understanding of biotoxin exposure in apex predators by vets, rangers, fieldworkers and technicians so that appropriate diagnostic samples are taken on mass during UME. To include two-day exercise with a mass mortality scenario, using the Incident Management System (IMS). The IMS is a standardized approach to emergency management encompassing personnel, facilities, equipment, procedures, and communications operating within a common organizational structure. IMS can be scaled according to the size of disaster and can be up or down scaled as appropriate.	Initiated in Year 1 and piloted in year 2, rolled out in Year 3 Year 3
5) Future Proofing:	Identify future funding avenues and collaboratively apply for funding to ensure the longevity of the working group and its outputs. Generate training materials and protocols, distributed to stakeholders and practitioners to enable continuation of work over medium time frames (4-10 years). With additional funding, generate materials for citizen science data collection (such as utilizing existing or modifying mobile phone apps) and public awareness materials, to facilitate monitoring along coastlines as well as increase vigilance in the public of the risks associated with consuming marine resources which may be affected by HAB/biotoxins.	Year 3

Deliverables

The working group will generate preparedness for toxic events affecting wildlife, aquaculture and fisheries in light of the climatic changes which are predicted to increase HAB occurrence and frequency across the globe. The expected outputs include knowledge transfer, training in optimal sampling and detection methodology, and collaboration on globally relevant scientific research articles. There will be a strong emphasis on science communication, providing training materials and information (printed and online media) for wildlife practitioners, fishers and aquaculturists, as well as the general public to facilitate citizen science data collection and raise awareness of potential human impacts of biotoxin exposure.

The data mining and expert option focus of deliverables 1-3 are expected to generate publishable papers, in high impact, international journals such as PLOS, Frontiers and Scientific reports. These are also intended to stimulate debate and further research in the impacts of biotoxins on marine top predators within the WG and further afield. By working together, the WG will summarize and pool resources and knowledge to facilitate analysis of stored samples and publication of research findings (such as that from the 2020/21 UME of Cape fur seals in the Benguela). Providing publically available data to will contribute to national and international policy making.

Between meetings, WG will engage in number of science communication activities including communications with (e.g. talks, posters, infographics), and reports to government partners, conservation and environmental managers (e.g. SAN Parks, CapeNature Conservation), tourism industry partners (e.g. Two Oceans Aquarium) and community partners (e.g. local communities in rural areas).

Capacity Building

The lack of capacity to investigate biotoxin exposure is a barrier to understanding its effects on the marine ecosystem in this region. This WG will generate capacity for scientists working in low and middle income countries in Africa, including the training of promising early and mid career scientists. The opportunity to provide training at the SCOR Research Discovery Camp in Namibia would further widen the impacts of the Working Group, and is achievable with moderate additional funding which we will apply for through SCOR.

To date, there has been no scientific research focus on the role of biotoxin exposure in mortality events throughout the affected regions (Pitcher & Louw, 2021). The processes of diagnosis are highly specialised and the WG will benefit from the expertise of the experienced members from the USA and UK. Identifying biotoxins in organic samples and the effects of domoic acid on animals requires specialist training in sampling and analysis. Domoic acid is water soluble and is excreted quickly with a with a half-life of roughly 12 hours. Despite this, effects are rapid and highly lethal but it means that finding residual DA in affected animals is fraught with difficulties and appropriate samples (such as stomach contents, amniotic fluids and excreta) must be collected and stored appropriately. Domoic acid exposure can have long lasting detrimental effects such as brain damage resulting in seizures (Gulland 1998). Without thorough, individual histories, these effects are difficult to link to exposure events so that in the wild the origin of these events can be difficult to trace. Additionally, biotoxin exposure could act synergistically with other impacts (lack of food resources, pollutants, bacteria or viruses) and cause behavioural change resulting in reduced foraging success, further complicating investigation and identification of proximal causes of death. The protocols of post mortem investigation differ from standard practices, requiring instead the collection of samples that focus on the gastro-intestinal tract for traces of DA, as well as amniotic fluid in pregnant females. Pathologically DA exposure is most characteristically observed in the brain and heart, requiring careful extraction of these organs whole to enable identification of lesions and abnormalities. Training in these best practices is essential for understanding the causes of death. Standard necropsy procedures used in South Africa and Namibia completely missed detection to date until linkages were made with specialists (Gullen ,Hall and Kershaw) who could advise on best practice. The WG will build on, and further this knowledge transfer between interested parties to facilitate appropriate data collection.

The monitoring of algal blooms and their consequences for marine life is important since such events are associated with both natural processes under the influence of climate change such as sea temperature, currents, etc. (Probyn et al 2000, Zingone and Enevoldsen 2000) and human impacts through anthropogenic loadings of the ecosystem (Sellner et al 2003). Impacts of HABs can be large in extent and significant at the population level, especially if combined with other ongoing population impacts such as disease and prey shortages. This comprehensive scientific investigation and collaborative working group will form the foundation of future long-term monitoring for biotoxins and their impacts on wildlife in the region. One of the key visions of this working group is to broaden knowledge and understanding of the role and impact of HABs and biotoxins on marine top predators; to train researchers, conservationists and members of the public to recognise early symptoms of exposure and develop and deploy appropriate biological sampling methods for detection. This will enable earlier detection and better and more proactive responses.

Understanding – Through working group discussions (annually in person, quarterly in virtual meeting spaces such as Microsoft Teams, weekly through email), literature reviews and data mining we will use available knowledge from better studied ecosystems and species to develop appropriate sampling and observation methods to detect biotoxins in upwelling ecosystems. Focus will be on transferring knowledge and skills across the WG and to associated early and mid career researchers and associated students.

Training – With the understanding of the needs identified above, WG members will develop methods and be trained in specific protocols for data and sample collection and analysis (e.g. the detection of domoic acid through the application of the most appropriate analytical methods). All skills training will be undertaken across the WG from early career researchers to pathologists, and the appropriate skills then extended to the broader marine conservation networks in the region (e.g. local vets, marine rangers, bird rehabilitators and citizen scientists) through seminars, hands-on training and involvement in ongoing research activities. Training will be led by highly experienced WG members (Gullen, Hall, Kershaw, Gardner) and if necessary we will apply for additional funding through the SCOR visiting scholar scheme for an extended trip for Gullen or Hall to provide in *situe* training in Namibia, South Africa and Mozambique.

Community Awareness – A key goal will be developing science communication materials (posters, flyers, videos, social media content) to provide information to the broader public about HABs and biotoxins – their causes, impacts and symptoms **-** with a focus on their potential effects on humans, pets and marine top predators within the context of broader environmental impacts.

Working Group composition (as table).

Full Members (no more than 10, please identify chair(s))

Name	Gender	Place of work	Expertise relevant to proposal
1) Dr Tess Gridley (co chair)	Female	South Africa/ Namibia	Marine mammal specialist working in Southern Africa and leading investigation into UME in pinnipeds
2) Dr Grant Pitcher	Male	South Africa	Active specialist and South African governmental researcher working on HABs in Eastern Boundary Upwelling Systems
3) Dr Brett Gardner (co chair)	Male	Australia	Veterinarian working in Australia and Southern Africa specializing in pinniped health
4) Mr Mwiitantandje Shaanika	Male	Namibia	Marine Conversationist within the Namibia Nature Foundation. Instructor for the Albatross Task force and important liaison person for linking NGO's and Government organizations.
5) Dr Simon Elwen	Male	Namibia	Director of the Namibian Dolphin Project and regional specialist on cetaceans - including coordinating strandings response in Namibia
6) Prof Ailsa Hall	Female	UK	Exceptional scientist with extensive knowledge of factors affecting marine mammal health. Leads research into the impact of domoic acid exposure in Harbour seal population recovery.
7) Dr Frances Gulland (co chair)	Female	USA	Chair of the Marine Mammal Commission for the USA. Globally the first to identify effects of HABs on top predators and continues to be a leader in the field of research. 20 years' experience in research into the impacts of domoic acid toxicosis in marine mammals off the California coast.
8) Dr Andrea Marshall	Female	Mozambique	Elasmobranch specialist and director of the Marine Megafauna Foundation - a wide ranging NGO in Mozambique.
9) Dr Joanna Kershaw	Female	UK	Marine mammal scientist with experience in laboratory methods for extraction and detection of DA in samples from marine top predators and prey species using ELISAs.
10) Dr Kathi Lefebvre	Female	USA	NOAA scientist focused on effects of naturally occurring marine seafood toxins on wildlife and human health

Associate Member (no more than 10)

Name	Gender	Place of work	Expertise relevant to proposal
1) Mr Mduduzi Seakamela	Male	South Africa	South Africa Governmental head marine mammal scientist
2) Dr Katrin Ludynia	Female	South Africa and Namibia	Sea bird specialist and project manager Southern African Foundation for the Conservation of Coastal Birds (SANCCOB)
3) Ms Lisa Mansfield	Female	South Africa	Technical skills in identification in DA vectors (molluscs) and interactions of Harmful Algal Blooms (HABs) and the effects on deoxygenation and ocean acidification
4) Mr Naude Dreyer	Male	Namibia	Director of Ocean Conservation Namibia, Extensive field skills working on multiple species.
5) Dr Andrea Marshall	Female	Mozambique	Director of the Marine Megafauna Foundation with 6 research stations in Mozambique. Lead author of the IUCN's Red List assessments for both species of manta rays and a member of the IUCN Shark Specialist Group
6) Ministry of Fisheries and Marine Resources Representative (as nominated internally)	na	Namibia	National knowledge on top predator abundance, distribution and health status
7) Ms Katie Reeve-Arnold	Female	Mozambique	Marine Research Director at All Out Africa in Tofo, Mozambique. Experienced in top predator ecology, sediment and water sampling as well as citizen science data collection
8) Dr Luca Mendes	Male	South Africa	Veterinarian based at the Two Oceans Aquarium and working on marine top predators, including seals and penguins

Working Group contributions

1) Dr Tess Gridley: Co Chair - convening meetings and ensuring momentum between meetings to achieve WG objectives. Marine mammal scientist coordinating investigation into unusual mortality of Cape fur Seals in South Africa and Namibia.

2) Dr Grant Pitcher: Expert in HAB research and part of the GEOHAB for many years. Prolific academic outputs and detailed knowledge of the Benguela ecosystem .

3) Dr Brett Gardner: Veterinary oversight and extensive knowledge of pinniped and seabird biology and health. Co-chair of working group, will facilitate cohesion of the group and meeting WG objectives.

4) Mr Mwiitantandje Shaanika: Marine Conservationist and strong community and social services professional with a Master's degree in Zoology from Nelson Mandela University, SA.

5) Dr Simon Elwen: Marine mammal scientist and coordinator of strandings data from Namibia. Additionally, independent consultant involved in Environmental Impact Assessments in both Namibia and South Africa and can feedback to policy makers.

6) Prof Ailsa Hall: Laboratory methods for the detection of DA in top predators and vectors. Risk assessment modeling of DA exposure to top predators.

7) Dr Frances Gulland: Detection of DA in top predators. Protocols and methodology. Dr Gulland is the co-editor of the CRC Handbook of Marine Mammal Medicine and has coauthored over 300 publications on marine mammal health and disease ecology. Co-chair of working group, will facilitate cohesion of the group and meeting WG objectives.

8) Dr Andrea Marshall: Marine scientist specializing in elasmobranchs. Works extensively in Mozambique and can facilitate training and data collection to widen geographical impact of WG

9) Dr Joanna Kershaw: Will provide training in these laboratory methods, as well as quality control / assessment checks and trouble shooting for capacity building in other labs. Will provide advice and interpretation of data.

10) Dr Kathi Lefebvre: Will advise and train on 1) pathways of trophic transfer of algal toxins through marine food webs, 2) assessment of acute and chronic exposure risks, 3) identification of physiological health impacts related to low level chronic exposure, and 4) development of biomarkers of chronic exposure and disease

Relationship to other international programs and SCOR Working groups

None currently - but likely links to GlobalHAB, co-funded by SCOR and the IOC – an infrastructural programme on harmful algal blooms as well as the SOOS (Southern Ocean Observing System) – for potential early detection of HABS

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Appendix

Dr Tess Gridley

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Dr Grant Pitcher

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