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FAO FISHERIES AND AQUACULTURE TECHNICAL PAPER

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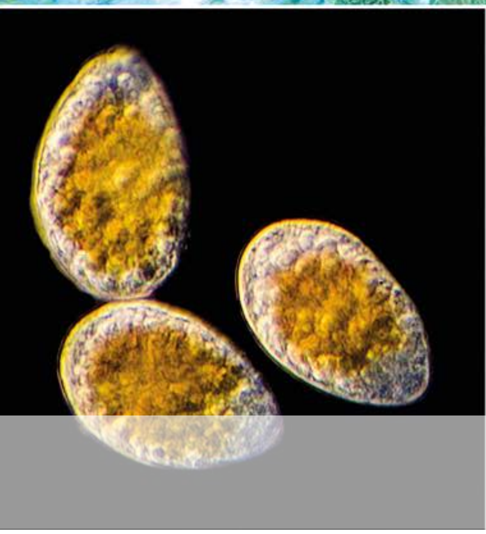
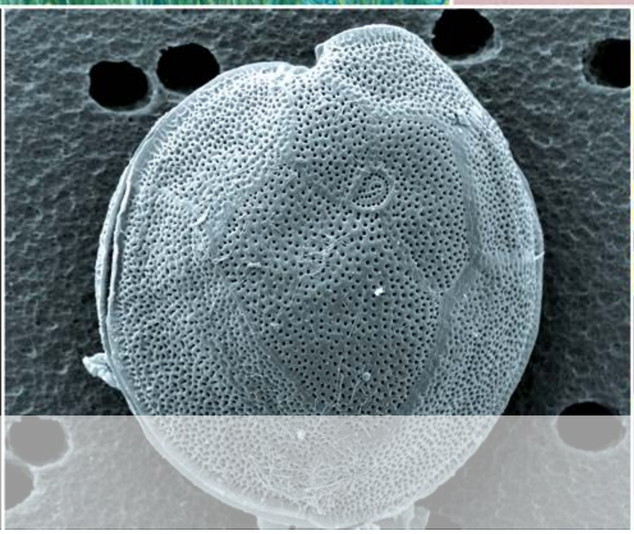
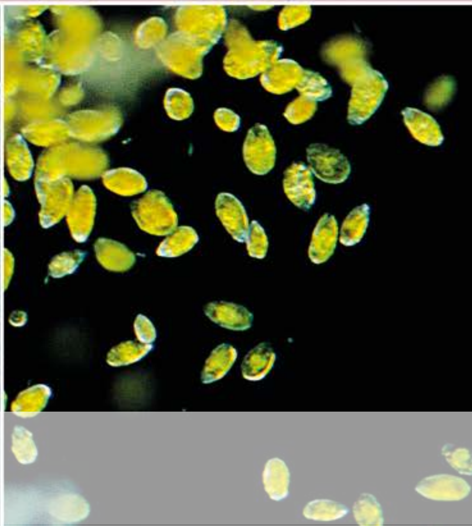
Joint FAO-IOC-IAEA technical guidance for the implementation of early warning systems for harmful algal blooms







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690

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Layout: Tomaso Lezzi

Preparation of this document

Over the past years, the participation of FAO in sessions organized by the Intergovernmental Panel on Harmful Algae Blooms (IPHAB) and other relevant expert meetings resulted in the identification of a number of issues and areas the needed attention.

One issue was for guidance in the implementations of early warning systems for HABs affecting food safety and food security. For this reason, FAO took the lead for the development of this joint Food and Agriculture Organization of the United Nations (FAO)-UNESCO's Intergovernmental Oceanographic Commission (IOC)-International Atomic Energy Agency (IAEA) International Atomic Energy guidance that was prepared by a multidisciplinary team of experts on harmful algal blooms.

The IPHAB Task Team on Early Detection, Warning and Forecasting for Harmful Algal Events served as a strategic advisory group for the development of this document.

Marie-Yasmine Dechraoui Bottein provided coordination support for the development of the document by liaising with experts, and by organizing the three involved agencies for the Expert Meetings that took place in October 2020 and January 2021.

Language editing, formatting was provided by Karen Englander, technical editing was provided by Esther Garrido Gamarro and Karen Englander. Layout was provided by Tomaso Lezzi.

Abstract

Harmful algal blooms (HABs) have significant impacts on food safety and security through contamination or mass mortalities of aquatic organisms. Indeed, if not properly controlled, aquatic products contaminated with HAB biotoxins are responsible for potentially deadly foodborne diseases and when rapidly growing, HAB consequences include reduced dissolved oxygen in the ocean, dead zones, and mass mortalities of aquatic organisms. Improving HAB forecasting is an opportunity to develop early warning systems for HAB events such as food contamination, mass mortalities, or foodborne diseases. Surveillance systems have been developed to monitor HABs in many countries; however, the lead-time or the type of data (i.e. identification at the Species-level, determination of toxicity) may not be sufficient to take effective action for food safety management measures or other reasons, such as transfer of aquaculture products to other areas. Having early warning systems could help mitigate the impact of HABs and reduce the occurrence of HAB events. In this regard, FAO took the lead in the development of a Joint FAO-IOC-IAEA Technical Guidance for the Implementation of Early Warning Systems for HABs. The document will guide competent authorities and relevant institutions involved in consumer protection or environmental monitoring to implement early warning systems for HABs present in their areas (marine and brackish waters), specifically for those affecting food safety or food security (benthic HABs, fish-killing HABs, pelagic toxic HABs, and cyanobacteria HABs).

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Abbreviations and acronyms

ANN	artificial neural networks
AOAC	Association of Official Agricultural Chemists
ARISA	automated ribosomal intergenic spacer analysis
ARPAL	Italian regional public agency for the protection of the Ligurian environment
ASP	amnesic shellfish poisoning
AZA	azaspiracid(s)
AZP	azaspiracid shellfish poisoning
BAWS	Baltic Algae Watch System (Sweden)
BEDI	benthic dinoflagellate integrator
BFAR	Bureau of Fisheries and Aquatic Resources (the Philippines)
BHAB	benthic Harmful Algal Bloom
BMAA	β-N-methylamino-L-alanine
CBA–N2a	neuroblastoma cell-based assay
CHABs	cyanobacterial harmful algal blooms
CP	ciguatera poisoning
CTD	conductivity, temperature and depth
CTX	ciguatoxin
DA	domoic acid
DAP	Department of Agriculture (the Philippines)
DISCO	Decision and Information System for the Coastal Waters of Oman
DSP	diarrhetic shellfish poisoning
DST	diarrhetic shellfish toxin
DTX	dinophysistoxin
EIA	enzyme inhibition assay
EFSA	European Food Safety Association
ELISA	enzyme-linked immunosorbent assay
ENSO	El Niño Southern Oscillation
ESA	European Space Agency
ESP	Environmental Sample Processor
EWEA	early warning early action
EWS	early warning system
EuroGOOS	European Global Ocean Observing System
FAO	Food and Agriculture Organization of the United Nations
FISH	fluorescence *in situ* hybridization
FLD	fluorescence detector

GIS	geographic information system
GOCI	Geostationary Ocean Color Imager
GOOS	Global Ocean Observing System
HACCP	Hazard Analysis Critical Control Point (United States of America)
HAB	harmful algal bloom
HABf INDEX	average water column ratios accounting for all harmful algae species and their weighted factor of risk to fish
HAB-Fish	fish-killing harmful algal blooms
HABON–NE	HAB Observing Network–New England
HAEDAT	Harmful Algae Event Database
HAIS	Harmful Algal Information System
HFR	high frequency radar
HPLC	high performance liquid chromatography
HTS	high-throughput sequencing
IAEA	International Atomic Energy Agency
IBM	individual-based models
ICES	International Council for the Exploration of the Sea
ICHA	International Conference on Harmful Algae
IEC	information education campaign
IFCB	Imaging FlowCytobot
Ifremer	Institut Français de Recherche pour l'Exploitation de la Mer (France)
IOC	Intergovernmental Oceanographic Commission of UNESCO
IODE	International Oceanographic Data and Information Exchange (IOC)
IOOS	Integrated Ocean Observing System (United States of America)
IPHAB	Intergovernmental Panel on Harmful Algal Blooms
IRD	Institute of Research for Development (New Caledonia)
ISO/IEC	International Organisation for Standardization/International Electrotechnical Commission
ISPRA	Istituto Superiore per la Protezione e la Ricerca Ambientale (Italy)
LC–MS	liquid chromatography–Mass spectrometry
LEK	local ecological knowledge
LFIA	lateral flow immunoassay
LGU	local government unit
LPTM	Lagrangian particle-tracking model
MBA	mouse bioassay
MERIS	Medium resolution imaging spectrometer
ML	machine learning
MODIS	moderate resolution imaging spectroradiometer
MS	Mediterranean Sea
N	nitrogen

NANOOS	Northwest Association of Networked Ocean Observing Systems
NASA	National Aeronautics and Space Administration
NEMO	Nucleus for European Modelling of the Ocean
NHABON	United States National Harmful Algal Bloom Observing Network
NOAA	National Oceanic and Atmospheric Administration (United States of America)
NODC	National Oceanographic Data Centre (International Oceanographic Data and Information Exchange)
NOAEL	no observed adverse effect level
NPZ	nutrient, phytoplankton, zooplankton
NSF	National Science Foundation (United States of America)
NSP	neurotoxic shellfish poisoning
NWS	National Weather Service (United States of America)
OA	okadaic acid
OBIS	Ocean Biodiversity Information System
ODA	Oregon Department of Agriculture (United States of America)
ODFW	Oregon Department of Fish and Wildlife (United States of America)
OLCI	The Ocean and Land Colour Instrument
ORHAB	Olympic Region HAB partnership (United States of America)
P	phosphorus
PADM	particle advection and dispersion model
PCM	prevention, control and mitigation (of HABS)
PHYSS	programmable hyperspectral seawater scanner
PICES	North Pacific Marine Science Organisation
PLTX	palytoxins
PNW	Pacific Northwest (United States of America)
POAS	Oceanographic and Environmental Program for Salmonids (Chile)
PRIMROSE	Predicting the Impact of Regional Scale Events on the Aquaculture Sector
PSMB	Programa de Sanidad de Moluscos Bivalvos or Bivalve Molluscs Sanitary Program (Chile)
PSP	paralytic shellfish poisoning
PST	paralytic shellfish toxins
PUFA	Polyunsaturated fatty acids
QGIS	Quantum Geographic Information System
qPCR	quantitative real-time polymerase chain reaction
RBA	receptor binding assay
RL	readiness level
ROMS	Regional Ocean Modelling System
ROS	reactive oxygen species
SEAFDEC	Southeast Asian Fisheries Development Centre
SEM	scanning electron microscope

SHA	sandwich hybridization assay
Si	silicon
SOOP	ship of opportunity program
SPATT	solid phase adsorption toxin tracking
SQL	Structure Query Language
SST	sea surface temperature
STX	saxitoxin(s)
TEK	traditional ecological knowledge
TEM	transmission electron microscope
TRL	technology readiness level
UV	ultraviolet
UW	University of Washington (United States of America)
VIIRS	visible infrared imaging radiometer suite
WDFW	Washington Department of Fish and Wildlife (United States of America)
WDOH	Washington Department of Health (United States of America)
WFO	Weather forecast office
WoRMS	World Register of Marine Species
WRF	Weather research and forecasting (University of Washington, United States of America)
YTX	yessotoxin(s)

1 Introduction

1.1 BACKGROUND AND MOTIVATION

Globally, there are 3 400 to 4 000 described species of marine microalgae but only 1 to 2 percent are considered to be harmful (Shumway *et al.*, 2018).

The generic term "harmful algal bloom"[1] (HAB) includes proliferations of microalgae in marine or brackish waters that can cause water discolouration and massive fish kills, contaminate seafood with toxins, or alter ecosystems and services in ways that humans perceive as detrimental.

The impacts and mass mortalities of marine species caused by harmful algae are not new and have been recorded for decades (Sellner and Rensel, 2018). However, there is growing concern that these events will increase due to accelerating global warming, climate change and anthropogenic activities.

Toxin-producing HABs, in particular, can contaminate seafood, drinking and recreational waters, or kill fish and wildlife. HAB events related with seafood biotoxins represent 48 percent of total HAB events globally (Hallegraeff *et al.*, 2021). Some HABs are responsible for potentially deadly food-borne illnesses, and some HAB toxins may be aerosolized and cause respiratory distress in susceptible or high-exposure populations (for example, aerosolized brevetoxins from *Karenia brevis* blooms in Florida, United States of America).

Six human syndromes are presently recognized to be caused by consumption of seafood contaminated with marine algal toxins:

- amnesic shellfish poisoning (ASP)
- azaspiracid shellfish poisoning (AZP)
- ciguatera poisoning (CP)
- diarrhetic shellfish poisoning (DSP)
- neurotoxic shellfish poisoning (NSP)
- paralytic shellfish poisoning (PSP)

In addition, cyanobacteria are identified as food-borne poisoning causative organisms.

The species considered harmful are catalogued in published texts (for example, in Lassus *et al.*, 2016) and now periodically updated in the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Lundholm *et al.*, 2021) and in AlgaeBase (Guiry and Guiry, 2021).

Non-toxin-producing but high biomass blooms, including proliferations of macroalgae, can also be harmful. These "nuisance" blooms can disrupt ecosystem services with impacts on the economy and human health; for example, desalination systems, the seafood industry, fisheries and aquculture activites and resources, the tourism sector, and recreation and maritime facilities such as ports. (Boerlage and Nada, 2015; Jasim and Saththasivam, 2017).

High biomass HABs cause environmental stress by reducing dissolved oxygen in seawater, causing hypoxia or anoxia and also expansion of dead zones leading to indiscriminate kills of marine life. Both toxin-producing and nuisance HABs result in the loss of livelihoods and food security issues for those dependent on seafood and other coastal or marine resources for their incomes or subsistence. Some HABs occurred in many regions of the world before coastal ecosystems were altered by human activities. Other terms that describe these naturally occurring phenomena are phytoplankton blooms, microalgal blooms, toxic algae, red tides, or harmful algae.

[1] Intergovernmental Oceanographic Commission. IOC HAB Programme. 2023. In: *The Intergovernmental Oceanographic Commission.* Paris, France. Cited 23 February 2023; http://hab.IOC-UNESCO.org

Monitoring and forecasting HABs can provide early warning of the presence of harmful algae or toxins, and the potential of a HAB event occurring, that allow regional authorities, industry, or individuals to take actions to minimize or mitigate public health, environmental, or economic impacts. A recent review that examined regional HAB observing programs in the United States of America, the European Union and Asia found that there is no global "one-size-fits-all approach", but regional responses and solutions can be reached through integrated and coordinated advances in scientific understanding of HABs (Anderson *et al.*, 2019). The following recommendations were provided by the review authors to advance "an integrative global ocean observing system optimized for HABs" building on the Global Ocean Observing System[2] and were considered in developing this guidance document:

- Deliver systems that are fit for purpose, with cost-effective and sustainable HAB forecasts that address the HAB-risk warning requirements of end-users, use (preferably automated) near real-time information to provide advanced HAB warnings and share knowledge of best practices across regions.
- Use a systems approach for sustained global ocean observing that incorporates earth observations/models with ecological knowledge/models to advance seasonal to decadal forecasts that will allow governments to plan and adapt to a changing marine environment while ensuring coastal industries are supported and sustained.
- Develop interfaces that provide mutual benefit to different sectors and address critical stakeholder needs, recognizing different priorities for regulators, industry, science and society, and provide complementary and accessible data sets.
- Promote the transformation of data into information that serves both science and societal needs including robust communication among stakeholders, partners and policymakers, and leveraging regional HAB observing systems to support technology comparisons.

In some countries, surveillance systems have been developed to monitor HABs; however, the lead-time or the adequacy of data (that is, identification and enumeration of phytoplankton at the Species-level, determination of seafood toxicity) may not be sufficient to effect timely and meaningful action to protect food safety or avoid costly fishery losses. Different approaches for HAB early warning systems (EWSs) that provide advanced warning of shellfish toxicity include the "traffic light" risk index approach used by the Scottish HABreports system (Davidson *et al.*, 2021) and the European Union Interreg Atlantic Area funded PRIMROSE project[3] that supports the aquaculture industry in Europe's Atlantic Arc region; near-real-time HAB risk mapping and warnings used by SoundToxins[4] and the Pacific Northwest HAB bulletin[5] in the United States of America; and the use of passive sampling devices for early warning of diarrhetic shellfish poisoning (DSP) toxins in shellfish (Hattenrath-Lehmann *et al.*, 2018).

Implementing regional or species-specific EWSs could reduce HAB impacts such as food contamination, fish kills or food-borne diseases. For this reason, the Intergovernmental Oceanographic Commission (IOC) HAB Programme's Intergovernmental Panel on Harmful Algal Blooms[1] (IPHAB, or the "Panel") established a Task Team on the Early Detection, Warning and Forecasting of HAB Events in 2019.

The Panel decided in 2021 to continue the Task Team to serve as a strategic and advisory

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[2] Intergovernmental Oceanographic Commission. 2023. Global Ocean Observing System. In: *The Intergovernmental Oceanographic Commission.* Paris, France. Cited 23 February 2023. www.goosocean.org

[3] Interreg Atlantic Area. 2023. Predicting the Impact of Regional Scale Events on the Aquaculture Sector (PRIMROSE). In: *European Regional Development Fund.* Potsdam, Germany. Cited 23 February 2023. www.shellfish safety

[4] SoundToxins. 2023. SoundToxins--a Puget Sound phytoplankton monitoring program. In: *Washington Sea Grant and Washington State Department of Health.* Washington, DC. Cited 23 February 2023. www.soundtoxins.org

[5] US Pacific Northwest HAB Bulletin. 2023. Harmful Algal Blooms. In: *HAB Forecasts Bulletins.* Washington, DC. Cited 23 February 2023. www.nanoos.org/products/habs/forecasts/bulletins.php

group for the establishment of guidelines, recommendations, and advancement of EWSs for HABs. Member state agencies were urged to support the implementation of EWSs for HABs to reduce the economic, social and human health risk of HAB impacts that affect seafood sustainability. As a follow-up action, the Food and Agriculture Organisation of the United Nations (FAO) jointly with IOC and International Atomic Energy Agency (IAEA) convened a group of experts to draft technical guidance to support managers in developing EWSs for HABs.

An EWS is "an integrated system of hazard monitoring, forecasting and prediction, disaster risk assessment, communication, and preparedness activities systems and processes that enables individuals, communities, governments, businesses and others to take timely action to reduce disaster risks in advance of hazardous events".[6] EWSs for impending hazards are not a new concept; ancient tribal communities observed signs in the oceans and the skies to warn of impending tsunamis and catastrophic weather events. The development of more advanced environmental hazard sensors and the instant communications afforded by modern technologies have allowed improved lead-times and accuracy for EWSs and an expanded list of hazards that are amenable to advanced warning. The importance of early warning to mitigate risk and reduce hazard impacts has been recognized by the global disaster management community explicitly in the Sendai Framework for Disaster Risk Reduction 2015-2030.[7] One of the nine targets of the Sendai Framework is to "substantially increase the availability of and access to multi-hazard EWSs and disaster risk information and assessments to people by 2030".[8] The international climate change community explicitly includes the need for EWSs that inform climate services and support decision-making in the Paris Agreement (Article 7, paragraph 7c).[9] This *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* aims to support countries or regions developing EWSs for HABs, and will advance broader EWS initiatives including multi-hazard EWSs that address HABs and their impacts to individuals and society.

1.2 OBJECTIVE AND SCOPE

Objective: This *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* will guide competent authorities and relevant institutions involved in consumer protection or environmental monitoring to develop and implement EWSs for HABs.

The intended goals of this technical guidance document, based on the targets of the Sendai Framework for Disaster Risk Reduction[8], are described in Figure 1.1. The overarching goal of a HAB EWS is to reduce individual and societal HAB impacts by enhancing response and preparedness to enable timely and coordinated action in advance of a HAB event by individuals, communities, governments, businesses or industries, and non-governmental partners.

An EWS for HABs should alert stakeholders of the occurrence and potential impacts associated with HABs. Impacts associated with HAB events include the presence of toxins in seafood or drinking or recreational waters affecting food safety and food security; ecosystem effects related to high algal biomass, mucus or foam; water discolouration;

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[6] United Nations Office for Disaster Risk Reduction. 2023. Shendai Framework for Disaster Risk Reduction 2015-2030. In: *Building Risk knowledge*. Geneva, Switzerland. Cited 23 February 2023. www.undrr.org/implementing-sendai-framework/what-sendai-framework

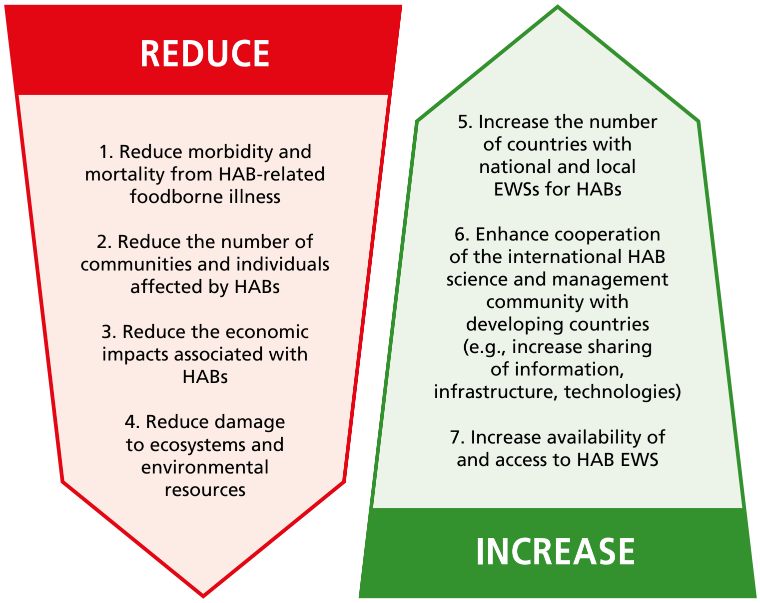
[7] United Nations. 2023. Office for Outer Space Affairs UN SPIDER Knowledge Portal. In: *Risks and Disasters, Early Warning Systems*. Vienna, Austria. Cited 23 February 2023. www.un-spider.org/risks-and-disasters/early-warning-systems#no-back

[8] United Nations Office for Disaster Risk Reduction. 2023. Shendai Framework for Disaster Risk Reduction 2015-2030. In: *Implementing the Shendai Framework*. Geneva, Switzerland. Cited 23 February 2023. www.undrr.org/implementing-sendai-framework/what-sendai-framework

[9] United Nations. 2023. United Nations Climate Change. In: *United Nations Framework Convention on Climate Change*. Bonn, Germany. Cited 23 February 2023. http://unfccc.int/sites/default/files/english_paris_agreement.pdf

and wildlife The different aspects of early warning of benthic HABs (BHABs), pelagic toxin-producing HABs, high biomass and fishkilling HABs, and cyanobacterial HABs (CHABs) are addressed. The EWS should include warnings of marine and brackish water HAB events so should include all marine HAB species and those from freshwater bodies that expand into estuaries and coastal areas. Different HABs have different impacts, and regional needs and response capabilities differ, so a HAB EWS should be regional and species-specific with the warning or endpoint based on the stakeholder or user's needs.

FIGURE 1.1
Harmful algal bloom early warning system targets



Source: Modified from Pearson, L. and M. Pelling. 2015. *The UN Sendai framework for disaster risk reduction 2015–2030: Negotiation process and prospects for science and practice.* Journal of Extreme Events. **2**(01): p. 1571001.

The guidance provides a roadmap for stakeholders on how to improve or implement an EWS for HABs and biotoxins, where appropriate. It is important to note that not all countries and institutions can implement the same level of EWS for HABs, and this guidance is intended mainly for those who seek to broaden existing early warning systems, or who are just beginning to consider putting a system in place. Prior to developing this *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms*, other complementary efforts have been produced and recognized:

- The IOC IPHAB's *Task Team on the Early Detection, Warning and Forecasting of HAB Events*[10] serves as a strategic and advisory group for the establishment of guidelines, recommendations, and advancement of Early Warning Systems, and as such, Task Team members have participated in the development of this *Technical Guidance* document.
- The GlobalHAB International Science Programme's *Theme 9: Observation, Modeling, and Prediction*[11] aims to advance the outcome of "Improved capabilities in early warning and real-time observation and prediction of HABs".

[10] Intergovernmental Oceanographic Commission. 2023. IOC-FAO Intergovernmental Panel on Harmful Algae Blooms (IPHAB). In: *The Fifteenth Session.* Paris, France. Cited 23 February 2023. https://ab.ioc-unesco.org/ioc-intergovernmental-panel-on-harmful-algal-blooms-iphab

[11] GlobalHAB. 2023. Global Harmful Algal Blooms. In: *GlobalHABs New Topics, Observation, Modeling and Predicting.* Oostende, Belgium. Cited 23 February 2023. www.globalhab.info/science/globalhab-new-topic/observation-modelling-and-prediction

- The *United States National Harmful Algal Bloom Observing Network* (US NHABON)[12] aims to efficiently and effectively integrate local, state, regional, and federal HAB observing capabilities and operational products in the United States of America to enable HAB forecasting and early warning.
- The International Conference on Harmful Algae (ICHA) hosted a special session on *Early Warning Systems (EWS) for Harmful Algal Blooms (HABs)* 14 October 2021 at the virtual ICHA meeting, jointly sponsored by FAO, IOC, and IAEA.[13] Presentations and informal discussion with experts allowed participants to further understand the process used to develop, implement and assess the accuracy of EWSs. At least eight country case studies on EWSs for HABs, representing diverse food safety and security foci and broad geographical representation, are published in the ICHA 2021 proceedings.

However, this *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* serves a specific purpose that is unique from the efforts listed. Not all countries and institutions can apply the same level of EWSs to HABs. As such, this Technical Guidance can be used as a roadmap for authorities and institutions in countries or regions to commence building an EWS or expand an existing system. This document provides guidance to develop and implement a system for early warning of HABs in marine and brackish waters and details HAB impacts that may affect food safety or food security.

1.3 CONTENTS OF THE TECHNICAL GUIDANCE

This *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* follows the general layout depicted in Figure 1.2.

FIGURE 1.2
Organisational structure of *technical guidance for the implementation of early warning systems for harmful algal blooms*

Guidance for early warning systems for HABs			
Chapter 2: Framework and shared concepts for HAB EWSs			
Chapter 3: Benthic HABs (BHABs)	Chapter 4: Pelagic toxin-producing HABs	Chapter 5: High biomass and fish-killing HABs	Chapter 6: Cyanobacterial HABs (CHABs)
For each HAB group (in Chapters 3-6): • HAB organisms and their impacts • Distribution, habitats and lifecycle • Dynamics and forcing factors • Monitoring, event response, and data considerations • Nowcasts, forecasts, modeling and prediction • Stakeholders and user needs • Case studies: HABs and EWSs in action			

Source: Elaborated by the authors.

[12] IOOS Association. 2023. National Harmful Algal Bloom Observing Network. In: *Implementing Strategy for a National Harmful Algal Bloom Observing Network.* Cited 23 February 2023. https://ioosassociation.org/nhabon

[13] International Society for the Study of Harmful Algae. 2023. International Conference on Harmful Algae (ICHA2021). In: *ICHA Conference Proceedings, Highlights and Abstracts.* Copenhagen, Denmark. Cited 23 February 2023. https://issha.org/publications-resources/conference-proceedings

Chapter 2 provides a framework for the planning and design of HAB EWSs (see Section 1.3.1 for a brief introduction). This framework allows the reader to situate themselves within the EWS development process. Chapters 3 to 6 each focus on a different group of HABs. Each HAB must be considered independently in an integrated HAB EWS due to the unique distribution, habitat or lifecycle of organisms within the group.

Chapter 3 addresses the novel challenges presented by benthic HAB (BHAB) species for an EWS. These benthic dinoflagellates are found on three-dimensional substrates or within the dynamic sediment–water interface at the seafloor. This chapter focuses on the toxin-producing BHABs *Gambierdiscus* spp. and *Ostreopsis* spp.

Chapter 4 addresses the pelagic toxin-producing HAB species that plague human health because of the consequence of shellfish consumption. As pelagic species, these HABs reside in open waters and produce various shellfish poisoning syndromes. Human food safety is threatened by these pelagic toxic HABs because the toxins produced by algae can accumulate in the food chain, in filter-feeding fish and in the bivalve molluscs and crustaceans that are harvested or farmed in most areas of the world.

Chapter 5 discusses the high biomass or high cell density HABs also found in the pelagic zone but that cause fish kills and other environmental impacts. Mortality of fish and invertebrates can be caused by mechanical action or by excretion of fish-produced toxins (icthyotoxins) and other bioactive compounds without harm to humans but may cause other environmental impacts. Along with pelagic toxic HABs (Chapter 4), high biomass HABs can have costly impacts on the seafood industry. They can cause marine faunal morbidity or mortality, including seabirds and marine mammals, by affecting the food-web or via a cascade of environmental stress.

Chapter 6 discusses instances when freshwater cyanobacterial HABs (CHABs) enter marine and brackish waters and the resulting impacts that can occur in the coastal environment.

As detailed below (Sections 1.3.2 to 1.3.6), each of Chapters 3 to 6 cover the following content for the different HAB groups:

- HAB organisms and their impacts;
- distribution, habitats and life cycles;
- dynamics and forcing factors;
- monitoring and events;
- data considerations;
- nowcasts, forecasts, modelling and prediction;
- stakeholders and user needs; and
- case studies of HABs and EWSs in action.

1.3.1 Early warning system development framework

Chapter 2 is dedicated to the principles and considerations of developing EWSs. Several key elements are common across EWSs that serve communities effectively. A people-centred approach, including local and regional stakeholders in the EWS planning and development process, is key to the success of EWSs for many different hazards. HAB EWSs must incorporate the following elements:

- Regionally specific knowledge of HAB risk, based on the systematic collection of data and HAB risk assessments.
- Observation, monitoring, detection, and forecasting of HABs and assessment of the risk of potential HAB-related impacts of concern.
- Dissemination and communication, by an official source, of authoritative, timely, accurate, and actionable warnings on the likelihood of HAB occurrence and the risk of potential HAB-related impacts of concern.

- Preparedness at all relevant levels to respond to early warnings with timely actions that reduce impacts to individuals, communities, industry, and society.

The development of an integrated EWS for HABs should follow the iterative stages of planning, implementation, use, and evaluation depicted in Figure 1.3(A). During planning, the user community should be directly involved in determining the purpose of the EWS, what knowledge exists, and what types of resources are possible for observation, monitoring, and detection of HABs and HAB toxins. Chapter 2 discusses the importance of considering stakeholders and user needs early and often in EWS planning and implementation. Implementation of a HAB EWS involves developing, implementing and refining models that can be used to predict the occurrence and potential impacts of a HAB event. Information must be disseminated to users in a planned and targeted manner to achieve the desired results – use of the information provided by the HAB EWS to inform response and preparedness. The HAB EWS should enable timely action in advance of an event by individuals, communities, governments, businesses and industry, and other partners. Feedback should be collected from users to evaluate the accuracy of EWS predictions (of both HABs and impacts) and inform improvements to the EWS. A detailed overview of how these processes form an integrated EWS for HABs is depicted in Figure 1.3(B).

1.3.2 Harmful algal bloom species and effects

This *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* addresses marine and brackish water HAB events. Thus, the EWS should consider all marine HAB species and those from freshwater systems that expand into connected estuaries and coastal waters. As noted above (Figure 1.2), the species addressed in this guidance fall into one of the four groups: benthic HABs (Chapter 3), pelagic toxin-producing HABs (Chapter 4), high biomass or high cell density HABs that kill fish and invertebrates by mechanical action or production of bioactive compounds and icthyotoxins (Chapter 5), and cyanobacterial HABs that occur in the freshwater-to-marine continuum (Chapter 6).

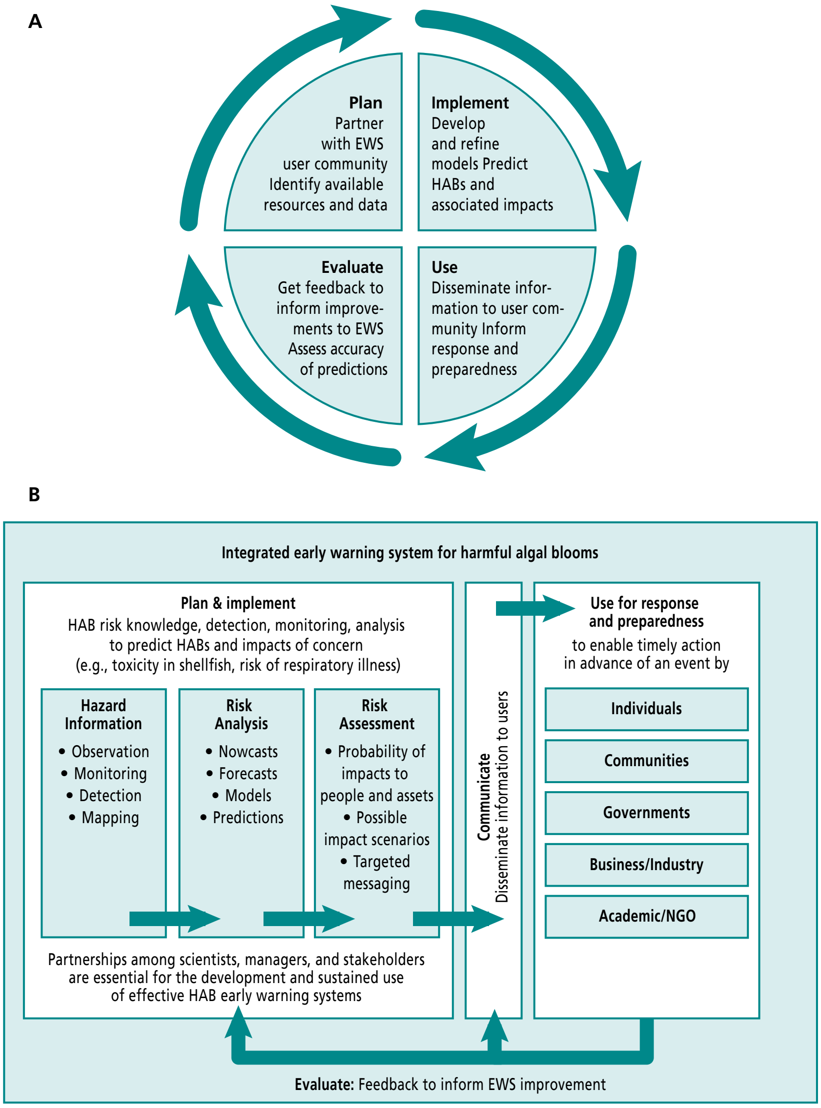
Early warnings for HABs with a direct impact on food safety and security (via contamination of food or of drinking water when desalination plants are involved) as well as those that affect human health (via aerosols or skin contact), disrupt ecosystems (hypoxia, anoxia, marine life kills), or the economy (through reduced fishing, fish kills, harvesting delays, product recall) should be considered.

1.3.3 Stakeholders and their needs

Consideration of stakeholders and user needs during the design and development of a HAB EWS is imperative. Only the user can advise on the purpose of the EWS and what information should be contained in targeted early warning messages. Users can be individuals, communities, governments, business or industry, or other partners (for example, academic scientists/researchers or non-governmental agencies). They are the group(s) that will use the information to act on preparation and response to HAB events. HAB EWS developments initiated by the scientific community must be driven by the needs of industry and regulatory authorities (Fernandes-Salvador *et al.*, 2021) to achieve sustained success.

The first question a user can ask is “early warning of what?” Knowledge of the occurrence of a HAB is useful in itself, but often the user wants to know whether HAB-related impacts of concern will occur. The HAB impacts of concern in an EWS should target one or more specific endpoints (for example, occurrence of a high biomass bloom, toxicity in fish or shellfish) or a broader array of information that informs a generalized response to a HAB event. Stakeholders can be users of any part of the EWS, users of the information provided by the EWS, or any individual or group that is adversely impacted by a HAB event. Stakeholder questions may include:

FIGURE 1.3
Harmful algal bloom early warning system overview



Source: elaborated by the authors
Note: (A) based on United Nations Office for Disaster Risk Reduction and adapted from World Meteorological Organization presentation on Multi-Hazard Early Warning Systems. (B) based on Global Ocean Observing System.

- If I fish or harvest shellfish recreationally or for cultural or subsistence purposes will the fish or shellfish be safe to eat?
- Will my business or livelihood be compromised due to the occurrence of a HAB?
- Is it safe to swim or be exposed to a body of water with a HABs?
- Does the government need to issue warnings or safety advisories due to the presence of toxins in-water or food sources?
- Will a HAB event have detrimental effects to an ecosystem and its marine life?
- Will a protected/threatened/endangered marine species experience illness or death as a result of a HAB?

HAB EWS users are also the group that can best advise on what types of data are available, or can be made available, within a country or region. They can comment on the degree of advanced warning that is necessary or desired to enable timely action. There is often a trade-off between cost (that is, the expense incurred by observing/monitoring/

detecting HABs or toxins), efficiency (the timeliness of early warning predictions), and the predictive accuracy of endpoints of concern. All should be considered carefully during design and implementation of a HAB EWS to best serve the needs of the stakeholder community.

1.3.4 Data considerations/requirements

There is no simple EWS model that fits all types of HABs. Data on HAB cells, toxins, fish and shellfish health, and ancillary environmental conditions can inform the development of an early warning system. Examples and case studies throughout this document highlight EWSs that utilize data from coastal resource monitoring programs, *in situ* sensor observations, remote sensing and modeling. For a wide application, a generic system, including agnostic data software should be considered. The use of open access software (SQL, QGIS) and the International Oceanographic Data and Information Exchange (IODE) National Oceanographic Data Centre[14] may be a good option globally. The important parameters to monitor, grouped as "core" and "supporting" data, must be defined for each type of HAB during the planning and design of an integrated HAB EWS framework. Best practices for the development of national or regional near real-time databases (with cloud storage/warehouse of data specific to the spatial scale at which the EWS is run, where possible) should be implemented. Data may be protected with a non-disclosure agreement if EWS output is open access, and long-term data should be broadly accessible via international data platforms such as the IOC–UNESCO Harmful Algae Information System (HAIS),[15] Harmful Algae Event Database (HAEDAT),[16] or Ocean Biodiversity Information System (OBIS).[17]

1.3.5 Nowcasts, forecasts, modeling and prediction

An EWS for HAB events must provide warning with sufficient lead-times (a minimum of 2 to 3 days in advance) to take effective action for management and mitigation. The EWS will need to be customized to adapt to the diversity and specificities of different HABs in different regions. Models may be appropriate in some locations, and development of models that work in different places instead of just one should be prioritized.

The degrees of advanced warning can vary substantially for the different types of HABs, the impact or endpoint targeted in the warning message, and differing availability of information/data. A nowcast informs the user about current conditions. It is a detailed analysis and description of the current situation. Satellite imagery, microscopic or molecular identification and cell counts of algae or in-water sensors for HAB cells and toxins provide "snapshot" data for nowcasts. A forecast involves predicting future conditions, typically using a model. Three types of HAB forecasts include industry alert "bulletin" reports compiled from multiple data sources including biotoxin or phytoplankton monitoring programs; particle tracking based systems that aim to identify production points of toxin-producing phytoplankton and track their dispersal using oceanographic models; and statistical models based on remote sensing that aim to predict toxic events under particular environmental conditions (Fernandes-Salvador *et al.*, 2021).

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[14] Oceanographic Intergovernmental Commission. 2023. International Oceanographic Data and Information Exchange (IODE). In: The National Oceanographic Data Centre (NODC). Silver Spring, Maryland. Cited 23 February 2023. About NODCs (iode.org)

[15] Oceanographic Intergovernmental Commission. 2023. The Harmful Algal Information System and Global HAB Status Report. In: *Data portal with species occurrence and event data.* Paris, France. Cited 23 February 2023. The Harmful Algal Information System – HAIS - Harmful Algal Bloom Programme (ioc-unesco.org)

[16] "International Oceanographic Data and Information Exchange" (IODE) of the "Intergovernmental Oceanographic Commission" (IOC). In: *Search Events.* Paris, France. Cited 23 February 2023. Harmful Algal Information System (iode.org)

[17] UNESCO. 2023. Ocean Biodiversity System. In: *Data access.* Oostende, Belgium. Cited 23 February 2023. https://obis.org

Nowcasts can inform forecasts when there is an understanding of the factors that govern the development and movement of HABs and their toxicity. The detection of HAB cells in the water column can provide early warning that shellfish may not be safe for harvest and consumption due to the presence of toxins. The first steps toward producing a forecast involve collecting baseline data on the presence/absence of HAB species and fish/shellfish toxicity and assessing what near-time data sources are available (Harley *et al.*, 2020). These nowcast data can be essential for protecting human health where recreational, cultural, or subsistence harvesting of potentially toxic fish and shellfish occur. They may provide the basis for models that advance forecasting efforts. Significant progress in HAB forecasting has been advanced in recent years with site-specific and region-specific models. In some regions, short-term and seasonal forecasts are used to predict the likelihood and severity of HABs that occur with annual or seasonal frequency. Predictive models require knowledge of the HAB species and its toxicity (including specific regional toxin profiles), a mechanistic understanding of how the bloom occurs within a region, and time-series of oceanographic and HAB data.[18]

This *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* and the case studies it reports provide an opportunity to evaluate what models and forecast systems are currently available and whether they are suitable for use in new locations. These systems may be of particular interest in countries or regions that do not currently have early warning for HABs or their impacts that may affect food safety or security.

1.3.6 Sustaining HAB EWS

The overarching goal and intended targets of this *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms* described in Section 1.2 and Figure 1.1 can only be achieved when HAB EWSs are sustained. Chapter 2 (Section 2.6) provides the following key requirements for a sustained HAB EWS:

- stakeholder engagement from the beginning;
- systematic and organized development and operation;
- operationality of individual components (for example, data sources, data platforms, models); and
- accountablity procedures (for example, feedback to inform EWS improvement, troubleshooting system failures, continual financial investment).

To ensure funding and other support for an operational HAB EWS and avoid data ownership issues, the EWS should be developed at a national level, or in selected cases, for several countries in a region with shared oceanographic boundaries and concerns. Stakeholders and users must be engaged early in the design process to ensure the system meets the needs of users, and citizen monitoring should be employed, where appropriate, in support of event reporting and environmental monitoring. The HAB EWS must be designed in a deliberate way to meet the specific needs of stakeholders and users within a country or region. It should provide advanced warning of the relevant HAB species or impacts of concern to allow preparedness and response that enables timely action by individuals, communities, government authorities, business/industry, or other partners. In cases where HAB EWSs are initiated by the scientific community, the needs of industry and regulatory authorities should be considered and addressed to sustain use of the system (Fernandes-Salvador *et al.*, 2021). Additionally, each component of the EWS should be operational independent of the integrated system. Procedures should be designed and documented for incorporating feedback to improve the EWS and for identifying and correcting inevitable system failures when they occur. The case studies provided in Chapters 3 to 6 provide examples of HAB EWSs that have been sustained successfully.

[18] U.S. National Office for HABs. 2023. Harmful Algae. In: *Prediction and Early Warning*. Cited 23 February 2023. https://hab.whoi.edu/response/prediction%20and%20early%20warning

1.4 CASE STUDIES

Incorporated into the HABs chapters are case studies of harmful algal blooms and EWSs in action (Table 1.1). They illustrate the initial problem, the local context and the processes towards a solution. Each case study answers the following questions for a specific type of HAB in a region where a HAB EWS has been established:

- What is the problem caused by the harmful algal bloom?
- Who are the stakeholders and what were their needs?
- What was the development status of the country or region in terms of monitoring?
- What approach/technology was taken to solve the problem? Monitoring?
- What forecast data were used?
- What early warning system was put in place?
- What were the results for forecast operation?
- What were the consequences of the early warning system for stakeholders
- What lessons were learned?

TABLE 1.1
Case studies discussed in this technical document

Chapter	HAB group	Phytoplankton species	Geographic location of case study
3	BHABs	*Gambierdiscus*	French Polynesia
3	BHABs	*Ostreopsis*	Mediterranean Sea
4	Pelagic toxin-producing	*Pyrodinium bahamense*	Philippines
4	Pelagic toxin-producing	*Pseudo-nitzschia* spp.	US Pacific Northwest
4	Pelagic toxin-producing	Multiple (*Alexandrium* spp.and *Gymnodinium* spp.)	Catalan, Mediterranean Sea
5	High biomass	Multiple (*Margalefidinium polykrikoides* and *Noctiluca scintillans*)	Oman
5	Fish-killing	Multiple (*Alexandrium catenella*, *Heterosigma akashiwo*, and *Pseudochattonella verruculosa*)	Chile
6	CHABs	*Anagnostidinema amphibium*, *Aphanizomenon flosaquae*, *Coelosphaerium kuetzingianum*, *Dolichospermum* spp., *Microcystis* spp., *Nodularia spumigena*, and *Planktrotrix asgardhii* (a more detailed list is found at Karlson *et al.* 2021, Chapter 6	Baltic Sea

Source: Elaborated by the authors.

1.5 ADDITIONAL RESOURCES

An appendix is included in this *Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms*. Appendix 1 provides resources biotoxin monitoring, management and regulation, based on monitoring of phytoplankton and on shellfixh toxicity testing. International organisation such as FAO, IOC and partners published resources focused on global events. Individual countries provide resources focused on their specific geographic region.

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Shumway, S.E., Burkholder, J.M., and Morton, S.L (eds) 2018. *Harmful Algal Blooms: A Compendium Desk Reference. First Edition. Introduction* (p. xxiii), 696 pp. ©2018 JohnWiley and Sons

2 General framework for developing an early warning system

When taking a general, conceptual view to Harmful Algal Bloom (HAB) Early Warning Systems (EWSs), it is important to first recognize the wide variety of HABs and how this variety influences the design of an EWS. This variety occurs with respect to the biology and ecology of different HAB species, to their impacts, stakeholder needs, and to funding. Structurally, EWS variety is also influenced by the data and tools available for visualisation, evaluation, dissemination, and prediction and forecasting. EWSs can range from simple rule-based systems that are easy to implement to varying degrees of intensive monitoring and forecasting systems that combine many sources of data and information in complex ways that require management. The framework outlined here should help orient an EWS developer to the steps needed and what a potential final product can look like, but it should be read with the understanding that HAB EWSs can come in many different forms.

The purposes of this chapter are to:

- Outline a general concept of a HAB EWS to guide the development of a system, using a System Overview approach.
- Orient the reader to where to situate their EWS in the development process, using the Technology Readiness Levels framework.
- Provide a framework that permits incorporation of the HABs data, as discussed in further detail in subsequent chapters.

While this chapter lays out a general conceptual framework and roadmap for building EWSs, the differences between different HABs and HAB management scenarios should be kept in mind. To give some orientation to the different types of EWSs that can result, this document discusses a range of specific case studies in the later chapters (Figure 2.1)

2.1 EARLY WARNING SYSTEM FUNDAMENTAL CONCEPTS

2.1.1 Variety of harmful algal bloom species and ecology

Dozens of species can cause HABs. They can occupy different aquatic habitats, or zones, of the water column and the benthos, and they can have different life cycles. Toxin production follows different environmental cues and drivers, and blooms have many different dynamics. The high diversity of HAB species is one of the main challenges to building a general and global infrastructure and expertise needed for HAB EWSs and requires a deep knowledge and understanding of toxin profiles and ecosystem dynamics. In this Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms we have divided HAB species into four main categories, discussed in Chapters 3 to 6: benthic, pelagic, high biomass fish kills, and cyanobacteria HABs (Figure 2.1).

2.1.2 Variety of impacts and stakeholder needs

HAB impacts are diverse. They have significant impacts on food safety and nutrition, through contamination of fish and shellfish, or massive kills of marine organisms, damage to ecosystems, and impacts on recreational activities. They are also a threat to freshwater security everywhere, including countries that depend on desalination plants for freshwater supply. They can occur in all oceans and seas of the world. Biotoxins associated with HABs, if not properly controlled, may be responsible for potentially fatal food-, water-, and air-borne illnesses and other severe impacts on human health. In addition to toxin production, where phytoplankton cell abundance increases rapidly, HABs can cause environmental stress by reducing seawater dissolved oxygen causing hypoxia and dead zones, with associated mortalities of macrofauna, and wild and farmed fish, causing habitat deterioration, a loss of ecosystem services, and reduction in income and livelihoods.

This wide range of potential effects of HABs means that there is a wide range of stakeholder needs in terms of EWSs. For an EWS to effectively identify the stakeholders and their needs, the process needs to actively involve local communities at-risk and must take into account monitoring and computational resources available to them. It must include public education and awareness of the risks, an effective means to disseminate warnings and alerts, and maintenance of a constant state of preparedness. A well-constructed, thorough and effective EWS must take into account risk analysis, monitoring and warning, dissemination and communication and possibly a response capability involving risk management decisions. This is best done through a process of knowledge co-production, involving stakeholders from the beginning. A more detailed discussion of stakeholder needs is provided in Section 2.4.

2.1.3 Variety of tools and data available

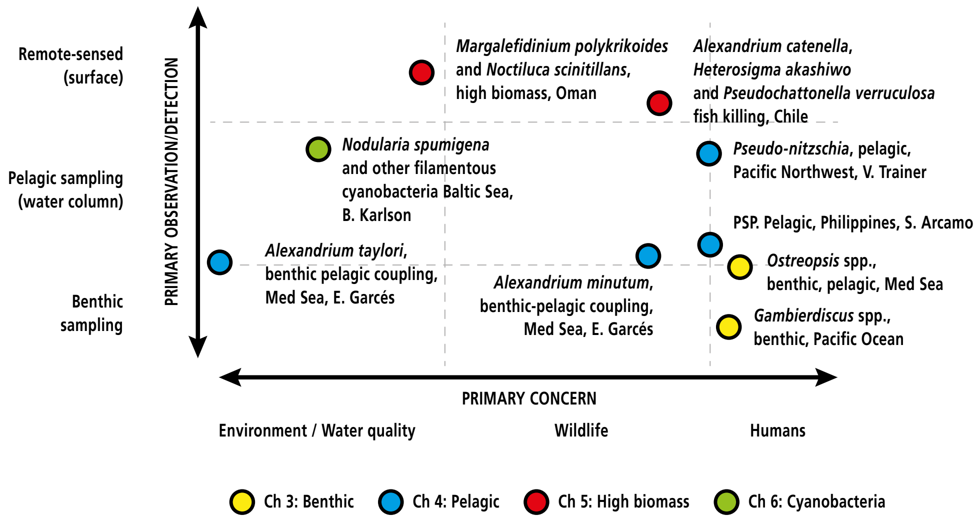
HAB EWSs are based on an understanding of how they respond to changing weather, climate, and ocean conditions, and often historical measurements of the HAB species or toxins themselves. Monitoring and forecasting of HABs often requires rapid, intensive, extensive and sustained *in situ* monitoring at sea due to the transient nature, complexity and scale of HABs. In addition to the traditional methods that rely on sampling and laboratories for chemical or biological analyzes, the use of remote sensing technologies and hydrodynamic models that operate autonomously in situ will develop comprehensive observation strategies for timely detection of HAB events. Many new approaches are now being used to enhance and automate time consuming methods and provide earlier, faster, cheaper and more accessible warnings. The choice of appropriate tools and data relies on the particular HAB species, associated toxicity profiles, the region, the impacts, and the stakeholder needs. Tools and technologies are reviewed in Section 2.5.

2.1.4 Developmental models

Users of this document may be at different starting points when it comes to developing an EWS. In some cases, there might be monitoring and/or basic knowledge of the HAB dynamics, and in other cases not. The Technology Readiness Levels (TRLs, Table 2.1) provide a chronological process for developing an EWS. A user of this guidance document should take some time to identify which step in the TRLs best describes where they are starting from. The subsequent TRLs then provide a roadmap for next steps. The system overview (Section 2.2, Figure 2.2) should be viewed as an endpoint – a fully developed EWS with its interrelated components. This is what the TRL process is building toward.

FIGURE 2.1

A few different HAB species and scenarios, mapped out based on their impacts and the tools for observation. The species shown here are those that are discussed in case studies in later chapters. Orienting a HAB species within this space helps to prioritize monitoring and stakeholder needs



Source: Elaborated by the authors.

2.2 SYSTEM OVERVIEW

We begin with an overview of what a fully developed and advanced EWS can look like. This can be viewed as the endpoint of the development process. It is not always realistic to expect to reach a fully developed system in a short timeframe. The next section discusses a stepwise process (that is, TRLs, Table 2.1) for building toward this kind of EWS from a range of different starting points.

Because of the variability across HABs, their impacts, and data availability, the EWSs in place take many different approaches. At a high level, however, EWSs strive for a common system design, such as shown in Figure 2.2. An EWS will have these key components: Data acquisition, data assimilation, modeling (that is, EWS production), product generation, dissemination, and review (that is, feedback and retuning). Finally, there is an overarching requirement for maintenance and improvement of the components, where feedback from users and analysis informs updating of the system.

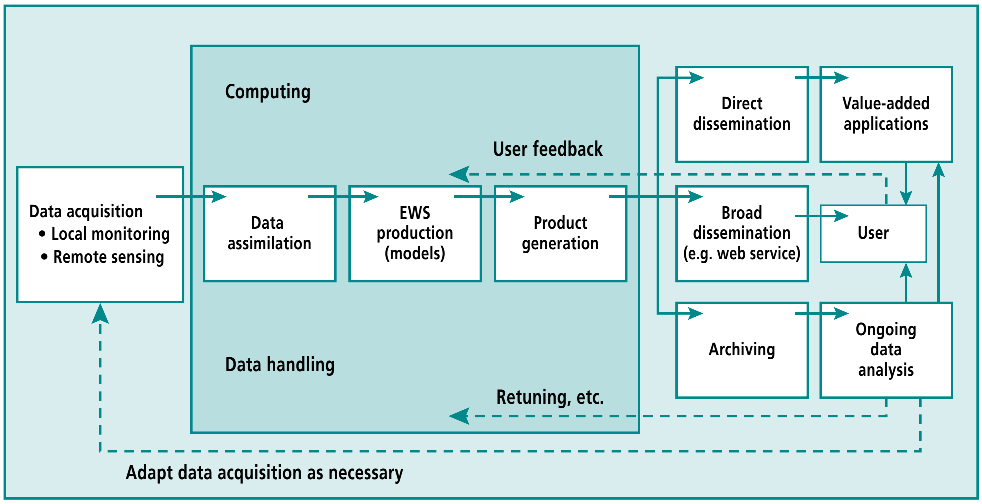
The activities for each of the components may originate in multiple agencies, and some may have been developed for purposes other than for harmful algal blooms. Simple examples of these include satellite data for environmental variables such as water temperature, and numerical hydrodynamic models that were developed for navigation. Both may be quite relevant and usable for HABs but were not necessarily designed for that purpose. Activities might may also originate in stakeholder communities, such as local or industry monitoring programs.

Data acquisition. Data acquisition is the most critical aspect of the EWS. For many HABs, if we do not know where they are, we can do nothing for an early warning. However, the question of what to measure and when derives ultimately from an identified HAB problem or an EWS user. Stakeholder input should be part of the process of planning data acquisition.

Acquisition will involve the observations of use for the forecast. Details of types of observations are covered in Section 2.5, Tools and Technology. Stumpf *et al.* (2010) described the types of data systems with examples:

- single point sampling, typically from water samples or shellfish directly;
- continuous point sampling from an automated instrument;

FIGURE 2.2
General schematic of an EWS for HABs



Source: adapted from Bauer, P., *et al.* 2021. *The digital revolution of Earth-system science.* Nature Computational Science. **1**(2): p. 104-113.

- transect sampling, such as may be collected from a ship or ferry; and
- synoptic sampling, such as from satellite.

The type of data needed will depend on the HAB in question and the local environment.

Data assimilation. The data must be brought into a reliable database that can support models and predictions. The challenge then becomes one of data assembly and assimilation for use in the EWS components. These several questions need to be answered.

- Who is responsible for data delivery?
- If water sample data, how does it get organized in order to be ingested into a database?
- If an automated system like a mooring or pier-based instrument, who is responsible for maintenance?
- Who is responsible for notifications when data flow stops?
- What happens when it stops working?
- How are the data sets, whether from water samples or instruments or satellites quality controlled?
- Who maintains the data assimilation system?
- What products are produced to help in inspecting and monitoring the data sets?
- If one component of software or hardware is updated does it affect the operation of the EWS?

The answers to these questions depend on the type of data and the local circumstances, but thinking through them carefully beforehand will help guide the setup of the system and avoid problems later on.

EWS production (models). The next step is EWS production, which typically involves some kind of numerical model. Models can range from simple statistical or rule-based associations to complex biological–physical coupled simulations. A model may be more tractable if it only requires one input. For example, the simplest EWS is either "sentinel" warning or "persistence" modeling. For example, detection of a bloom at a sentinel site might provide an alert to expect it at another sensitive location.

In such cases, the group responsible for data assimilation can often produce the models, products, and distribute. In some cases, more complex models are needed; Anderson *et al.* (2015) identify the variety of models that might be used for HAB EWS systems. The more complex the model, the more likely multiple groups are involved in the process. Output from a weather model may be needed for the forecast, or a numerical hydrodynamic model may be required.

Just as with data acquisition, similar questions can guide the system development:

- What happens when the model stops working (computer systems fail, models are dependent on other models), or the data stops being provided (upgrades to the model, changes in formats, changes in access)?
- Who is contacted?
- What are their responsibilities to get the model running again?
- What products are produced to help in inspecting the model output used in the EWS forecast?

Product generation. Output from models is sometimes considered a "forecast", but it is more appropriate to describe it as "model guidance", as is done in the weather forecast community. If we consider weather forecasts for extreme events, there are multiple numerical weather models. A forecast is the synthesis of these from an expert. While weather science has decades of experience in modeling and forecasting, the HAB community lacks a similar framework; however, the concept is the same. An expert has to decide on what the early warning will be. This will come down to the products that are generated. Even if the products are generated automatically, they require routine inspection and evaluation by a knowledgeable expert to identify when they are suspect or wrong. As an example, many HAB species are flagellates and swim vertically, or cyanobacteria and have buoyancy control. If there is different circulation at surface and bottom, a simple assumption that surface currents will explain transport away from the coast can be quite wrong (Rowe *et al.*, 2016), and give the impression that a bloom will leave the coast, when it will not.

Product generation is one of the key areas where stakeholder engagement is important. Model output alone is not always usable for stakeholder decision-making. There are numerous examples of products for forecast systems. Anderson *et al.* (2016) has reviewed some of these. In all cases they need review and inspection regularly by both the producers and users.

- Do the products reflect changes in the models?
- Do the users need alternative ways to receive warnings?
- Is the communicated information as easy to understand as originally considered?

Dissemination, direct and broad. Product dissemination results from the merging of the products and stakeholder needs. This may be done through an automated system, or semi-automated, or a manual notification. The approach to dissemination should consider the likely technology used by the stakeholder and their level of understanding and their ability to interpret the product and provide advisories. For a web-based system, the dissemination should be smartphone friendly and where possible easily updatable as technology and devices are updated. An important part of dissemination is drawing attention to the products.

- Are there multiple paths to the products?
- Do user groups get the information?
- Are underserved communities being reached?

Feedback and retuning. Finally, the system requires review, and a method for implementing changes (see TRL 9). We list this here as a final component, but it should really be an ongoing process through all components, as indicated in the description above. The review should identify failures, gaps, weaknesses, and potential improvements, in that order. Critically, the review needs to incorporate information to the appropriate communities: management, monitoring, and user. What are the failures and what are potential solutions? It may be that the failures are hard to solve. Perhaps a better computer system or monitoring technology is needed, or a key observation system is difficult to maintain. A failure could also be in the mode of communication and how a stakeholder is understanding the information. And it may take some significant resources to make those critical observations. Other failures may be more solvable, perhaps an adjustment of a model. Gaps and weakness will tend to fall together. For example, some areas cannot be forecasted reliably because we don't have the critical observations. Perhaps more frequent observations are needed. New research, technologies and datasets may be relevant to the problem. However, in all cases there needs to be some assessment of the potential impact of the change: "If we make this change, that problem will be addressed, which will have a benefit of this."

Building to a fully developed EWS, as described above, can be a lengthy and sometimes non-linear process, with adjustments made along the way. Recognizing that the path from any given starting point to a completed operational EWS is not simple, the next section described a roadmap that can help EWS developers determine the right path forward.

2.3 TECHNOLOGY READINESS LEVEL ROADMAP

To reach the point of a fully operational EWS, there are a series of steps in the development process, beginning with the early stages of research, and ending with a deployed and a fully operational EWS. Parties interested in developing an EWS could be beginning from a range of different starting points. For example, there could be some monitoring in place already, or nothing in place yet; there could be historical knowledge of the HAB in question, or it could be a a previously undocumented species. The objective of this section is to outline the stages of development as a sequential "roadmap" (Table 2.1) so that one can identify the level of an EWS at any given time and map out the logical next steps to move to the next level.

The roadmap is based on the Technology Readiness Levels (TRL) framework. NOAA has established a process for identifying, transitioning, and coordinating research and development output to operations, applications, commercialization, and other uses (NAO–NOAA Administrative, 2016). This process grew from the NASA and European Union technology readiness levels (TRL, Héder, 2017), and it provides a step-by-step sequence for bringing a technology from a basic or conceptual level all the way to an operational deployment. The TRL framework has been useful for a range of applications and has been modified here to apply specifically to HAB EWSs.

The objectives of using a TRL framework are:

- orient the EWS developer to a logical sequence of steps based on the starting point;
- understand how close/far an EWS is from being ready to use (i.e. operational);
- Assess the costs (at each level) to achieve an EWS;
- facilitate the difficult transition from research and development to operationalisation; and
- provide a common language and framework across the diversity of HAB ecologies and circumstances.

For each of the nine TRLs, outlined below, there are guiding questions and action items to transition to the next step in the process. The description of each step below includes references to the sections where more details are provided.

TABLE 2.1
A nine-phase technology readiness level (TRL) roadmap for developing an EWS

	NOAA TRL	HAB EWS TRL
1	Basic research	Background/basic understanding of phenomenon of concern Local/traditional knowledge
2	Applied research	Stakeholder involvement Identification of user needs/concerns Synthesis of knowledge
3	Proof of concept	Monitoring, data analysis Demonstrated predictive relationships
4	Validation of system in a test environment	Hindcast exercise of prediction
5	Validation of system in relevant environment	Hindcast analysis of full EWS system Stakeholder feedback
6	Demonstration in a test environment	Running in operational mode privately
7	Demonstration in a relevant environment	Running in operational mode with focus group Stakeholder feedback
8	Demonstration in the actual environment	Running in operational mode with larger/full user community Stakeholder feedback
9	Deployment and regular use	Deployment with sustained support and maintenance Continued incorporation of feedback, updated data, and retuning

Note: The left column shows the TRL framework outlined by NOAA for operationalising research and development. The column on the right provides steps for applying this framework to HAB EWSs. The colours demark the transitions from research and development, to testing, to deployment.

TRL 1: Basic research. This level includes any background and ongoing research on the HAB species of concern, as well as the local or regional human–natural system. Basic knowledge can include life history, distribution, and toxicology of the HAB species, oceanographic and climate dynamics, and study of the social and economic systems that could be affected.

Questions:

- What is the basic and background knowledge on the HAB species in question, the associated toxicity profiles and impacts on human health and food safety, the local or regional environment, impacts on aquaculture production as well as local ecological knowledge?
- Who are the local, regional and national stakeholders?

Actions:

- Review and compile relevant knowledge and information (literature, available data, traditional knowledge, local expertise, human impacts).
- Identify the communities, industries, and management organizations who could be involved in the development or use of an EWS.
- If no prior knowledge of HAB is documented, identify personnel, resource and measurement needs and provide assistance to commence monitoring activties.

TRL 2: Applied research. This level begins to transition the basic and background knowledge toward an EWS that is aimed at achieving a particular solution. This is a key step for involving stakeholders, to avoid unintended consequences (for example, Hobday *et al.*, 2017) or "parachute science" (Stefanoudis *et al.*, 2021). It is helpful to identify at an early stage in the development process the types of information (for example, toxin versus cell counts), the lead-time scales for prediction, and the types of information that are actionable for EWS users. These questions can be revisited periodically, but the answers will guide the design of the system moving forward. Details on stakeholders and stakeholder needs are detailed in Section 2.4.

Questions:

- What observational data are available, and what observational data are needed?
- What problems do the stakeholders want to solve?
- What are the most important time and space scales for an EWS?
- What specific piece(s) of information should the EWS provide and in what format?
- What concerns do stakeholders have, and how do they want to be involved in the process?

Actions:

- Collect and compile available observational data. Some preliminary thought can go into database design or data storage.
- Survey of stakeholders and/or meetings with stakeholders. Funds might be required for convening.
- Estimate costs of the needed monitoring and strategize long-term funding options.

TRL 3: Proof of concept. Not all species or time scales or problems will be amenable to an EWS. The purpose of this TRL is to test the monitoring and analysis to determine if there are predictive relationships in the data that can provide the target actionable information identified in TRL 2. A survey of monitoring and analysis tools and technologies is provided in Section 2.5.

Questions:

- What are the data needs? This is discussed in detail in Section 2.5.
- Are there relationships in the data that show potential for prediction/early warning? This is where a wide range of modeling approaches could apply, from very simple to very complex. See the discussion of models and algorithms in Section 2.5.4.

Actions:

- Monitoring: Establish or continue monitoring programs for data collection, that is, building the "data acquisition" component of Figure 2.2. Some consideration of funding and resources needed to maintain data infrastructure.
- Analysis: Testing data and early warning algorithms for predictive potential, addressing needs identified in TRL 2. Funding and resources here go toward the time needed to run analysis, test algorithms, etc. There may be computational resources as well, depending on the complexity of the model.

TRL 4: Validation in test environment. A standard practice for building forecasting systems like EWSs is to run the forecast in retrospective, or "hindcast" mode where predictions are made at each time step using only data collected up to that point, that is, as though the EWS was in place at each time step. This TRL focuses on the development of the predictive algorithm. Details on algorithm development are provided in Section 2.5.4.

Questions:

- How would the EWS have performed in the past, based on historical data?
- What issues are observed with the results of the hindcast?

Actions:

- Iterative model skill assessment and refinement. Continued need for computational resources and expertise.
- Resolve identified issues observed with hindcast validation.

TRL 5: Validation in relevant environment. This level considers the EWS in the context of the full system (Figure 2.2), from monitoring of the environment to the dissemination of the early warnings to forecast users. This is another stage at which stakeholder feedback is essential.

Questions:

- What is the best mode of early warning information dissemination?
- How do stakeholders respond to the communication of the early warning information?
- What format and visualization tools are required by the stakeholder?
- Is the data and forecast prediction relevant and useful to the stakeholder to enable risk management decisions and actions

Actions:

- prepare EWS output and communication tools; and
- survey or convene stakeholders to gather feedback on the form of early warning communication and/or dissemination and potential unintended consequences.

TRL 6: Demonstration in test environment. This level represents a transition from analyzing the EWS in a hindcast or retrospective mode to running the system in real-time. Here, monitoring data is assimilated in real-time, and predictions are made. The transition from retrospective analysis to real-time prediction often uncovers unforeseen challenges in the assembling of all of the EWS components. At this TRL, the predictions are not yet released, so that technical problems can be worked out before the EWS is disseminated broadly. At this stage, something close to a fully functioning EWS, as outlined in Section 2.2, should be in place for testing.

Questions:

- Are the data inputs and technical components in place for the EWS to be able to function in an operational mode?
- Are these tools easily updatable in the future to account for technological advances and changes in sources of data?
- Are stakeholders able to understand the data being disseminated and, if not, what changes are required?

Actions:

- running the full EWS in real-time, from data collection to prediction (though not yet dissemination);
- continued analysis of prediction skill;
- troubleshooting any unforeseen problems; and
- future-proofing of technological components and data sources.

TRL 7: Demonstration in relevant environment. At this level, the EWS is run in operational mode, often with a subset of users (that is, stakeholders) who can provide feedback. This is viewed as a trial phase for identifying unintended consequences, difficulties with communication and dissemination, or other unforeseen challenges.

Questions:

- Is the EWS functioning as planned?
- Are there unintended consequences?
- Are early warnings providing actionable information?
- Are there any knowledge gaps or could the information generated be improved?

Actions:

- running the full EWS in real-time, from data collection to prediction;
- identify a subset of users/stakeholders who will provide feedback on the EWS;
- continued analysis of prediction skill; and
- review and improve where required.

TRL 8: Demonstration in actual environment. This level represents the transition of the EWS to full operational mode. At this point, the full group of users and stakeholders are receiving the early warnings. Developing an EWS is usually an iterative process, so earlier steps can be revisited, making adjustments and improvements as appropriate.

Questions:

- Is sustained support, buy-in, and funding established to make the transition to TRL 9?
- Is the EWS adequately staffed?

Actions:

- continued analysis of prediction skill;
- continued revisiting/retuning of the system components, from data collection to early warning dissemination and feedback gathering; and
- securing long-funding for operations.

TRL 9: Deployment and regular use. Maintaining a system in regular use has challenges somewhat distinct from the development of the system. These include continued sustained support and funding for continued maintenance and refinement of the system, as well as adapting to changing social, environmental, and technological circumstances. Strategies for sustaining an EWS are discussed in Section 2.6.

Questions:

- How can the system be sustained over the long-term?
- Is there long-term buy-in from stakeholders?
- How does the system respond to and implement stakeholder requirements? Is the EWS adaptable to future needs?
- Are the decision-making needs of stakeholders met, presently and in potential future circumstances?

Actions:

- maintaining feedback from stakeholders to validate, retune, and improve the system;
- ensuring the operational structure and backbone of the EWS is updated with technology software updates as well as training of personnel; and
- transition to a long-term funding model.

Three key aspects of these TRLs – stakeholder engagement, tools and technologies, and planning for a sustainable EWS – are discussed in detail in the remaining sections of this chapter.

2.4 STAKEHOLDERS AND THEIR NEEDS

One theme that runs throughout many of the TRLs is the need for stakeholder input. Stakeholders should be involved early in the process and throughout the development of an EWS. Working with the stakeholders to address their needs may include exploring questions as they may not connect what information is needed for advice to answer their specific application. Identifying those decisions, and the right type of information to inform those decisions, should be done early in the process (see TRL 2, Table 2.1). Different stakeholders often have different needs and respond differently to information and predictions about the environment. The type of product or the communication of the early warning must be tailored to the needs and goals of the end user, particularly regarding effective timelines for action. Ultimately, stakeholders want to be able to use an early warning to inform a decision, or multiple decisions, that they have to make. These are also issues that should be revisited periodically, through multiple TRLs, and EWSs can be refined to meet changing needs.

Stakeholders can be classified into four categories: industry, regulators, health, and society (Table 2.2, Figure 2.3). Each of these categories may include members of Indigenous and local communities that often co-manage ocean resources with state, provincial, and federal managers. Their input is highly valued and an important voice to include at the beginning of EWS development. More details on inclusion of Indigenous and local communities are highlighted in the Local and Traditional Knowledge subsection below.

The industry category includes all coastal industries and businesses (for example, aquaculture, desalination plants, tourism, entertainment sector, and so on) that may be impacted by HABs. The regulators category includes scientists, policy makers, resource management and monitoring agencies who are responsible for HABs monitoring and forecasting and issuing warnings. The health category includes the health sector and managers of water and food security who are responsible for monitoring food safety, water quality conditions, and human health. Also, stakeholders include the category of society, which involves community members, fishermen and tourists who may be affected by HABs while practicing their activities in the sea, such as fishing and recreation. These four categories of stakeholders are often interlinked with each other.

The main information that stakeholders typically need from an early warning system in order to reduce negative economic, health, and environmental effects are:

- prediction of HAB or toxicity onset, magnitude, spatial extent, and/or duration.
- type of species or biotoxin present and the risk associated.
- identification of hot spots where HAB is expected to occur or be particularly intense.
- alert to the time of HABs occurrence in the neighboring areas.

There are other types of information that stakeholders may want or need, however, and identifying these needs should be part of the process of involving stakeholders.

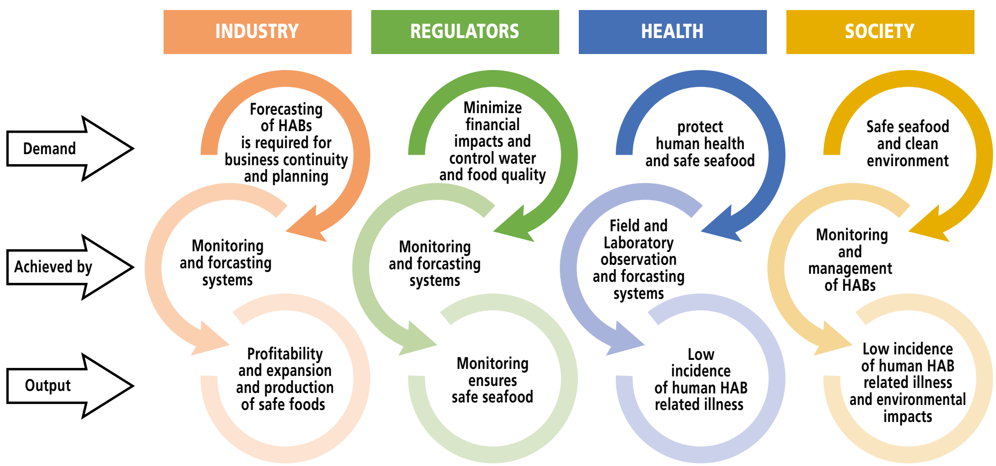
One of the major challenges that proponents of early warning systems for HABs continue to have is communication and/or dissemination of data and information and forecasting predictions to the stakeholders, that is, how to package data, model output, products. In many instances, stakeholders do not have the computational resources to access the data, or even if they do, it is presented to them in a format that is not readily understandable. Models themselves represent a form of guidance that requires translation to match the needs of any specific user group. Recent improvements in information and network technology tools offer the possibility of circumventing this challenge. These technologies now make it possible to disseminate data and information via smartphones and tablets in a variety of automated data visualisation formats, that wasn't possible until a decade ago. However, these platforms might not be the best tool for every stakeholder audience and require a separate type of expertise to develop and maintain. Addressing the issue of comprehension of the data/information being delivered to the stakeholder will require a convergent designing approach that solicits input and takes into account the needs of communities facing threats of HABs. Finally, the dissemination of the data has to be timely and clearly communicate issues of uncertainty.

TABLE 2.2
Co-development framework for developing EWSs with stakeholders

	Industry	Regulators	Health	Society
WHO	• Aquaculture industry • Tourism and recreational sector • Desalination operation • Fishing industry	• Resource management agencies • Water and food security managers • National authority • Monitoring Labs • Enforcement agencies and legislators	• Health sector • Water and food security managers	• Citizens and/or communities • Fishers • Tourists
NEED	• Early notification of harmful/toxic bloom and their spatial extent • 2–3-day notice at a minimum • Easy to interpret results	• Monitoring and predicting HABs onset and spatial extent • Support the scientific advice given to regulatory bodies	• Alert of toxic bloom • Data on HABs toxins concentration and related illness	• Alert of harmful/ toxic bloom in beaches
OUTPUT	• Help to minimize financial impacts • Help to improve supply logistics (e.g. ability to manage customers' needs) • Help to reduce mortalities	• Help to take necessary actions, develop strategies for minimizing economic losses and mitigating environmental impacts	• Help to protect public health and control water and fish quality	• Help toavoid fishing, swimming and diving in affected area
EWS PRODUCT	• *In situ* HAB and biotoxin products • Remote sensing and GIS applications • Numerical models and forecasting systems	• *In situ* HAB and biotoxin products • Remote sensing and GIS applications • Numerical models and forecasting systems	• Observation systems (sampling, HAB and biotoxin products, and laboratory works • Remote sensing and GIS applications	• Remote sensing and GIS applications • Community science monitoring programs

Source: modified from CoClime. 2023. Co-development processes. In: *Adapting to a changing marine ecosystem.* Galway. Cited 14 February 2023. www.coclime.eu/Co-Development-Processes www.coclime.eu/Co-Development-Processes

FIGURE 2.3
Different needs of different stakeholder groups, and the different configurations of EWSs that might best serve those needs



Source: modified from CoClime. 2023. Co-development processes. In: Adapting to a changing marine ecosystem. Galway. Cited 14 February 2023. www.coclime.eu/Co-Development-Processes

2.4.1 Local and traditional knowledge

Information obtained from local and traditional knowledge can be an important component of data collection, particularly when considering remote and/or new HAB impact locations. For example, in Canada, historical records from the early seventeenth century indicate that Indigenous Peoples along the coasts of the Bay of Fundy (Nova Scotia and New Brunswick), and the Gulf of St. Lawrence (Quebec) were aware of the hazards associated with the consumption of blue mussels and would not eat them during the summer months (McKenzie *et al.*, 2021). Local commercial and recreational shellfish harvesters, as well as Indigenous Peoples, have a wealth of generational knowledge about their environment, habitat and seasonal variations that clearly has a role in developing an effective EWS for specific areas or regions.

Many Indigenous communities view oceans as a living entity to which they have a sacred responsibility (for example, Wilson *et al.*, 2018). Indigenous approaches that highlight a balanced strategy to ocean resource harvest and protection was not considered in past ocean management plans. However, in an example in Washington State, United States of America, responsible management was recognized by the landmark "Boldt Decision" court case, which reaffirmed the treaty-fishing rights of Washington State tribes while also recognizing individual tribes' sacred histories of management and conservation. Several native tribes in the region were recognized as "self-regulatory" because of their demonstrated history of self-management and conservation of salmon stocks. A biocultural framework can help incorporate Indigenous understanding into management while supporting tribal and Indigenous sovereignty through co-management of ocean resources (for example, Caston, 2013). The co-development of EWS, to include Indigenous knowledge, such as that demonstrated by the development of the Pacific Northwest HAB Bulletin (www.nanoos.org/products/habs/forecasts/bulletins.php) will help to improve the knowledge base of EWSs and help to ensure that systems are meeting stakeholder needs and are sustainable and inclusive (Varanasi *et al.*, 2021).

2.5 TOOLS AND TECHNOLOGY

The observing systems that support HAB EWSs can encompass a wide spectrum of approaches and data types, and the observational tools and technologies required to support a given EWS will depend on the strategy adopted for a target region or location (for example, Anderson *et al.*, 2019; Stauffer *et al.*, 2019). Selection of the appropriate strategy will be dictated by many factors, including type of data needed, spatio-temporal resolution of information required, and cost-benefit analysis of protocols under consideration.

2.5.1 Overview

The HABs to be managed by EWSs fall into a few categories: biomass blooms, toxin-producing blooms, and benthic blooms. There is some overlap; for example, some toxin-producing blooms may become high biomass blooms. There are categories of measurements and models as seen in Table 2.4 that apply to various degrees across these three types of blooms.

The water samples or field observations may involve collection of water for cell counts and toxins, or of shellfish for toxins, or of observations of bottom covered by the benthic blooms. For both biomass and toxic blooms, any method of reliably identifying cell presence would also be useful, whether a more expensive system such as deploying the Imaging Flow Cytobot (see Section 2.5.2 for further discussion). For high biomass blooms, other options also exist, such as deployed instruments. This is discussed in detail below.

TABLE 2.3
Types of tools and technologies needed for EWSs associated with HAB categories, with priority levels from low to high indicated. As noted previously, the priority level can change depending on particular stakeholder needs or uses

Tool/Technology	HAB type			
	Benthic	Pelagic	High biomass	Cyano
In situ abundance measurements	High	*High*	*High*	High
In situ toxicity measurements	High	High	*Low*	High
Aircraft remote sensing	High	Low	*Medium*	Medium
Satellite remote sensing	Medium	Medium	Medium	High
Sophisticated mechanistic model	Medium	Medium	Medium	Medium

Source: Elaborated by the authors.

2.5.2 Tiered approach to monitoring

Cost is always a consideration, and one way to be efficient is to take a tiered approach to monitoring. The idea is to deploy the most frequent, informative, and expensive sampling effort at the most sensitive times, and to gradually reduce effort during low-risk times.

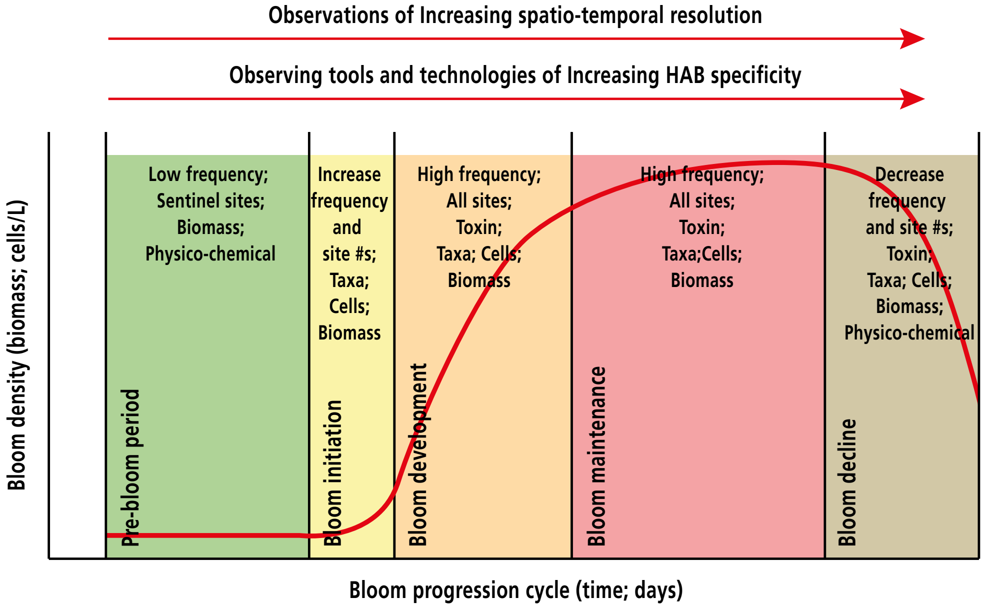
Tools/technologies (and their corresponding data) of increasing specificity, resolution, and often expense can be used with increasing frequency in advance of a bloom season, with the goal of avoiding, or at least minimizing, sole reliance of the EWS on only the most costly solution(s). As data are evaluated, one can look for predictive relationships that could serve in an EWS (see TRL 3).

Taking a closer look at such a tiered system, first consider the specificity of the data in terms of observations and/or measurements that are readily available in relation to what is needed. HABs occurring in certain regions may be characterized by a well-established sequence or progression of changes in the physico-chemical properties of the water column (including the surface) that have a high likelihood of promoting the growth, accumulation and/or transport of both innocuous and harmful algal or cyanobacterial populations. These changes, as well as an associated increase in biomass, may be related to hydrographic and/or meteorological phenomena that can be detected by relatively low-cost, commercially available devices or sensors (hand-held or autonomous) or by readily accessible satellite/airborne imagery of sea surface properties (for example, temperature, salinity, colour). Such features may include upwelling relaxation, thermal stratification, rainfall (and potential nutrient runoff) events, and so on, which can promote the appearance of increasing algal or cyanobacterial biomass. However, such data might provide little or no insight as to the nature or composition of a potentially developing bloom, although certain diagnostic pigment signatures can be used in the assignment of higher-level taxonomic groupings. Therefore, the intrinsic value of this information in an EWS is to signal the possibility of a bloom developing in, or moving into, a location. This information could then signal implementation of more HAB-specific observations at a greater spatio-temporal resolution and initiate steps towards mitigating potential impacts. This tiered approach to monitoring is shown in Figure 2.4.

As an example of the tiered approach to monitoring, if the target lead-time for notification of an impending bloom is considered to be three to five days, the temporal resolution of observational data should be, if not real or near-real time, not more than ~24 hours. In this case, tools and technologies that facilitate rapid acquisition of actionable data (regardless of data type) represent critical components of an EWS. In the context of spatial resolution, geographically broad, synoptic/continuous observations and measurements are best, but are not always possible (with the exception of some remote sensing imagery). Thus, the ability to strategically locate fixed-position observing nodes, hand-sampling sites, or mobile platform deployments, requires an in-depth understanding of the dynamics of a system as well as the underlying seasonal variability of phenomena that can potentially influence HAB initiation and growth.

FIGURE 2.4

Schematic representation of a bloom progression cycle overlaid with a tiered approach to monitoring. Schematic representation of a bloom progression cycle overlaid with a tiered approach to a HAB EWS comprising a sliding scale of observations being made at increasing spatio-temporal resolution using increasingly HAB-specific tools and technologies, generally accompanied by rising costs. Sampling frequency and sites, as well as examples of target variables, are given for each of the color-coded bloom phases.



Source: original figure designed and drawn by Gregory Doucette

2.5.3 Field observation methods

In terms of HAB-specific field observation that forms the centerpiece of an EWS, there are three broad data categories:

1) HAB organism-based data, including biomass (using pigments as a proxy) levels and cell concentrations of known taxa;
2) HAB toxin concentrations in particulate and dissolved fractions; and,
3) HAB toxin levels in vector species, primarily molluscan shellfish, crustaceans, and finfish.

Observations and measurements of cells and toxins are of highest importance for most HAB EWSs. In the case of HABs that cause adverse effects via production of high biomass levels and their associated impacts (for example, shading, hypoxia, clogging of gills or water intakes, and so on), monitoring of cell concentrations or proxies for biomass (for example, photosynthetic pigments) is often sufficient. However, for toxigenic species, although acquiring information on cell abundance is important, determination of toxin levels associated with these cells (that is, cellular toxicity or toxin quota) is essential, as cell counts maybe be decoupled from toxin levels. Since a bloom population can range from minimally toxic (or even non-toxic) to highly toxic (even over the course of a single event) depending on environmental factors as well as the presence and/or absence of toxigenic strains, determination of both cells and toxins provides the most complete and useful dataset to inform early warning systems. Similarly, toxin accumulation in vector species can be decoupled from the aforementioned measurements, so it is often important to measure toxin levels in vector species (for example, Abraham and Rambla-Alegre, 2017; Grasso *et al.*, 2019)

A wide range of well-established, conventional methods as well as more advanced, and in some cases autonomous, technologies, have been used to monitor levels of HAB cells and toxins (Zhang and Zhang, 2015; Doucette *et al.*, 2018). The latter represents a rapidly emerging area for development of HAB detection and surveillance tools, which has been the subject of several recent reviews (for example, Vilariño *et al.*, 2013; Doucette and Kudela, 2017; Gilbert *et al.*, 2018). In the context of designing and implementing observing networks required to support a given HAB EWS, the methods and assets adopted must be fit to the specific purpose, with cost-effectiveness, ease-of-use and suitability (for example, spatio-temporal resolution) of the data among the primary considerations. In Table 2.4, a list of diverse methods, tools, and technologies *currently available* for detection, identification, and/or measurement of HAB cells and toxins, and/or their concentrations, is provided and separated into three major categories:

- laboratory-based test methods and analytical tools;
- field-deployable tools and rapid commercial tests; and
- *in situ* or autonomous technologies.

Along with the brief descriptions of the detection method and target(s) provided, it should be noted that the cost associated with a particular tool or technology (that is, capitalization and acquisition, operation and maintenance costs) can vary considerably and generally increase along a spectrum ranging from simple diagnostic tools or kits to advanced analytical and automated, in situ instrumentation. Taking this information under consideration will assist in determining where a given approach might fit within a tiered EWS approach in terms of cost-effectiveness.

TABLE 2.4
Methods, tools, and technologies available for detection, identification, and/or measurement of HAB cells and toxins, as well as other HAB-related information

Tool/ Technology	Detection method	Detection target(s)	Data type	Reference/Website
Laboratory-based test methods and analytical tools				
Microscopy (light, epifluorescence)	Discrete; images	Genus and/or species	Cell concentration; relative abundance	Utermöhl, 1958; Sournia,1978; Hallegraeff *et al.*, 2003; Karlson *et al.*, 2010
Sandwich hybridization assay (SHA)	Molecular probes	Genus and/or species	Cell concentration	Karlson *et al.*, 2010; Medlin and Orozco, 2017
Fluorescence in situ hybridization (FISH)	Molecular probes	Genus and/or species	Cell concentration	Karlson *et al.*, 2010; Medlin and Orozco, 2017
Quantitative real-time PCR (qPCR)	qPCR primers	Genus and/or species	Cell concentration	Karlson *et al.*, 2010; Medlin and Orozco, 2017
Automated Ribosomal Intergenic Spacer Analysis (ARISA)	PCR primers	Genus and/or species	Relative taxon abundance	Hubbard *et al.*, 2014; Clark *et al.*, 2019
Mouse bioassay (MBA)	Whole organism toxicity	Toxin pharmacologic activity	Toxic activity equivalents	Hallegraeff *et al.*, 2003; Doucette *et al.*, 2018
Receptor binding assay (RBA)	Pharmacologic receptor binding	Toxin pharmacologic activity	Toxic activity equivalents	Van Dolah and Ramsdell, 2001; Dechraoui Bottein and Clausing, 2017; Doucette *et al.*, 2018
Enzyme inhibition assay (EIA)	Enzymatic activity	Toxin pharmacologic activity	Toxic activity equivalents	Van Dolah and Ramsdell, 2001; Doucette *et al.*, 2018

TABLE 2.4

Methods, tools, and technologies available for detection, identification, and/or measurement of HAB cells and toxins, as well as other HAB-related information

Tool/ Technology	Detection method	Detection target(s)	Data type	Reference/Website
Cytotoxicity assay	Cellular toxicity	Toxin pharmacologic activity	Toxic activity equivalents	Fessard, 2017; Doucette *et al.*, 2018
Enzyme-linked immunosorbent assay (ELISA)	Antibodies	Toxin structural epitope	Toxin equivalent concentration	Vilariño *et al.*, 2013; Zhang and Zhang, 2015; Doucette *et al.*, 2018
Lateral Flow Immunoassay (LFIA)	Antibodies	Toxin structural epitope	Toxin equivalent concentration	Anfossi *et al.*, 2013; Doucette *et al.*, 2018
High performance liquid chromatography (HPLC)	UV or fluorescence spectroscopy	Toxin chemical structure	Toxin concentration	Quilliam *et al.*, 1995; AOAC, 2005; Vilariño *et al.*, 2013; Zhang and Zhang, 2015; Doucette *et al.*, 2018
Mass spectrometry; tandem mass spectrometry (MS; MS/MS)	Mass spectroscopy	Toxin chemical structure	Toxin concentration	Villar-González el al., 2011; Vilariño *et al.*, 2013; Zhang and Zhang, 2015; Suzuki *et al.*, 2017; Doucette *et al.*, 2018
Field-deployable tools and rapid commercial tests				
Solid Phase Adsorption Toxin Tracking (SPATT)	Adsorbent resin of choice coupled with appropriate back-end toxin detection method	Most algal and cyanobacterial toxin classes	Quantity or equivalent amount of toxin adsorbed per gram of resin per unit time	MacKenzie *et al.*, 2004, 2010; Kudela, 2017; Roué *et al.*, 2018
Abraxis Marine and Freshwater Test Plates	Antibody probes; colorimetric ELISA plate	Domoic acid/ ASP; saxitoxins/ PST; okadaic acid/ DST; brevetoxins/ NST; microcystins; cylindrospermopsin; anatoxin-a	Toxin equivalent concentration	abraxis.eurofins-technologies.com/ home/ products/ rapid-test-kits/algal -toxins/algal-toxin elisa-plate-kits/
Zeulab Okatest	Inhibition of serine/ threonine protein phosphatases (PP1 and PP2A) activity; colorimetric plate	Okadaic acid/DST	Toxin equivalent concentration	www.zeulab.com/ en/producto/water -and-marine-toxins/ enzymatic-water-and-marine-toxins/ okatest/
Abraxis Freshwater Strip Tests	Antibody probes; colorimetric test strip	Microcystins; cylindrospermopsin; anatoxin-a	Toxin presence	abraxis.eurofins-technologies.com/ home/ products/ rapid-test-kits/algal -toxins/algal-toxin-test-strip-kits/
AmpliFire	Molecular probes; NASBA/isothermal amplification	Karenia	Cell concentration	www.puremolecular. com/ products
CyanoDTec and DinoDTec	Molecular probes; real-time qPCR	Cyanobacteria and toxin genes (microcystin, cylindrospermopsin, nodularin, saxitoxin)	Quantity of toxin -specific genes	www.phytoxigene. com/products
LightDeck® System	Antibody probes; fluorescence	Microcystins; domoic acid; saxitoxins/PSTs; okadaic acid/DSTs	Toxin equivalent concentration	https://lightdeckdx. com/
Liberty16	Molecular probes; real-time qPCR	Species for which probes are included	Cell concentration	www. ubiquitomebio.com/ products
Mercury Science, Inc.	Antibody probes; colorimetric plate ELISA and "SPOT" test	Domoic acid; saxitoxins	Toxin equivalent concentration or toxin presence	www. mercuryscience. com/ products.html
Neogen Reveal 2.0	Antibody probes; lateral flow immunoassay	Saxitoxins/PST; domoic acid/ASP	Toxin presence	www.neogen.com/ categories/seafood -testing/

TABLE 2.4
Methods, tools, and technologies available for detection, identification, and/or measurement of HAB cells and toxins, as well as other HAB-related information (cont.)

Tool/Technology	Detection method	Detection target(s)	Data type	Reference/Website
Scotia Rapid Test/ now AquaBC Chile	Antibody probes; lateral flow immunoassay	Saxitoxins/PST; domoic acid/ASP; okadaic acid/DST	Toxin presence	www.aquabc.cl/
In Situ **and/or autonomous technologies**				
CytoSense	Flow-through; images	Species for which it is trained	Cell concentration	www.cytobuoy.com/ products/
Environmental Sample Processor (ESP)	Molecular probes (SHA); antibody probes (ELISA)	*Pseudo-nitzschia*/ DA; *Alexandrium*/ PST; *Microcystis*/ MC -NOD; *Heterosigma*	Cell and toxin concentration	www.mclanelabs. com/ environmental -sample-processor
FlowCam	Discrete or flow -through; images	Species for which it is trained	Cell concentration	www.fluidimaging. com
Imaging FlowCytobot (IFCB)	Discrete or flow-through; images	Species for which it is trained	Cell concentration	www.mclanelabs. com/imaging-flowcytobot
HABscope	Discrete; images/ swimming behaviour	Species for which it is trained (currently, *Karenia* spp.)	Cell concentration	https://habscope. gcoos.org/
Programmable Hyperspectral Seawater Scanner (PHYSS)	Pigment absorption profile; spectrophotometry	*Karenia* spp.	Probability that chlorophyll is *Karenia*	https://mote.org/ research/ program/ ocean-technology-research; Shapiro *et al.*, 2015

Source: Elaborated by the authors.

2.5.4 Remote sensing measurements

In addition to direct biological and chemical measurements, HAB EWSs typically also make use of available oceanographic, weather and climate data. Remote sensing satellite data, data from local weather stations and buoys, in situ data collected by industry and management, real-time weather model output, and other field sampling programs can help inform early warnings. It is helpful to undergo a survey of available data at the outset of the EWS design. Some useful resources for familiarizing with available data are:

- Copernicus: www.copernicus.eu/en
- NASA OBPG: https://oceancolor.gsfc.nasa.gov/data/overview/
- Google Earth Engine: https://earthengine.google.com/

Aircraft can be helpful for screening for high biomass blooms in small areas, provided the blooms discolor the water. Imaging systems may be useful for some benthic blooms, although this will require more effort to fully automate. Satellite ocean color imagery is used effectively for high biomass blooms, provided the area of interest is not routinely cloudy. For benthic blooms, the newer Sentinel 2 data set may have resolution sufficient to detect changes indicative of blooms. Higher resolution commercial satellites may be more suitable but pose a potentially significant cost issue. Some toxic blooms can achieve high biomass, in which case those blooms are detectable by satellite.

Satellites can show sea surface temperature, which may aid in environmental models. Some blooms have strong temperature dependencies. Temperature can also indicate water masses associated with blooms, or indicate events like upwelling that may introduce blooms. Using temperature signals has been shown effective in work in Ireland with *Karenia mikimotoi*, and PSP producing *Alexandrium* off the Iberian coast. Temperature may also indicate conditions that are unfavorable to development, as harmful algal species generally have both high and low temperature limits.

2.5.5 Models and algorithms

A decision must be made regarding which model(s) or algorithm(s) to employ to make the needed predictions. These can be one of three types, or some combination. The decision will depend on a variety of considerations, such as HAB type, data availability, decision support needs and other stakeholder needs, and particulars of the local environment (for example, see Chapter 4, Section 4.6 for detailed explanation of these models relevant to pelagic HABS).

1) **Empirical or rule-based models** typically use statistical or threshold relationships to make predictions. The advantage of a model like this is in its simplicity – there are usually low data and computational requirements, and sustaining this type of system is easier. The main disadvantage is that there is usually not a strong mechanistic understanding of HAB dynamics built into the model, so if environmental conditions or biological dynamics change over time, the model can lose its ability to make good predictions.
2) **Mechanistic or process-based models** combine known biological, chemical, and physical mechanisms, such as reproductive rates and advection/transport, into a coupled simulation of ocean and HAB dynamics. An example of a sophisticated mechanistic model is the *Alexandrium catenella* population model for the Gulf of Maine, United States of America, which is a three-dimensional coupled biological–physics model. Mechanistic models can also be simpler, such as one-dimensional vertical models, or dynamical time-series models. The advantage of mechanistic models is that with known mechanisms, the model can potentially be predictive over a very wide range of different environmental conditions, and when predictions are off, it is possible to identify exactly where monitoring or modeling needs to be improved. The disadvantage is that such systems are usually complex, with intensive monitoring and computational efforts that must be sustained. They generally require higher-levels of sustained funding.
3) **Machine learning and deep learning algorithms** are similar to statistical and rule-based models, in that they are basically empirical, but as these algorithms have become more sophisticated, they have become able to detect very subtle predictive signals in data and have become more flexible in changing conditions. Deep learning neural networks are gaining widespread use in all fields of prediction and are easy to implement on personal computers. They have had success in predicting toxicity in shellfish (e.g. Grasso *et al.*, 2019) and can be inexpensive to run and maintain. In many cases, however, machine learning algorithms are used without a solid foundation of known mechanisms, and there are some of the same pitfalls as with statistical or rule-based EWSs.

These approaches can be combined as well, depending on the needs of the program and the availability of data. For example, machine learning can be used to parameterize areas of uncertainty within mechanistic models.

Whichever model or algorithm is developed, it is advised to use an open-source platform, such as R (www.r-project.org/) or Python (www.python.org/) programming languages and to maintain code in an open repository such as GitHub (https://github.com/). Use of these platforms supports reproducibility and accountability and is free to the developer. Additionally, packages and libraries for building predictive models are freely available (for example, the "forecasting" and "keras" packages in R, or the "scikit-learn" library in Python, as well as many others), and there are online communities available to help troubleshoot code.

It should also be remembered that the model typically provides a quantitative measure, such as the likelihood of an event, but it is up to the forecaster or EWS designer to decide how to evaluate and convert this information into an early warning. This decision is informed by the broader needs and requirements of stakeholders and the EWS project.

2.6 SUSTAINING EWS

Transitioning an EWS from research and development to something that is used and sustained (TRL 9) is often a challenge. Yet sustaining the system is the ultimate goal in order to have a positive impact for stakeholders over the long-term. The following recommendations will help position an EWS for long-term sustainability.

Stakeholder engagement from the beginning. As mentioned previously (TRL 2, and Section 2.2), two-way communication and knowledge co-production will ensure that the demands, issues and concerns of stakeholders are a fundamental part of the EWS design. Additionally, the iterative process of refining the forecast based on feedback (Figure 2.2) will help focus the dissemination of the EWS product on the type of information and the disseminated product that stakeholders can use. User (stakeholder) feedback should be solicited and reviewed on an ongoing basis, including questions, complaints or positive feedback. Stakeholders should be invested in the EWS program.

Systematic and organized. EWSs can be daily or weekly operations. Being deliberate and clear about the system design (that is, the components in Section 2.2), even from the demonstration phases (TRL 3–8), helps to ensure a robust and sustainable system. There should be good documentation, so that multiple people can be easily trained on the use, continued development, and deployment of the system. Regular interaction during the development phase between developers and stakeholders helps to build commitment even during the demonstration phase.

Operational. Each component of the system needs to be sustainable to maintain operationality. The datasets on which predictions depend must be sustainable. The documentation, software, and training must be available so that the system is personnel independent. Stakeholder engagement must be maintained. There should be an expectation that updates and improvements will be needed. And finally, some mode of funding must be available long-term to support all of this.

Accountability. It is unlikely that any EWS will get every prediction correct. Stakeholders will develop their own understanding of how reliable the system is over time. When a bad prediction is made, or when the system goes down for a period of time (for example, if a data stream becomes unavailable), it is useful to have planned procedures for troubleshooting a process for identifying where in the system something went wrong, and how it is being addressed, and how it might be minimised or mitigated against in the future Furthermore, communicating these steps to stakeholders, and listening to and incorporating feedback, will help maintain trust in the system.

2.7 SUMMARY

The chapter outlines a general concept of a harmful algal bloom (HAB) early warning system (EWS) to guide the development of a system, using a System Overview approach.

The Technology Readiness Levels framework orients the reader to where to situate their EWS in the development process.

There are many considerations that vary a great deal between different EWSs: stakeholder needs, HAB species and environmental particulars, monitoring options and costs, and options for sustainability, all of which should be considered when designing a HAB EWS.

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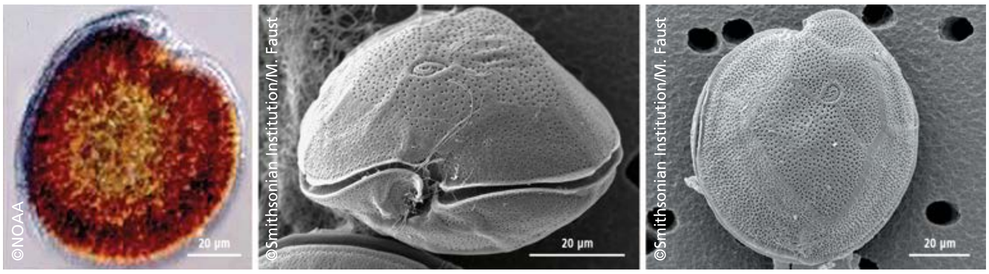
3 Benthic harmful algal species

In comparison to planktonic species, the distribution and ecology of harmful benthic dinoflagellates (BHABs) are poorly understood. Much of this uncertainty is attributable to the complex habitats in which benthic dinoflagellates live – neither suspended in the water column nor buried in the sediment. The benthic species are instead associated with three-dimensional biotic and abiotic substrates or with the sediment–water interface (Figure 3.1). Two genera of BHABs are of special concern because they produce toxins that affect marine food-webs and human health. The increased numbers of adverse incidents associated with both *Gambierdiscus* and *Ostreopsis* species have generated strong research interests since the beginning of the twenty-first century, particularly with regard to taxonomy, toxin characterization and protocols for cell-based monitoring systems. Information on species, toxins and cell abundance can serve as early warnings of human health risks.

FIGURE 3.1
Complex benthic habitats of (A) mangrove roots, (B) tropical reefs that support *Gambierdiscus* and (C) Macroalgae in a rocky substrate covered by an *Ostreopsis* cf. *ovata* blooming in the Mediterranean Sea

Some species of *Gambierdiscus* produce ciguatoxins (CTXs), lipid-soluble compounds that are odorless, tasteless, heat stable and present at very low (typically <ppb) levels in contaminated seafood. This makes their detection and quantification challenging without advanced detection methods (see FAO–WHO, 2020). Ciguatera poisoning (CP) is a form of food poisoning most common in tropical, marine environments. It is endemic between latitudes 35° north and 35° south, but it occurs globally due to the international fish trade and other seafood products. Ciguatera poisoning is the most common non-bacterial seafood intoxication associated with fish consumption and a historically significant public health issue in the tropical Pacific Ocean (Chinain *et al.*, 2021) and the Caribbean Sea (Tester *et al.*, 2010). Records are sparse in other areas but a recent review of ciguatera in the Indian Ocean and Arabian Sea has helped fill data gaps (Habibi *et al.*, 2021). New regions of concern include Micronesia and Japan (Nishimura *et al.*, 2014; Bravo *et al.*, 2019; 2020).

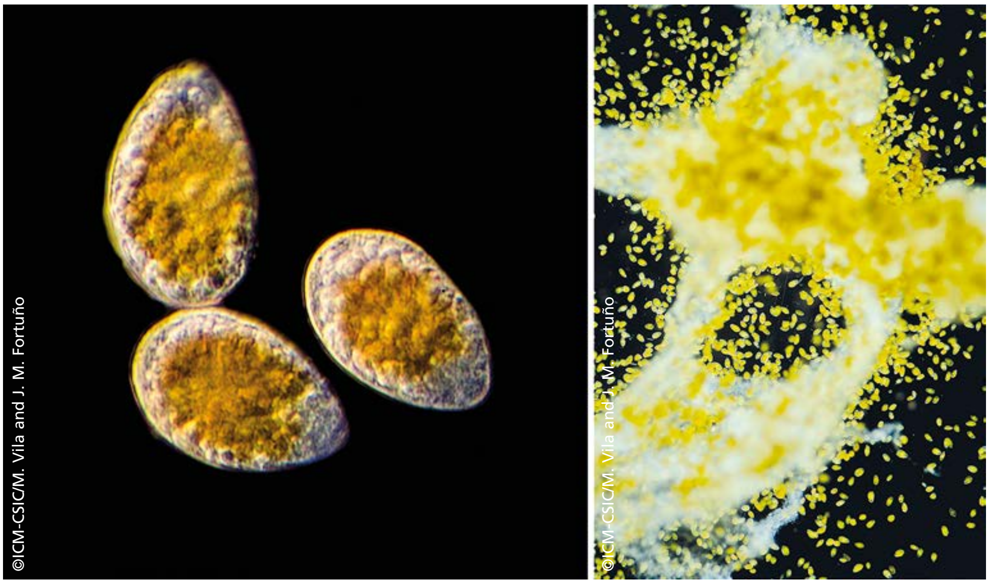
FIGURE 3.2
Images of *Gambierdiscus* spp., the benthic dinoflagellate genera that causes ciguatera poisoning



In the last decade there has been a resurgence of interest and research focused on the benthic the dinoflagellate genera *Gambierdiscus* that causes CP (Figure 3.2). Over that period several thousands of CP cases have been reported, but this number is greatly underestimated. The true incidence of CP is difficult to quantify because there is widespread failure to recognize its symptoms thereby limiting available epidemiological information (Friedman *et al.*, 2017). Despite this, globally, CP is recognized as a major health issue and is especially problematic in endemic areas. However, new areas of concern are being identified in more northerly latitudes, possibly associated with climate change (Tester *et al.*, 2020).

A second benthic genus of interest is *Ostreopsis* (Figure 3.3). *Ostreopsis* species produce a series of water soluble, highly toxic compounds including palytoxins (PLTX). In humans and other mammals, PLTX and associated toxins are powerful vasoconstrictors targeting the ATPase Na+/K+ pump, a transmembrane enzyme that plays a role in maintaining the resting potential of nerve, muscle and heart cells. Of particular concern in coastal areas of the Mediterranean are aerosols responsible for febrile respiratory syndromes as well as respiratory and skin irritations. There is growing evidence that toxins produced by *Ostreopsis* species can be transmitted through the food-web to humans by eating bottom-feeding fishes, shellfish or sea urchins.

FIGURE 3.3
Examples of the benthic dinoflagellate species, *Ostreopsis* cf. *ovata*



3.1 *GAMBIERDISCUS* AND CIGUATERA

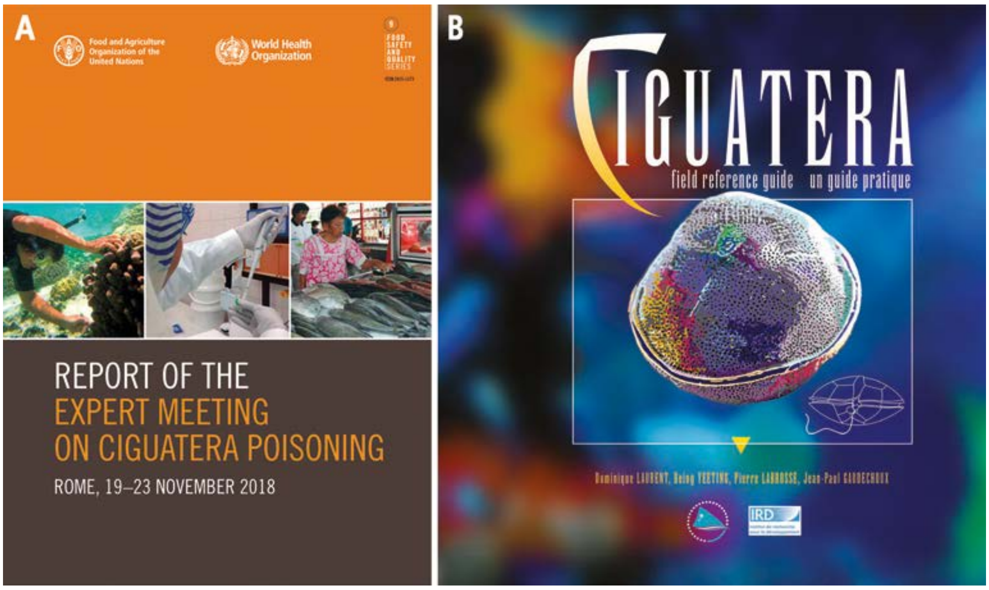
3.1.1 Ciguatera poisoning, human health effects and impacts

CP affects not only human health but has global socioeconomic consequences. Health threats and negative publicity around CP events often lead to a reduction of commerce in reef fish in island communities and jeopardizes reef fish exports. Consequently, a cascade of changes in lifestyles result in dietary shifts in favor of imported, canned meat products (Rongo and van Woesik, 2012). Economic impacts are felt when bans are placed on the sale of high-risk fish species. When 55 nations and island territories were designated "Ciguatera at-risk destinations" by the International Association for Medical Assistance to Travellers in 2020, loss of tourism resulted, and recreational fishing activities were discouraged (www.iamat.org/risks/ciguatera-fish-poisoning).

Global occurrences and trends in CPs have been comprehensively reviewed (Friedman *et al.*, 2017; Chinain *et al.*, 2021). Paired with -species-specific maps of *Gambierdiscus* distribution and summarized information on human illnesses presented by regions, recent reviews provide an excellent starting point for the background and justification for early warning systems (EWSs).

Beyond the reviews by Friedman *et al.* (2017) and Chinain *et al.* (2021) mentioned above, there are other valuable resources available for management and monitoring programs. One is the FAO elearning Academy course "Monitoring and Preventing Ciguatera Poisoning". It was released in December 2020 and provides tools, approaches and strategies for the design and implementation of environmental, food safety and epidemiological monitoring, with a view to developing a well-informed ciguatera risk management plan (https://elearning.fao.org/course/view.php?id=648). Laurent *et al.*, (2005) developed the *Ciguatera Field Reference Guide* (Figure 3.4A) specifically written for the South Pacific but it includes universally useful information on ciguatera risk assessment and management. This is an excellent starting place for those responsible for developing a monitoring plan for *Gambierdiscus*.

FIGURE 3.4
(A) FAO/WHO (2020) Report of the Expert Meeting on Ciguatera Poisoning (https://elearning.fao.org/course/view.php?id=648) and (B) Ciguatera Field Reference Guide (https://repository.oceanbestpractices.org/handle/11329/969)



Source: Elaborated by M. Vila and J. M. Fortuño (ICM-CSIC), and Ruben Duro (Science into Images)

In addition, the joint FAO/WHO (2020) *Report of the Expert Meeting on Ciguatera Poisoning* includes a full evaluation of known CTXs (toxicological assessment and exposure assessment), including geographic distribution, epidemiology, toxicity and methods of detection. It serves as a reference document, and based on guidance provided by this report, risk management options were developed (Figure 3.4A) (www.fao.org/3/ca8817en/ca8817en.pdf).

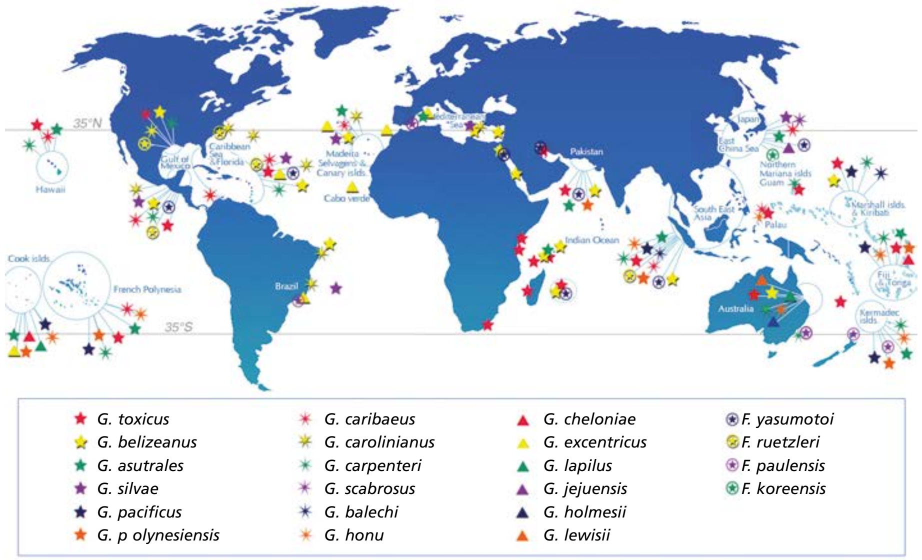
3.1.2 *Gambierdiscus* distribution

Currently there are 18 described species of *Gambierdiscus* (and four species in the sister genus *Fukuyoa*), 13 of which are known to produce ciguatoxin(s) (Figure 3.5, Table 3.1, Table S3.1). The tables in Litaker *et al.*, (2017), Chinain *et al.*, (2021), FAO/WHO (2020) and Tester *et al.*, (2020) provide extensive species-specific information on environmental parameters, growth rates and toxicity.

3.1.3 *Gambierdiscus* habitats

Overall, interactions among environmental factors and *Gambierdiscus* (and *Fukuyoa*, hereafter in Chapter 3 "*Gambierdiscus*" includes both genera), growth and settlement allow them to occupy habitats in complex, diverse, tropical and subtropical regions. Cell abundances are highly variable and patchy with species compositions varying over time. In a study by Lee *et al.*, (2020), they noted *Gambierdiscus* achieved the highest abundance in turf algae dominated microhabitat (255 cells 100 cm^{-2}) followed by hard corals and lower abundances in habitats dominated by fleshy algae. In the same study, comparisons made for *Ostreopsis* indicate it has broader habitat preferences with hard coral, turf, fine sand, microbial mats and flesh macrophyte habitats being favored in that order. Most data on *Gambierdiscus* cell abundances show there are fewer than 1 000 cell g^{-1} wet weight of substrate, but, rarely, cells may reach densities of more than 100 000 cells g^{-1} (Litaker *et al.*, 2010).

FIGURE 3.5
Global species distribution of the genera *Gambierdiscus* and *Fukuyoa*



Source: Chinain, M., *et al.*, 2020. *Ciguatera-causing dinoflagellates in the genera Gambierdiscus and Fukuyoa: Distribution, ecophysiology and toxicology.* Dinoflagellates: Morphology, Life History and Ecological Significance; Subba Rao, DV. p. 405-457.

TABLE 3.1
Toxic *Gambierdiscus* species, toxins and harmful effects

Causative organism(s)	Toxins	Clinical symptoms	Syndrome	Selected reference
Gambierdiscus and *Fukuyoa* spp. *F. koreansis** *G. australes* *G. balechii* *G. belizeanus* *G. caribaeus* *G. carolinianus* *G. carpenteri* *G. excentricus* *G. pacificus* *G. polynesiensis* *G. scabrosus* *G. silvae* *G. toxicus*	Ciguatoxins	Gastrointestinal, neurologic, and cardiac symptoms	Ciguatera poisoning	Friedman *et al.*, 2017, Chinain *et al.*, 2021 FAO, WHO, 2020 See Supplementary Table S3.1 for additional information, species-specific assays available for monitoring activities to support early warning programs. References are included.

Source: Chinain, M., *et al.*, 2020. *Ciguatera-causing dinoflagellates in the genera Gambierdiscus and Fukuyoa: Distribution, ecophysiology and toxicology.* Dinoflagellates: Morphology, Life History and Ecological Significance; Subba Rao, DV. p. 405-457.
*possibly toxic

Gambierdiscus grow relatively slowly, so one to five months may be necessary for significant increases in cell abundances. The relationships between cell abundance, toxicity and illness are still poorly documented largely because of the paucity of species-specific cell abundance data. However, the best data available are from French Polynesia and will be detailed in the case study below (Section 3.2).

The development and bloom dynamics of *Gambierdiscus*, whether associated with a substrate, or in certain cases, swimming in the water column, are regulated by numerous factors that may be related in a complex manner and that are difficult to separate (for example, Nakada *et al.*, 2018). They include temperature, substrate, salinity, light, water motion, nutrient load, as well as anthropogenic and natural disturbances.

TABLE 3.2

Species of the genus *Gambierdiscus* (and sister genus *Fukuyoa*) with selected reference and published sequence data, PCR and/or qPCR assays. Species designated with (*) are known to produce ciguatoxins as assessed by MBA, RBA, CBA-N2a and/or LC–MS/MS.

Species	Geographic origin	Selected references	Sequence, PCR or qPCR assay
Gambierdiscus australes (*) Chinain *et al.*, 1999a	Australes Archipelago, South Pacific Ocean	Chinain *et al.*, 1999a; Litaker *et al.*, 2009	Nishimura *et al.*, 2016; Darius *et al.*, 2017
G. balechii (*) Fraga *et al.*,2016	Celebes Sea, Pacific Ocean	Fraga *et al.*, 2016	Fraga *et al.*, 2016
G. belizeanus (*) Litaker *et al.*, 2009	Belize, Central America, Caribbean Sea	Faust 1995; Litaker *et al.*, 2009	Vandersea *et al.*, 2012
G. caribaeus (*) Litaker *et al.*,2009	Belize, Central America, Caribbean Sea	Litaker *et al.*,2009	Vandersea *et al.*, 2012; Darius *et al.*, 2017
G. carolinianus (*) Litaker *et al.*, 2009	Continental Shelf, North Carolina, USA	Litaker *et al.*, 2009	Vandersea *et al.*, 2012
G. carpenteri (*) Litaker *et al.*,2009	Belize, Central America, Caribbean Sea	Litaker *et al.*, 2009	Vandersea *et al.*, 2012; Darius *et al.*, 2017
G. cheloniae Smith *et al.*, 2016	Cook Islands, South Pacific Ocean	Smith *et al.*, 2016	*G. cheloniae* Smith *et al.*, 2016
G. excentricus (*) Fraga *et al.*, 2011	Canary Islands, Northeast Atlantic Ocean	Fraga *et al.*, 2011	Litaker *et al.*, 2019
G. holmesii Kretzschmar et al 2019a	Great Barrier Reef, Australia	Kretzschmar et al 2019a	Kretzschmar *et al.*, 2019a
G. honu Rhodes *et al.*, 2017b	Cook Islands, South Pacific Ocean	Smith *et al.*, 2017	Rhodes *et al.*, 2017b
G. jejuensis Nishimura *et al.*, 2013	Jeju Island, Korea, East China Sea	Nishimura *et al.*, 2013	Nishimura *et al.*, 2013
G. lapillus Kretzschmar *et al.*, 2017	Great Barrier Reef, Australia	Kretzschmar *et al.*, 2017	Kretzschmar *et al.*, 2019a
G. lewisii Kretzschmar et al 2019b	Great Barrier Reef, Australia	Kretzschmar *et al.*, 2019b	Kretzschmar *et al.*, 2019b
G. pacificus (*) Chinain *et al.*, 1999b	Tuamotu Archipelago, South Pacific Ocean	Chinain *et al.*, 1999b; Litaker *et al.*, 2009	Darius *et al.*, 2017
G. polynesiensis (*) Chinain *et al.*, 1999b	Australes and Tuamotu Archipelago, South Pacific Ocean	Chinain et al., 1999b; Litaker *et al.*, 2009	Darius *et al.*, 2017
G. scabrosus (*) Nishimura *et al.*, 2014	Kashiwa-jima Island off southern Honshu, Japan	Nishimura *et al.*, 2014	Nishimura *et al.*, 2016
G. silvae (*) Fraga and Rodriguez 2014	Canary Islands, Northeast Atlantic Ocean	Fraga and Rodriguez 2014	Litaker *et al.*, 2019
G. toxicus (*) Adachi and Fukuyo 1979	Gambier Islands, French Polynesia, South Pacific	Adachi and Fukuyo 1979; Litaker *et al.*, 2009	Darius *et al.*, 2017
Fukuyoa paulensis Gomez *et al.*, 2015	Ubatuba, Southern Brazil, South Atlantic Ocean	Gomez *et al.*, 2015	
F. ruetzleri (previously *G. ruetzleri*) Gomez *et al.*, 2015	Belize, Central America, Caribbean Sea	Litaker *et al.*, 2009; Moved to new genus Gomez *et al.*, 2015	Vandersea *et al.*, 2012; Darius *et al.*, 2017
F. yasumotoi (previously *G. yasumotoi*) Holmes 1998	1998 Singapore Island off Pulau Hantu, Southeast Asia	Holmes 1998; Litaker *et al.*, 2009; Gomez *et al.*, 2015	
F. koreensis (corrected from *F. koreansis*) Li *et al.*, 2021	2021Korean coastal waters	Li *et al.*, 2021	

Note: Also see Lyu et al., 2017 (RFLP assays); Smith et al., 2017 (metabarcoding approach); Pitz et al., 2021 (FISH probes).
MBA: mouse bioassay, RBA: receptor binding assay, CBA: cell-based assay.
Source: Elaborated by the authors.

Temperature. The occurrence of *Gambierdiscus* is correlated with the water temperature, and blooms may be developing above a certain threshold, up to 31 °C. Careful examination of the thermal preference for species of interest can be useful in deciding when to sample.

Substrate. In decreasing order of preference, substrates range from turf algae, hard corals, flesh algae, sand to coral rubble.

Salinity. *Gambierdiscus* growth is optimal between 25 and 35 with optimum varying within species. They have a poor tolerance to land runoff.

Light. *Gambierdiscus* cells live in tropical environments with high light intensities although they the equivalent of 10 percent of ambient surface irradiance (230 to 250 umol photons m^{-2} s^{-1}) is sufficient for optimal growth.

Water column stability. Water movement such as current or waves may affect cell growth and settlement. There is generally a negative correlation between water motion and *Gambierdiscus* abundance. Highest cell abundances are recorded in relatively stable water column conditions, where cell dispersion is reduced; however, they may also be found in shallow reef crests exposed to wave action.

Nutrient loading. The influence of nutrients (nitrogen, N/P ratio) on *Gambierdiscus* growth and toxin production is ambiguous. The information available is mostly limited to laboratory culture settings and may not be directly convey to the natural settings. Nutrient availability may cause between 2- to 9-fold increase in toxicity although this effect is likely to be strain- and species-dependent.

Environmental disturbances. Natural events such as hurricanes, severe storms, salinity fluxes due to heavy rain, invasive crown-of-thorn starfish feeding on corals, coral bleaching or earthquakes are all natural events that may directly or indirectly impact cell growth. And in a similar manner, disturbances from anthropogenic origin that impact the ecosystems may also impact *Gambierdiscus* growth; they may include constructions, shipwrecks, dredging of shipping channels, environmental pollution (including plastics), destructive fishing practices, sedimentation or bomb testing.

To summarize, for *Gambierdiscus* EWS design, the following environmental conditions are known to favor *Gambierdiscus* spp. in tropical and sub subtropical waters:

- optimal temperatures for *Gambierdiscus* species are between 20 °C and 28 °C, and the maximal growth temperatures usually are >25 °C;
- tolerance salinity range 20 to 40;
- *Gambierdiscus* photosynthesis saturation at low light levels;
- preferred, natural substrate: turf algae, hard coral, sand and to a lesser extent fleshy macrophytes; and
- calm water on the lee side of islands, in shallow bays.

3.1.4 *Gambierdiscus* bloom dynamics and toxin transfer

In contaminated seafood, CTXs vector from the toxin-producing *Gambierdiscus* cells to edible fish and shellfish (Figure 3.6). The lipid-soluble CTXs are readily transferred through the food-web from algae to herbivorous organisms feeding either on turf algae or macrophytes hosting *Gambierdiscus* or coral rubble. The CTX are subsequently bioaccumulated and distributed in fish tissues (Ledreux *et al.*, 2014; O'Toole *et al.*, 2012; Clausing *et al.*, 2018; Leite de Prado *et al.*, 2021), biomagnified (Lewis and Holmes, 1993; Lehane and Lewis, 2000) to higher trophic level carnivorous predators, and ultimately reach human diets as a contaminated food. CTXs produced by *Gambierdiscus* reach hundreds of marine species, from lower trophic level fish or shellfish to large predators and marine mammals (Chinain *et al.*, 2010; Chateau-Degat *et al.*, 2005a; Chateau-Degat *et al.*, 2005b; Bottein *et al.*, 2011: Gaboriau *et al.*, 2014).

FIGURE 3.6
Lag time for ciguatoxins to enter marine food-web

A lag of one month occurs between increased *Gambierdiscus* cell abundances and the detection of toxin in fish. (Clausing *et al.*, 2016) while a three-month lag time is seen between peak *Gambierdiscus* densities and a peak in ciguatera cases (Chateau-Degat *et al.*, 2005b).

Credit: ©M. Vila and J. M. Fortuño (ICM-CSIC, Barcelona and Ruben Duro / Science into Images).

Lag times are complicating factors in modeling toxin transfer. What is the timeline between an environmental perturbation or trigger to transfer from toxic cells to herbivorous fish? A study to address this included a 14-month field effort with weekly sampling and toxin analysis of 270 *Gambierdiscus* samples and 465 specimens of *Centochaetus striatus* (maito or striated surgeon fish). Comparisons of *Gambierdiscus* abundance and toxicity of herbivorous fish provided predictive models of human intoxication (Chinain *et al.*, 2010; Chateau-Degat *et al.*, 2005a; Chateau-Degat *et al.*, 2005b) (Figure 3.6). Results suggested the toxin transfer time from peak *Gambierdiscus* to herbivorous fish was one month and an additional two months between peak cell densities and increasing CP cases. Together field data of fish toxicity and (limited) experimental projects have provided some insight into CTX transfer efficiency and velocity as well as biomagnification in marine ecosystems (Chinain *et al.*, 2019; Chateau-Degat *et al.*, 2005a, 2005b; Clausing *et al.*, 2018; Holmes *et al.*, 2021).

Current models of CTX trophic transfer are limited to a conceptual nature (for example, Cruz-Rivera and Villareal 2006; Lewis and Holmes, 1993; Holmes *et al.*, 2021). Consequently, *Gambierdiscus* abundances on the reef are not easily associated with potential for CTX risk. Additional uncertainty may be introduced by a series of biological, chemical and ecological factors that influence the transfer of CTXs from primary producers to high trophic levels. These may include:

- The *Gambierdiscus* cells densities may vary from few cells to thousands per gram of substrate (Litaker *et al.*, 2010).
- The species of *Gambierdiscus* involved which have different toxin profiles and which toxin production may vary by over 1000-fold (and some are non-toxic) (Roeder *et al.*, 2010; Litaker *et al.*, 2017; Pisapia *et al.*, 2017).
- The palatability/tastiness of the host macrophyte (Cruz-Rivera and Villareal 2006; Rains and Parsons 2015) determining the consumption by herbivorous fish.
- The chemical profiles of CTXs in fish and the microalgae differ because each has a specific partition coefficient which will affect its individual trophic transfer and toxicokinetics in fish.
- The assimilation efficiency of the CTX which is influenced by the food quantity and quality, the partitioning of CTX in food particles, and the digestive physiology of the predator species.
- The rate of elimination or excretion out of the predator organism which varies within species.
- The toxic effect of CTX on predator species as CTX may induce behavioural and morphological changes in sensitive organisms, which could either be preyed upon or killed more easily, making part of the food-web more vulnerable.

- The organism behaviour, including foraging and feeding activities of predators, the rate of dietary intake, migration and home range are also factors that influence food-web transfer of CTXs.
- The number of trophic interactions involved in the ecosystem will define the rate of food-web transfer (Lewis and Holmes, 1993; Lehane and Lewis, 2000; Clausing *et al.*, 2018).
- Environmental conditions such as higher seawater temperature also have been linked to greater fish toxicity. It is unclear if this is due to changes in fish migration or a bias due to increased fishing activities, or to seasonal variability in the abundance or toxicity of *Gambierdiscus*.

3.1.5 *Gambierdiscus* sampling methods

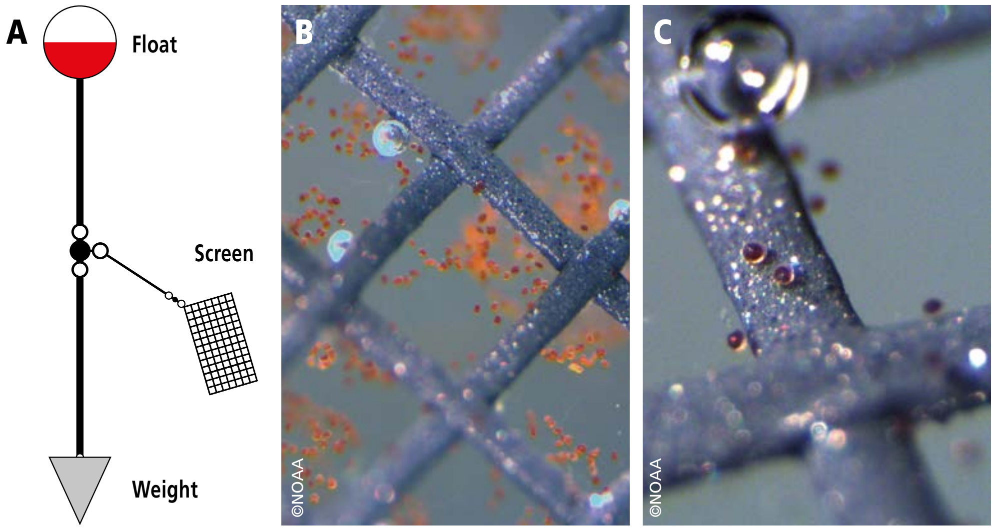
It is likely, for the benthic HAB genera *Gambierdiscus/Fukuyoa* (and *Ostreopsis*, see Section 3.2.4), cell-based monitoring will be the basis of an EWS for the near future. Remotely sensed information is of limited use and a toxin based ESW is too expensive for most programs to implement and maintain. Cell-based monitoring for *Gambierdiscus* is currently used in French Polynesia under the auspices of the Institut Louis Malardé and in the Canary Islands, Spain (Observatorio Canario de algas nocivas (Universidad de Las Palmas de Gran Canaria y Gobierno de Canarias, http://observatoriocanariohabs.com). In each of these programs much consideration has been given to how, when, and where to monitor. Total *Gambierdiscus* abundances are the data default with species identification more difficult to achieve if only light microscopy is available. However, we should not lose sight of the end goal of a mature monitoring program because it is the toxin level in food that matters. Cells monitoring is a support to toxin analysis if it available.

Fortunately, the decade between 2010 and 2020 has been a productive one for discovery of new *Gambierdiscus* species, fostered by a renewed interest in the genus and the development of molecular assays to help detect and quantify cells. Species-level identification is important because of the 22 described *Gambierdiscus* and *Fukuyoa* species, 13 are known to produce ciguatoxins (Table 3.1) and inter and intraspecific toxicity is variable (Litaker *et al.*, 2017). Since multiple species co-occur in many habitats, species identification and enumeration can be efficiently achieved using quantitative qPCR (or other high-throughput methods) that scale well for multiple replicates (Table S3.1). The importance of knowing abundances of the most toxic species in areas of interest are clearly demonstrated in 2014 during a bloom of *G. polynesiensis* in French Polynesia. Five *Gambierdiscus* species were present in the three bays on Nuku Hiva Island; however, CP was reported only in the bay with 85 percent of the cells identified as *G. polynesiensis*, the most toxic species (See case study in Section 3.2).

Detailed sampling methods for both *Gambierdiscus* and *Ostreopsis* are available (Tester *et al.*, 2014; Moreira and Tester, 2016; Vassalli *et al.*, 2018; Jauzien *et al.*, 2018; Tester and Kibler, 2018). Traditionally, *Gambierdiscus* cells were collected by shaking BHAB cells from macrophyte substrates. As a preliminary survey method, using macrophyte substrates to determine the species in the area or for food-web studies is understandable (for example, Tudó *et al.*, 2020). However, as a quantitative method, macrophyte substrates are inadequate. This method does not normalize cell abundance to surface area but rather to biomass which is not reliable among different macrophyte substrates or from various locations or seasons (Tester *et al.*, 2022).

The tedious exercise of measuring the surface of macrophytes to normalize to macrophyte surface area is not practical for a monitoring method. Tester *et al.*, (2014) introduced the idea of a standardized artificial substrate as a sampling method that could be used universally (Figure 3.7A–C). Cells are normalized to surface area, not biomass. It is non-destructive, so sampling can be accomplished in protected areas without denuding sampling sites when high numbers of replicates or repeated samples are required. Importantly, artificial substrates support robust statistical sampling designs because artificial substrates can be placed in any location, especially those without macrophytes. Cells collected from the artificial substrate (for example, window screen mesh) are clean and free of debris that could inhibit PCR species identification. The artificial substrate method was developed in the Caribbean (Tester *et al.*, 2014) and has been tested and adapted in the Mediterranean, the Atlantic coast of France for *Ostreopsis* collection (Jauzein *et al.*, 2018), in the Canary Islands (Fernández-Zabala *et al.*, 2019) and in the Pacific (Lee *et al.*, 2020).

FIGURE 3.7
(A) A carefully measured piece of window screen is suspended between a weight and a float on a short line, so the entire sampling device is suspended sub surface to avoid any disturbance from waves and swells. For sampling high concentrations of *Ostreopsis*, a frame can be added to help support the screen, and in high energy areas frames are useful to keep the mesh screen from folding back on itself. (B) and (C) *Gambierdiscus* cells on artificial substrate (fiberglass window screen mesh) at two different magnifications



Gambierdiscus monitoring method requirements:

- A standardized sampling method should be unbiased and universal so statistically robust data may be normalized to surface area allowing comparisons over time and location.
- Adequate replicate samples are required to reduce the high coefficient of variation associated with the patchy distribution of *Gambierdiscus* and other BHABs found in complex environments.
- Sampling, when possible, should be integrated over 24 hours using artificial substrate. This is especially important when cell abundance is low.
- *Gambierdiscus* species identifications need to be confirmed by qPCR. Microscopy, generally, will not suffice to identify *Gambierdiscus* to the Species-level. Species identification can be achieved using molecular methods that scale up well for faster sampling processing.
- Definitive species identification is immutable because interspecific toxicity differences in *Gambierdiscus* are as high as 1000-fold.

3.1.6 Monitoring cells, toxins, food-web organisms

Prior to establishing a monitoring program, it is advised to identify high-risk species and fishing locations. If resources and infrastructure allow, conduct an initial random survey of *Gambierdiscus* cells accompanied by an evaluation of CTX accumulation in a variety of organisms across multiple food-web components, if possible. These data can, in turn, be used to characterize potential risk factors, both biological and environmental, associated with the probability of catching fish containing CTXs in each area.

- Efforts to monitor *Gambierdiscus/Fukuyoa* species diversity in benthic assemblages of CP-prone areas have significantly increased at a global scale. The use of molecular tools such as qPCR assays to rapidly screen environmental samples for specific (toxic) species, is advocated for a higher taxonomic resolution and accurate determination of species.
- The utility of solid phase adsorption toxin tracking (SPATT) technology as a complementary strategy to CTX evaluation in microalgal populations has been demonstrated both in the laboratory (Caillaud *et al.*, 2010; 2011) and in the environment in ciguatera endemic areas (Darius *et al.*, 2018; Roué *et al.*, 2018a; 2020).
- Implement cost-effective, rapid, field monitoring tools such as high-throughput sequencing (HTS) metabarcoding to screen ciguatoxic biotopes for the presence of co-occurring species (for example, other dinoflagellates and cyanobacteria) in benthic communities, and related toxic classes not directly involved in CP. Using HTS metabarcoding approach in tandem with PCR assays, Smith *et al.* (2017) were able to characterize the microbial communities from both New Zealand warm–temperate waters (Northland) and subtropical waters (Kermadec Islands). This was followed by qPCR assays for the *Gambierdiscus* and *Fukuyoa* positive samples. They were able to alert managers in the Kermadec Islands of the emerging risk posed by the identification of *G. polynesiensis* (the most toxic of all *Gambierdiscus* species) in benthic samples around the islands.
- Areas of high biodiversity of *Gambierdiscus* and *Fukuyoa* species are not necessarily high-risk areas for CP if most species/strains that are present in benthic assemblages are non-CTX producing strains.
- Toxin production can greatly vary among the toxic species in these two genera, from fg to pg CTX3 C eq cell^{-1}, with *G. polynesiensis* and *G. excentricus* displaying the highest toxic potential so far (Litaker *et al.*, 2017; Rhodes *et al.*, 2017a; Larsson *et al.*, 2018; Longo *et al.*, 2019; Rossignoli *et al.*, 2020). But species such as *G. scabrosus*, and *G. silvae* should not be overlooked, depending on the region of interest.
- It should be possible to identify areas where CP risk is greatest by monitoring only *G. polynesiensis* and *G. excentricus* abundance (potential risk biomarkers) using species-specific molecular assays (Litaker *et al.*, 2017; Darius *et al.*, 2017).
- Herbivorous fish species (for example, scaridae, acanthuridae and kyphosidae) are major contributors to ciguatera poisoning events in the South Pacific Ocean region (Darius *et al.*, 2007; Chinain *et al.*, 2020a; Rongo and van Woesik 2011; Chinain *et al.*, 2021), unlike what is observed in other CP endemic areas such as the western Pacific Ocean (Oshiro *et al.*, 2010; Chan 2015), the Indian Ocean (Quod and Turquet, 1996), the Caribbean Sea (Pottier *et al.*, 2001) and the eastern Atlantic Ocean (Nuñez *et al.*, 2012);
- Several species of marine invertebrates which also represent a valuable source of protein and revenue for many island communities (for example, giant clams, sea urchins, lobsters, crabs, octopus) also can naturally bioaccumulate ciguatera toxins in their tissue (Earle, 1980; Rongo and van Woesik 2011; Mak *et al.*, 2013; Roué *et al.*, 2018b, 2018c; Darius *et al.*, 2017, 2018). Therefore, these organisms should be monitored and tested, along with fish, in ciguatera control programs.
- As of now, there is no conclusive evidence as to whether fish size, weight or

estimated age can serve as reliable indicators of ciguatera risk in CP-prone areas (Mak *et al.*, 2013; Gaboriau *et al.*, 2014; Sanchez-Henao *et al.*, 2020).
- Data on the combined environmental requirements of *Gambierdiscus* and *Fukuyoa* spp. (temperature, salinity, irradiance, wave energy, pH, nutrients) can be used to define latitudinal ranges and species-specific habitats, as well as to inform predictive/risk models (Kibler *et al.*, 2012; Yoshimatsu *et al.*, 2014; Tawong *et al.*, 2016; Xu *et al.*, 2016; Vacarizas *et al.*, 2018; Longo *et al.*, 2019).

3.1.7 Data considerations and outreach

Implementing relevant tools likely to improve the nearly universal under-reporting of CP cases observed in most of the affected areas appears critical in a perspective of EWS. Thorough, timely reporting of CP cases can help trace back to the toxic source quickly and thus help anticipate the potential emergence of CP outbreaks.

The website www.ciguatera.pf developed by the Institut Louis Malardé is an excellent example of CP data for French Polynesia and surrounding regions. It provides a platform for health professionals to report anonymously and opportunities for the public to self report CP illnesses. In addition to its educational emphasis, this website allows users to map CP incidence by location through time. IT-based tools such as websites can be used to foster the online reporting of CP cases (www.ciguatera.pf). A similar unified, reliable effort and website is greatly needed in the Caribbean region to report CP and share CP -related information. Efforts there have not been consistent, and CP is not getting the attention it deserves.

In addition, periodic updates on regional research and monitoring efforts are effective tools to keep local populations informed of the impacts and progress of cell-based monitoring programs. (See pages 42–51, https://coastfish.spc.int/en/publications/bulletins/fisheries-newsletter/469-spc-fisheries-newsletter-150)

As noted, field campaigns involving environmental investigations combined with toxicological analyses are useful in assisting local populations in identifying contaminated reefs and fish deemed not edible. However, community outreach and communication actions also play a prominent role in ciguatera risk management and are critical for increased awareness and sustainable results. Community outreach can be conducted in the form of public meetings and/or educational interventions targeted at school audience in the frame of a citizen science initiative or "Week of Science". Additional communication strategies tailored to local situations to provide continuous information and prevention messages on ciguatera risk (especially in remote, isolated locations) can also prove useful in reducing the number of CP incidents in a significant way. This is well illustrated by the case studies of Raivavae and Rapa Islands in French Polynesia (Chinain *et al.*, 2010, 2020a, 2020b, 2021; see case study in Section 3.2) where these communication actions were achieved through the dissemination of guidebooks, flyers and posters made available in both official and native languages and displayed at various strategic spots such as town halls, post office, medical centres and schools. In parallel, information and education actions targeted at the healthcare workers occupational group should not be overlooked, as it may help overcome the high under–reporting rate of CP cases currently observed worldwide (Chinain *et al.*, 2021).

3.1.8 Nowcasts, forecasts and modeling

Satellite-derived sea surface temperature (SST) data from Aqua Moderate Resolution Imaging Spectroradiometer (MODIS) have been used to project regional rates of growth for four *Gambierdiscus* and one *Fukuyoa* species in the Caribbean Sea. Dinoflagellate growth rates were modelled using experimental temperature versus growth equations and projected bottom temperatures with light penetration and bathymetry masks. Daily projected growth rates for each species were used to calculate monthly, yearly and multiyear averages between 2003 and 2013. The resulting projections were then used to characterize patterns of regional *Gambierdiscus*/*Fukuyoa* abundance and compare these to CP incidents in the greater Caribbean region. Model output indicated the highest growth potential was in the shelf waters of the Caribbean Sea, with moderate growth in the Bahamas, southern Florida (United States of America) and Gulf of Mexico. The lowest growth potential was in the northern Gulf of Mexico and along the south Atlantic coast of the United States of America. Mean projected growth rates generally coincided with distribution of ciguatera fish poisoning incidents in the region, with some exceptions in the southwestern Caribbean Sea. The results of this study indicate spatial differences in *Gambierdiscus* and *Fukuyoa* growth likely play a prominent role in governing the occurrence of ciguatera fish poisoning in the greater Caribbean region. Growth and distribution models may prove to be an effective tool for ciguatera risk assessment (Kibler *et al.*, 2017).

3.1.9 Stakeholders and their needs

At a minimum stakeholders deserve geographical and species/size consumption advisories (i.e., fish or invertebrate species that are safe and those that are risky for harvesting for consumption) that include seasonally updated maps and traceability procedures. The plan of the Hazard Analysis Critical Control Point (HACCP, United States of America, www.fda.gov/food/hazard-analysis-critical-control-point-haccp/seafood-haccp) states that primary seafood processors should avoid purchasing fish species associated with causing ciguatera from established or emerging areas linked with CP. Developing a CTX screening and monitoring plan, regardless of the origin of the sample, involves detailing specifications and standards, including defining maximum permissible levels allowed in seafood, and identifying official and routine methods of analysis (FAO/WHO, 2020).

FIGURE 3.8
Velvet leaf soldier bush (*Heliotropium foertherianum*) is native throughout the Indo-Pacific region including China, Madagascar, northern Australia and most of the atolls of Micronesia and Polynesia. It has been introduced in Hawaii

Another stakeholder need is treatment for CP. While traditional medicine provides only symptom-based palliative care for CP patients, the effectiveness of traditional remedies for relieving the symptoms of ciguatera have been assessed (Bourdy *et al.*, 1992; Rossi *et al.*, 2012; Braidy *et al.*, 2014). Many South Pacific communities use traditional remedies as a matter of course, particularly on remote islands and atolls where no healthcare services are available. Ethnobotanical research by the Institute of Research for Development (IRD) in New Caledonia has produced an inventory of nearly 100 plants used in traditional medicine for ciguatera treatment or prevention. Brews, teas or extracts are prepared from roots, leaves, bark and fruit, whether pure or mixed in varying proportions and dosages based on household recipes handed down through the generations. Further research narrowed the efficacy down to a single plant, *Heliotropium foertherianum* (velvet leaf soldier bush or octopus bush) and identified its main active ingredient, rosmarinic acid, isolated from the plant's leaves (Figure 3.8). As a result, a patent was registered in 2010 relating to the use of rosmarinic acid and its related derivatives in treating ciguatera.

To date there are no effective local remedies for CP used in the Caribbean region. There, reporting CP is required in a few countries, but generally, illness is regarded as the Caribbean "flu" and expected if locally sourced fish is a part of the diet. Unlike French Polynesia, there is no coordinated web-based reporting of CP cases among the Caribbean countries for regional events.

3.1.10 Baseline risk assessment

The FAO and WHO experts' report on ciguatera (FAO/WHO, 2020) and Laurent *et al.*, (2005) provide robust guidance on both the risk assessment and management of CP that should prove useful seeking to establish a risk assessment plan. Too, a number of reviews on CP and human health, both regional and global, provide a background and inform early efforts in viewing risk assessment in context (Chinain *et al.*, 2010; 2019; 2021; Daneshiam *et al.*,2013; Dickey and Plakas, 2010; Friedman *et al.*, 2017; Goater *et al.*, 2011).

Key information for risk management includes data on:

- Where was the seafood harvested?
- Identification of meal remnant to genus or species if possible;
- What symptoms were reported?
- Identification, concentration and distribution of toxin in seafood;
- How was seafood processed and cooked (head, gonads, intestines intact)?
- How much was eaten? By whom (male, female, age, weight, contributing health factors including previous CTX intoxication)?

Farrell *et al.*, (2017) suggest that until a "cost-effective, reliable, routine test for ciguatoxins" is developed it is incumbent upon regulators and industry "to improve existing strategies including communication campaigns for consumers, improved catch traceability, improved regulation of high-risk catch sites and the support of targeted research to improve testing capabilities". See Laurent *et al.*, (2005) and FAO/WHO (2020) for specific guidance (Figure 3.4A and 3.4B).

3.2 CASE STUDY – FRENCH POLYNESIA

What is the problem caused by the harmful algal bloom?

In June 2014, a mass -poisoning outbreak involving nine tourists in Nuku Hiva Island (Marquesas archipelago) following the consumption of *Tectus niloticus* was reported in the frame of the country-wide epidemiological surveillance network established since 2007 in French Polynesia. All patients exhibited clinical symptoms typical of CP (Gatti *et al.*, 2018).

Who are the stakeholders and what were their needs?

In this case study both the tourists and island residents were stakeholders. -The study that followed this CP event in Nuku Hiva was the first to describe the medical signature of *T. niloticus* poisoning in French Polynesia. It alerted local authorities about the potential health hazards association with the consumption of this highly prized gastropod (Gatti *et al.*, 2018).

What was the development status of the country or region in terms of monitoring?

The incident in Nuku Haiva Island was reported in the context of a country-wide epidemiological surveillance network established since 2007 in French Polynesia and supported by the Institut Louis Malardé.

What approach and technology were used to solve the problem?

The qPCR assays carried out on samples collected using artificial substrate (window screen, see Figure 3.7A) devices to survey for *Gambierdiscus* relative cell abundance and species distribution showed a high species biodiversity in the Nuku Hiva Island study site. Up to five distinct species were detected, with *G. polynesiensis* as the dominant species in Anaho Bay (~82 percent) versus *G. carpenteri* in Taipivai and Taiohae (90 percent and 88 percent, respectively).

The neuroblastoma cell-based assay (CBA-N2a) and LC–MS/MS toxicity analyses conducted periodically on *T. niloticus* showed only specimens collected from Anaho Bay were toxic, and that, despite a progressive 19-fold decrease in CTX contents over time, the residual toxicity monitored in *T. niloticus* remained well above the safety limit recommended for human consumption even 28 months after the poisoning event (Darius *et al.*, 2018).

In parallel, several *Gambierdiscus* spp. cultures were also successfully established in the laboratory. Although the culturing approach gave a highly biased representation of the community species composition in the three study sites as assessed by the artificial substrate method, it confirmed only one strain, a *G. polynesiensis* isolate, out of the 17 clonal strains examined, produced CTXs at levels around 1.20 ± 0.14 pg P-CTX-3C equiv/cell. Conversely, none of the *G. carpenteri* isolates showed toxicity in in vitro conditions.

Based on LC–MS/MS data, four distinct P-CTX analogs were detected in toxic *T. niloticus* tissues, namely CTX3B, -3C, - 4A and -4B, with CTX3B as the major congener. Since these four analogs are consistently found in *G. polynesiensis* culture extracts (Longo *et al.*, 2019), it can be concluded that *G. polynesiensis* populations, which predominate in Anaho Bay benthic assemblages, are the primary source of the CTX analogs detected in neighboring *T. niloticus*. Interestingly, these findings are consistent with the results of a field survey conducted in this same area by means of SPATT technology, which also showed evidence of the presence of CTX3B and -3C in the environment (Roué *et al.*, 2020). Of note, in addition to CTXs, okadaic acid and dinophysistoxin 1 were also detected in SPATT extracts using a LC–MS/MS-based multitoxin screening approach, thus highlighting the usefulness of passive samplers in the routine surveillance of CP and other -phycotoxin-related risks in -ciguatera-prone areas.

What forecast data were used?

Forecast data for benthic harmful algal species are generally environmental (temperature, seasonal monsoons or dry periods) or from either natural (tropical storms, hurricanes) or anthropogenic disturbances (dredging, installation of rip rap, breakwaters) that create new habitat space. Long-term data sets are rare for benthic species, but for the locations where records are kept, these are exceptionally valuable. When increases in *Gambierdiscus* cell abundance are paired with the approximate lag times for ciguatoxins to vector into human diets, a testing horizon emerges for managers to work within for more intensive sampling and toxin testing.

What early warning system was put in place?

This CP incident was reported within the context of ongoing sampling to provide early warnings of increased *Gambierdiscus* cell abundance supported by the Institut

Louis Malardé. The implication of this novel gastropod vector of high value for local populations in a CP incident prompted a two-year field survey involving the monitoring of *Gambierdiscus* species diversity and environmental toxins by means of passive samplers, the toxicity screening of in vitro cultures of *Gambierdiscus* spp. established from wild material, as well as a follow-up survey of the toxicity in *T. niloticus* specimens in three distinct fishing sites off Nuku Hiva Island (i.e. Anaho Bay, Taipivai Bay and Taiohae Bay).

What were the results for forecast operation?

This incident triggered by the consumption of invertebrates, rather than the traditional vector of CP (fish), has expanded the scope of sampling for ciguatoxins in the environment in French Polynesia. The data from the two-year field survey of the Nuku Hiva Island bays added to the information base and enhanced forecasting possibilities.

What were the consequences of the early warning system for stakeholders?

Stakeholders in CP-prone areas live with the possibility of becoming ill. Many will experience CP during their lives and some more than once. Having additional information, that invertebrates, as well as herbivorous and carnivorous fish may be CTX vectors, is a significant finding. Warnings to tourists and notification of regional clinics can be expanded for better protection of seafood harvesters.

What lessons were learned?

Considering the high poisoning risk characterizing Anaho Bay, as evidenced by the bioaccumulation of substantial amounts of CTXs in a variety of other organisms across multiple food-web components, including sea urchins in the species *Tripneustes gratilla* (Darius *et al.*, 2018) as well as herbivorous and carnivorous fish, a ciguatera alert and a fishing ban were issued in this area in 2018 in close coordination with local authorities.

Summary

EWS-based field studies targeted at *Gambierdiscus* currently exist in several regions of the world. By way of example, the case study of Nuku Hiva Island in the Marquesas archipelago (French Polynesia) is a good illustration of the benefits that an EWS approach can provide to efforts to reduce the risk of exposure of local populations to ciguatera toxins in CP-prone areas (see www.ciguatera.pf/index.php/en/).

3.3 *OSTREOPSIS*

3.3.1 *Ostreopsis* blooms, human health effects and environmental impacts

the genus *Ostreopsis* grows in shallow and well illuminated waters, attached to biotic (macroalgae, corals, bryozoans, mussel shells) and abiotic (rocks, sands) surfaces. Its life cycle includes a benthic and a planktonic phase. Cells attach to the surfaces by producing mucilaginous filaments and creating a matrix embedding high cell numbers. Modulated by a combination of internal migration rhythms and water circulation dynamics, cells detach from the substrates and become part of the plankton. Also, dense aggregates of *Ostreopsis* cells are found in the water surface. The *Ostreopsis* genus is known in tropical latitudes accompanying *Gambierdiscus* and other benthic microalgae.

The main concern posed by *Ostreopsis* spp. is that toxins synthesized by some species (ostreocins, mascarenotoxins and ovatoxins are chemical analogues of the potent palytoxins) (PLTX) can be isolated from the tropical soft coral genus *Palythoa*. PLTX-c ontaminated seafood (fish and crustaceans) was identified as the cause of a few fatalities in the tropics (Deeds and Schwarz, 2010; Tubaro *et al.*, 2011), and some studies suggested that *Ostreopsis* was the biogenic origin of the toxins involved in these foodborne poisonings (for example, Taniyama *et al.*, 2003). However, as discussed by Tubaro *et al.* (2011), this link has not been clearly established yet.

At the beginning of the twenty -first century, blooms of *Ostreopsis* spp. (mainly

O. cf. *ovata*) appeared suddenly in the Mediterranean Sea, New Zealand and other temperate regions (for example, Mangialajo *et al.*, 2011; Neves *et al.*, 2018; Kuzat *et al.*, 2021; Zingone *et al.*, 2021). Since then, the range of occurrence has expanded, and blooms have become recurrent in certain beaches coast of the Mediterranean Sea. Different ovatoxins and isobaric PLTX were reported in mussels, sea urchins or fish collected in the Mediterranean coasts (see literature data in Table 3.3 of Pavaux *et al.*, 2020) at concentrations exceeding the safety alert threshold of 30 µg of PLTX-equivalent per kg of fresh flesh recommended by the European Food Safety Authority (EFSA, 2009). Luckily, no cases of seafood poisoning have been reported yet in the Mediterranean region.

However, in some cases, people exposed to marine aerosols during the *Ostreopsis* spp. blooms have experienced mild but acute respiratory illness and skin and mucosa irritation by direct contact with the seawater containing high *Ostreopsis* cell concentrations (for example, Gallitelli *et al.*, 2005; Kermarec *et al.*, 2008; Vila *et al.*, 2016; summary Table 2 of Pavaux *et al.*, 2020). Some chronic effects have also been documented (Berdalet *et al.*, *Harmful Algae*, under revision). PLTX-like compounds have been postulated as the toxins causing these disorders (see for example, laboratory experiments by Poli *et al.*, 2018) although the direct implication has not been completely demonstrated yet. Some *Ostreopsis* spp. blooms have also been linked to massive benthic fauna mortalities in the Mediterranean Sea, the coast of New Zealand and Brazil (for example, Sansoni *et al.*, 2003; Vila *et al.*, 2008; Shears and Ross, 2009; Neves *et al.*, 2018). Overall, the human health and environmental risks posed by the *Ostreopsis* blooms stimulated the regular monitoring of these events in some areas, leading to occasional beach closures (Lemée *et al.*, 2012; Funari *et al.*, 2015). Other toxic substances produced by *Ostreopsis* spp., likely different from ovatoxins and ostreocins, and the mucilaginous matrix, seem to be involved in negative effects observed on marine organisms (Giussani *et al.*, 2015; Ternon *et al.*, 2018). The overall risks posed by *Ostreopsis* blooms have a potential economic cost in touristic areas, such as the Mediterranean zone.

Available data suggest that human health effects may occur at thresholds values of 5 x 10^5 cells g^{-1} wet weight of macroalgae and/or 3 x 10^4 cells/L of seawater (Lemée *et al.*, 2012, Funari *et al.*, 2015, Mangialajo *et al.*, 2017). Symptoms may not occur along the whole duration of the bloom but during certain periods (Vila *et al.*, 2016; Berdalet *et al.*, under revision). Environmental effects depend on the duration and surface extension of the bloom event. In the field, massive mortalities of certain macrofauna have been observed at 1.4 x 10^6 cells g^{-1} fresh weight of macroalgae in New Zealand (for example, Shears and Ross, 2009) or 250 x 10^6 3 x 10^9 cells/L of seawater in Italy (Sansoni *et al.*, 2003). Data on threshold cell abundances having toxic effects have been obtained from experimental ecotoxicity tests on different organisms (for example, Giussani *et al.*, 2015, 2017). The responses are variable, depending on experimental conditions and the tested organism.

Currently, and in the context of designing EWS for *Ostreopsis*, establishing threshold values is one of the fundamental issues, as it is discussed in Section 3.3.8.

The problems posed by *Ostreopsis* proliferations have, by now, a smaller relevance than *Gambierdiscus* and *Fukuyoa* blooms and CP in the world. Uncertainties include identification of the toxic compounds and harmful mechanisms involved in human and environmental health problems, the toxin transfer through the food-webs and the potential risk of foodborne intoxications, and the future trends of the blooms under a general global change and warming scenario. Meanwhile, the benthic nature of *Ostreopsis* and their blooms in temperate latitudes and different habitats, and the fact that it is part of the benthic assemblages of CP-causing species offer the possibility to approach the challenges of addressing CP and other potential benthic HABs.

3.3.2 *Ostreopsis* distribution, habitats and toxins

At the time of writing this document, 11 species described and six ribotypes are under study (Tester *et al.*, 2020). Based on that review, detailed information on the species geographical range is provided along with references of the species-specific produced toxins, and temperature, salinity and light requirements are provided here (Table 3.2, Figure 3.9). Additional information about *Ostreopsis* synthesized toxins and their impacts can be obtained in the reviews by Ciminiello *et al.* (2011) and Pavaux *et al.* (2020).

Rhodes (2011) showed a first map on the worldwide distribution of *Ostreopsis* species at that time, warning about the expansion of the microalgal group. More recently, Tester *et al.* (2020, world map in Figure 5) updated the information corroborating the trends already pointed out by Rhodes (2011).

In the Mediterranean Sea, three blooming species have been identified, namely, *O.* cf. *ovata*, *O.* cf. *siamensis* and *O. fattorussoi*. The toxin profiles of the three species characterized by LC–MS/MS indicate that *O.* cf. *ovata* is the most toxic species (Accoroni *et al.*, 2016; Ciminiello *et al.*, 2013; Tartaglione *et al.*, 2016), producing different ovatoxins and isobaric (or putative) PLTX. Mediterranean Sea and Atlantic Ocean *O.* cf. *siamensis* strains had a lower toxin content compared to the strains isolated in Japan. *O. fattorussoi* synthesizes mainly ovatoxin a, but in low amounts compared to *O. ovata*.

In French Polynesia, four species have been identified so far, namely, *O. lenticularis*, *O.* cf. *ovata*, *O. rhodesae* and *Ostreopsis* sp. 6 (= *O.* cf. *siamensis*), most of them being nontoxic. Only *Ostreopsis* sp. 6 strains proved toxic in culture, with a toxin profile dominated by ostreocin-D as confirmed by LC–MS/MS (Chomérat *et al.*, 2019; Chomérat *et al.*, 2020a, 2020b).

TABLE 3.3
Toxic *Ostreopsis* species, toxins and harmful effects

Causative organism(s)	Toxins	Clinical symptoms	Syndrome	Selected reference
O. cf. *ovata* *O.* cf. *siamensis* *O. fattorussoi*	Putative palytoxins (ovatoxins,, ostreocins, mascarenotoxins) Ovatoxins Ovatoxins	Mild but acute respiratory illness and skin and mucosa irritation; massive benthic fauna mortalities; beach closures due to dense mucilage	*Ostreopsis* spp. algal syndrome	Accoroni *et al.*, 2016; Ciminiello *et al.*, 2013; Tartaglione *et al.*, 2016

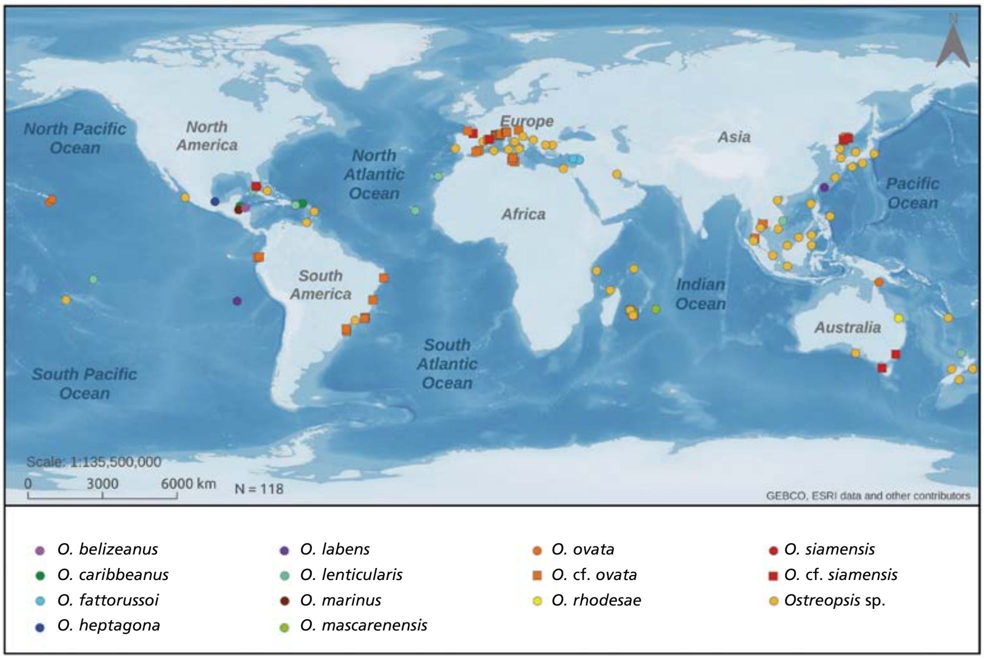
Source: C. Gatti and M. Chinain, ILM.
*possibly toxic

3.3.3 *Ostreopsis* blooms dynamics, ecophysiology and forcing factors

Understanding the biotic and abiotic forcing factors determining *Ostreopsis* bloom occurrence and modulating its ecophysiology are essential in bloom prediction and in developing EWSs (monitoring and forecast).

In the Mediterranean Sea, *Ostreopsis* blooms occur mainly in the summer–fall period, in relative wide ranges of temperature (22 °C to 30 °C) and salinity (20–40), attached to macroalgal turf formations and pebbles in shallow, well illuminated areas (Accoroni and Totti, 2016; Mangialajo *et al.*, 2011). The available data indicate that this genus is well adapted to grow on habitats subjected to anthropogenic pressures, including eutrophic conditions (Ungano *et al.*, 2010; Fricke *et al.*, 2018; Meroni *et al.*, 2018). In tropical environments, *Ostreopsis* also proliferates in natural habitats disturbed by natural (for example, hurricane and extreme events) or anthropogenic (that is, infrastructure constructions) disturbances.

FIGURE 3.9
Distribution of the species in the genera *Ostreopsis*



Source: Modified from Tester, P.A., R.W. Litaker, and E. Berdalet. 2020. *Climate change and harmful benthic microalgae.* Harmful algae. **91**: p. 101655.

The capacity of cells to incorporate organic nutrients and feeding mixotrophically are important considerations in bloom ecophysiology. In culture, toxin production increases from the exponential to the stationary phase. High water temperatures, high irradiance and high nutrient availability are major factors that converge to create an environment favoring *Ostreopsis* spp. and other benthic harmful algal blooms (Fraga *et al.*, 2012). These aspects are briefly presented next.

Bloom period. *Ostreopsis* blooms occur mainly in the summer–fall period in temperate areas (Mangialajo *et al.*, 2011; Pistocchi *et al.*, 2011). In the coasts of the Mediterranean Sea, *O.* cf. *ovata* blooms occur from mid-July to early November, with peaks in summer and/or autumn, although timing varies geographically (Accoroni and Totti, 2016).

Temperature. Seawater temperature is one of the primary drivers of *Ostreopsis* blooms (see for example, review by Pistocchi *et al.*, 2011; Drouet *et al.*, 2022) and blooms occur in a relatively wide range of temperature (22 °C to 30 °C). Sea surface temperatures exceeding 19.5 °C for three months can trigger blooms in the Atlantic coast of Spain and Portugal (David *et al.*, 2012), or above 20 °C maintained for about 7 to 10 days and reaching ≥25 °C in the northwest Mediterranean Sea (Cohu *et al.*, 2011). In the Adriatic Sea, bloom development usually occurs when temperature decreases (for example, Totti *et al.*, 2010). Interestingly, once initiated, *Ostreopsis* cf. *ovata* can continue to bloom independently of a potential decrease in seawater temperature. Recent experiments (Gémin *et al.*, 2021) showed an optimal growth of *Ostreopsis* cf. *ovata* strains isolated in the northwest Mediterranean Sea in the 21 °C to 25 °C range, with a decreased growth above this temperature. Thus, *a priori*, an excess warming of Mediterranean seawater may not favor *Ostreopsis* blooms, although the progressive adaptation of the species cannot be ruled out.

Salinity. *Ostreopsis* has a wide range of salinity tolerance (20–40), with growth favored by higher salinity in some cases, while toxicity was lowest at the highest salinity value (that is, 40) (Pistocchi *et al.*, 2011; Carnicer *et al.*, 2015; 2016).

Light. Irradiance (photon flux density and daylength) may be a major driver explaining the seasonality of *Ostreopsis* blooms in temperate regions. However, the available field and experimental data about the light dependence on *Ostreopsis* growth are contradictory (Accoroni and Totti, 2016). This could be because light cannot be separated from depth, and in turn, this parameter depends on the preferences of the macroalgae host species. *Ostreopsis* blooms occur in very shallow waters., between 10 cm and 3 m in the Mediterranean Sea coasts, despite cells found at 30 m depth (Cohu and Lemée, 2012). In these habitats, cells tolerate a wide range of light intensity variations (between 50 and 1800 µmol photons m2 s-1) and may rapidly adapt to contrasting shade and light natural conditions during the day (Fraga *et al.*, 2012). Irradiance could modulate mucus production which, together with the gas bubbles formed, contribute to the floating and drifting of cell aggregates (Heil *et al.*, 1993). Decreased light availability would also decrease toxin content (Lee *et al.*, 2020).

Substrate. *Ostreopsis* grows attached to a variety of substrates (see review by Accoroni and Totti, 2016), as epilithic (on sand and rocks), epizoic (on Bryozoa, mussel shells, corals) and, more often as epiphyte (of macrophytes and phanerogams) with a preference for three-dimensional, flexible and high surface area macroalgae. Substrate availability may depend on cascade effects in the ecosystem dynamics. Decreased sea urchin predation pressure may facilitate a shift from a coral-dominated to a macroalgae-dominated system and hence increase the suitable habitat for *Ostreopsis* spp. (Shears and Ross, 2010). Turf formations in anthropogenically affected habitats are also a preferred substrate for *Ostreopsis* spp.

Water motion. Relatively calm weather favors *Ostreopsis* proliferation. However, wavy conditions facilitate cell release from the substrate and dispersion of aggregates containing high cell densities which can colonize new areas (Mangialajo *et al.*, 2008; Fraga *et al.*, 2012). Indeed, high *Ostreopsis* spp. abundances have been reported in different habitats exposed to turbulent conditions in temperate ecosystems (Vila *et al.*, 2001; Giussani *et al.*, 2017) and coral reefs (Richlen and Lobel, 2011). In these environments, *Gambierdiscus* spp. seem to prefer calmer waters. Unfortunately, the quantitative characterization of natural and experimental turbulence is difficult, and in general, it is lacking. This may hamper the detecting the species-specific preferences concerning turbulent conditions and the comparison of data from different environments.

Nutrients. Nitrogen (N) and phosphorus (P) are necessary not only for *Ostreopsis* to grow but also for the macroalgae to develop. A balanced proportion between N and P is required for the bloom to start (Accoroni *et al.*, 2015) and then blooms need to be fuelled by high nutrient supply. Some data indicate that the microalga tolerates or benefits from increased nutrient levels (Ungano *et al.*, 2010; Pistocchi *et al.*, 2011 and references therein) in urbanized sites. However, *Ostreopsis* also grows on pristine waters, suggesting its capability to ingest microorganisms, that is, to be mixotrophic and to use remineralized nutrients from the sediment (Pistocchi *et al.*, 2011; Fraga *et al.*, 2012). *Ostreopsis* cf. *ovata* can use different N sources, with a preference for NH4+, followed by NO3- and N-urea (Jauzein *et al.*, 2017).

Links with bacteria. Bacteria associated with the surfaces or extracellular matrices of *Ostreopsis* spp. correlated with the high cell toxicity during the stationary phase of the cultures (references in Pavaux *et al.*, 2020; Ashton *et al.*, 2003). In turn, *Ostreopsis* blooms could provide biological surfaces (fragments of thecae or mucilage) for bacterial growth.

3.3.4 Monitoring *Ostreopsis* cells and toxins

The benthic stage constitutes the reservoir or stock of *Ostreopsis* cells that determines the duration and maintenance of the blooms, and the potential risk of effects on human health (Mangialajo *et al.*, 2011, 2017). Planktonic cells and aggregates released from the benthic substrate facilitate the dispersion of the bloom and the colonization of new sites and may be directly involved in respiratory impacts due to the aerosolization of toxic compounds at the water surface. The cells concentration in the plankton are more affected by site-specific hydrodynamics (Meroni *et al.*, 2018) and *Ostreopsis* vertical migration (Mangialajo *et al.*, 2017) and exhibit higher variability than cells in the benthos. However, collecting water samples a few dozen centimeters from the macroalgal assemblages (surrounding water, as in Mangialajo *et al.*, 2017) to estimate plankton cells concentrations, is easier than sampling macrophytes to estimate the benthic (epiphytic) stock. Still, benthic and plankton cells abundances are correlated well on a logarithmic scale. For these reasons, risk assessment is conducted in some areas combining plankton concentrations and the hydrodynamics of the sampling site (Funari *et al.*, 2015). Nevertheless, more studies are needed to monitor the *Ostreopsis* benthic population to have a more consistent risk assessment.

The most common method to sample benthic *Ostreopsis* is the collection of the dominant macroalgae in the area that appear covered by the mucilaginous biofilm containing *Ostreopsis* cells as described for example, by Moreira and Tester (2016) and Tester and Kibler (2018). By this method, cell abundance is referred to as cells per gram of macroalgae fresh weight. Pebbles and invertebrate shells can also be sampled, and the concentration is expressed as cells cm^{-2} of these substrates. The procedure is destructive, and the obtained value depends on the sampled substrate, with marked differences observed as a function of the collected macroalgae (for example, Cohu *et al.*, 2013; Meroni *et al.*, 2018; Gémin *et al.*, 2020). It has been noted that *Ostreopsis* does not attach to Ulvaceae (indicative of eutrophication), has a poor affinity for the *Cystoseira* family and a preference for the Corallinaceae and turf formations. With the aim to overcome these limitations and to standardize and compare data among sampling sites, the artificial substrate by Tester *et al.* (2014) has been examined and adapted in the Mediterranean Sea and the Atlantic coast of France for *Ostreopsis* collection by Jauzein *et al.*, (2018) and in the Pacific Ocean by Lee *et al.*, (2020). For the design of an EWS, the selection of the sampling method should be discussed, and the best strategy selected, taking into consideration logistic, technical and human resources in the area.

Cell counts in Lugol or formalin fixed samples are usually conducted by microscopy following the Utermöhl method with sedimentation chambers on inverted microscopes or -Sedgewick Rafter chambers. The identification at Species-level is difficult because the taxonomy is mainly based on the plate pattern, which is very similar in most species. Molecular PCR and qPCR analysis are facilitating the species-specific cell counts (for example, Battocchi *et al.*, 2010; Perini *et al.*, 2011; Casabianca *et al.*, 2013; Hariganeya *et al.*, 2013). New techniques are also being explored to augment the earlier, specific and easy to use detection methods for *Ostreopsis* species. Electrochemical biosensors (Toldrà *et al.*, 2019a) and colourimetric DNA-based assays (Toldrà *et al.*, 2019b) offer promising techniques.

Monitoring cell abundances is accompanied by recording other environmental parameters that can modulate the *Ostreopsis* bloom (Section 3.3.4), such as temperature, salinity, wind intensity and direction, general meteorology and sea conditions, collected substrate (macroalgae identification at genus level) and nutrients.

3.3.4.1 Toxin detection

The toxicity of palytoxin-like compounds is much higher than earlier thought. The chronic no observed adverse effect level (NOAEL) is as low as 0.03 µg/Kg (EFSA, 2009; Boente Juncal *et al.*, 2020). This fact, combined with the potential extreme chemical diversity of this family of compounds (2^{64}) potency isomers for the palytoxin structure), makes detection a major challenge. Precise detection and determination can be achieved by mass spectrometry. However, the fundamental drawback of this methodology is the lack of analytical standards in general, and of palytoxin analogues, in particular. Hence, functional methods are a better option (Fraga *et al.*, 2016) which, given the mode of action of these molecules (Tubaro *et al.*, 2014), should be based on the interaction with the Na^+ -K^+ ATPase (Alfonso *et al.*, 2013; Bignami, 1993; Pelin *et al.*, 2016; Boscolo *et al.*, 2013; Ledreux *et al.*, 2009). Unfortunately, the cytotoxic assays or ATPase based methods have several limitations as well, in terms of sensitivity or high-throughput since antibodies cannot guarantee a fit cross reaction to so many potential palytoxin analogues.

In the context of an EWS, the factors affecting toxin production should be understood. Some field studies noticed that the intracellular toxin content is negatively correlated with cell abundances in the water (Carnicer *et al.*, 2015) while salinity decreases cell toxin content (Pistocchi *et al.*, 2011).

Nutrients can also modulate toxin production, although the available data are sometimes contradictory. In culture, both N and P limitation induced a decrease in cell toxicity (Pistocchi *et al.*, 2011) with a negative correlation between the abundance of *O.* cf. *ovata* and nitrate concentrations.

It has been suggested that toxicity is linked to bacteria associated with the *Ostreopsis* blooms. The reduction of associated bacteria *Pseudomonas* and *Alteromonas* (Ashton *et al.*, 2003) on *O. lenticularis* cultures was concurrent with a decrease in toxin content. Recently, Gémin *et al.*, (2021) found higher toxin content in strains maintained at 23 °C than at 27 °C or 30 °C.

Higher toxin levels were found on cells attached to *Halopteris scoparia* than in Dictyota spp. epiphytes with the lowest toxic cells detected on *Padina pavonica* (Gémin *et al.*, 2021). The underlying mechanisms (competition for nutrition, pH or allelopathic interaction) remain yet to be determined.

Given the poor knowledge about the modulation of *Ostreopsis* toxins production and the technical difficulties to detect and quantify them, their determination cannot be included in an EWS. More work should be done to overcome technical limitations and find a reliable and cost-effective method. Also, more studies should be conducted to elucidate the toxic mechanisms involved in the harmful effects on humans and marine organisms, as well as the mechanisms of toxin aerosolization. Nonetheless, our suggestion is to define an *Ostreopsis* cell-based EWS, based in part on the foundation of the monitoring programs established in France, Italy, Monaco and Catalonia (northeast coast of Spain), which are discussed in Section 3.3.4.

3.3.5 Data considerations

Routine *Ostreopsis* spp. monitoring in summer is conducted in some Mediterranean countries, combining research purposes and surveillance actions to prevent human health impacts by *Ostreopsis* blooms:

- In Italy, monitoring is conducted by ISPRA (Istituto Superiore per la Protezione e la Ricera Ambientale) and ARPAL (Italian regional public agency for the protection of the Ligurian environment) (Funari *et al.*, 2015). Plankton and benthic samples are collected along with fundamental environmental parameters (temperature, salinity, macroalgal species identification).
- In France, monitoring is conducted by the Observatoire Oceanologique de Villefranche (Sorbonne University) and L'Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) in coordination with the Marseille Poison Control Centre that facilitates identification of *Ostreopsis*-related health symptoms. These coordinated institutions constitute the French Mediterranean *Ostreopsis* Surveillance Network (Kermarec *et al.*, 2008; Berdalet *et al.*, under review), under the French General Health Administration.
- In French Polynesia, the Institute Louis Malardé and Ifremer Concarneau as part of the TATOO program (https://wwz.ifremer.fr/lerbo/Activites-Missions/Programmes-de-recherche/TATOO) are in charge of *Ostreopsis* monitoring.

- In Spain, monitoring is conducted on plankton only, while increasing attention is given to the presence of *Ostreopsis* in the last ten years. In Catalonia, benthic monitoring and research studies have been conducted (Institute of Marine Sciences–Spanish National Research Council [CSIC]) since 2004, in coordination with the Catalan Water Agency and the Public Health Agency and concurrent epidemiology studies are described in Berdalet *et al.* (under review).
- In Monaco, monitoring supported by Monegasque funds has allowed collection of a long time-series (10 years) of data on the beach at Monaco. In addition, the RAMOGE agreement is facilitating the coordination of the *Ostreopsis* data collected by Italy, France and Catalonia (Spain) (see Section 3.1.7).

Health impacts are difficult to be monitored, in part because of the lack of specificity of the *Ostreopsis*-related symptoms, which are usually misdiagnosed as a light flu. Only when an exceptionally high number of people have noticed respiratory health symptoms and general malaise at a single beach and the potential link with the sea is suggested, and if subsequent samplings conducted after some elapsed time identify the presence of high *Ostreopsis* cell numbers, the link between the microalgal bloom and the human health symptoms is established. The accumulated experience in Italy, France, Spain and Algeria and some simultaneous epidemiology and ecology studies (Vila *et al.*, 2016; Berdalet *et al.*, under review) constitute a basis for the design of coordinated human health and *Ostreopsis* monitoring as part of the EWS. Note that while in Italy, France and Monaco alerts are determined based on the cell abundances in the water and/or in the benthos, in Catalonia, alerts are set by the combination of the abundances and reported cases of human health disorders (Berdalet *et al.*, 2022).

3.3.6 Nowcasts, forecasts and modelling

According to the model by Accoroni *et al.* (2015), *Ostreopsis* cf. *ovata* blooms appear to be triggered by a combination of optimal temperature and available nutrients, provided that suitable hydrodynamic conditions also exist. A water temperature threshold of 25 °C would favor the germination of *O.* cf. *ovata* cysts and therefore bloom onset. Then, an N:P ratio around 16:1 Redfield value is a necessary condition to allow cell proliferation. The synergy of higher temperatures and optimal N:P ratios would result in a higher net growth rate of *O.* cf. *ovata* cells. Bloom decline will occur when temperatures drop below 18 °C. The net effect of the synergy between local hydrodynamic conditions, temperature, and N and P availability may help to explain why blooms in the northern Adriatic Sea occur differently from those in other Mediterranean regions.

Asnaghi *et al.*, (2017) modeled meteorological data as input features to predict the concentration of *O.* cf. *ovata* in seawater. Ten meteorological features were used to train a Quantile Random Forests model, which was then validated using *Ostreopsis* cells concentration field data over the course of a summer sampling season. The proposed model was able to accurately describe *Ostreopsis* abundance in the water column in response to meteorological variables.

3.3.7 Stakeholders and their needs

In the Mediterranean Sea, monitoring of *Ostreopsis* spp. is necessary in the summer vacation period given the risk of respiratory and cutaneous irritations in beach users. When the first cases occurred in Italy, France, Spain and Algeria, beaches were closed to the public and a high number of alarmed beach users (100 to 200 people) simultaneously looked for health support in hospitals and primary health care emergency facilities. To avoid these impacts, regular monitoring is conducted, when possible, at the most popular beaches, where previous blooms and impacts on health have been documented with the highest spatial and temporal resolution and with fast sampling and cell numbers estimation. Coordination between health, environmental and scientific entities is essential. The general public is always informed, trying to avoid alarming messages, but keeping in mind that the best prevention is beach closure, although this measure can entrain economic consequences to the tourist sector. It is important to note that, in the Mediterranean region, the scope and goal of the *Ostreopsis* EWS is justified as a tourism problem, not a toxic seafood problem.

The priority of the EWS is twofold: 1) avoiding closing beaches to the public and, 2) preventing the collapse of the primary health care and hospital emergency facilities. Concerning treatment, acute *Ostreopsis*-related health symptoms usually disappear in the subsequent few hours (6 to 24 h, rarely 72 h) after leaving the beach. Anti-inflammatory drugs and, if necessary, bronchodilators can be administered. Climatization also helps to reduce inflammation of the respiratory system. Recent studies suggest that chronic exposure may also have health impacts (Berdalet *et al.*, under review), although they are not well characterized yet.

3.3.8 Baseline risk assessment

The risk assessment is based on the reported cases of people potentially affected by *Ostreopsis*-related symptoms (Table 3.4) and a few epidemiology studies conducted in parallel to the monitoring of *Ostreopsis* blooms (Vila *et al.*, 2016; Berdalet *et al.*, under review). Clinical information is already available to identify the symptoms (first described by Tubaro *et al.*, 2011). Currently, citizen science can also contribute to monitoring *Ostreopsis* in addition to non-governmental organisations (as conducted in France by Surfrider) or private companies.

TABLE 3.4
Reports of mild respiratory, cutaneous and/or general malaise symptoms in humans after exposure to aerosols in *Ostreopsis* spp. blooms

Year	Species	Location	Affected people (n)	Reference
1998, 2004	*Ostreopsis* sp.	NW Mediterrean (Spain)	74 (estimated ~200)	Vila *et al.*, 2008
1998, 2000, 2001	*Ostreopsis ovata*	Tirrenian (Italy)	~100	Sansoni *et al.*, 2003
2001, 2003, 2004	*Ostreopsis* sp.	S Adriatic (Italy)	28	Gallitelli *et al.*, 2005
2005, 2006	*Ostreopsis ovata*	Ligurian (Italy)	228, 19	Brescianini *et al.*, 2006
2005	*Ostreopsis ovata*	Genoa (Italy)	209	Durando *et al.*, 2007
2006-2009	*Ostreopsis ovata* and *O. siamensis*	France	47	Tichadou *et al.*, 2010
2006	*Ostreopsis* sp.	SW Mediterrean (Spain)	57	Barroso García *et al.*, 2008
2009	*Ostreopsis* sp.	SW Mediterrean (Algeria)	150–200	Illoul *et al.*, 2012
2010	*Ostreopsis* cf. *ovata*	Adriatic (Croatia)	7	Pfannkuchen *et al.*, 2012
2013	*Ostreopsis* cf. *ovata*	NW Mediterrean (Spain)	13	Vila *et al.*, 2016
2014, 2015, 2016, 2017, 2018, 2019	*Ostreopsis* cf. *ovata*	NW Mediterrean (Spain)	66	Berdalet *et al.*, 2022

Source: Elaborated by the authors.

3.4 CASE STUDY – MEDITERRANEAN BASIN

Since the first health outbreaks potentially related to *Ostreopsis* blooms occurred in Italy, France, Spain and Algeria at the beginning of the twenty-first century, scientists were aware that an emerging HAB problem was a common threat in the Mediterranean basin. Fortunately, national and international funds for research facilitated not only research at local level, but also fostered the coordination of research among researchers of different Mediterranean countries.

What is the problem caused by the harmful algal bloom?

The genus *Ostreopsis* grows in shallow and well illuminated waters, mainly attached by mucilage to biotic (macroalgae, corals, bryozoans) and abiotic (rocks, sands) surfaces. Cells may also detach from the substrates and become part of the plankton, forming dense aggregates of *Ostreopsis* cells at sea surface during the blooms.

The palytoxin analogues produced by some *Ostreopsis* species are high molecular weight compounds. They are determined by LC–MS/MS although standards for the different toxins are lacking. Ovatoxins and isobaric PLTX were reported in mussels, sea urchins or fish collected in the Mediterranean coasts (reviewed by Pavaux *et al.*, 2020) at concentrations exceeding the safety alert threshold of 30 µg of PLTX-equivalent per kg of fresh flesh recommended by the European Food Safety Authority (EFSA, 2009) but, luckily, related seafood poisonings have not been reported in the Mediterranean region yet. Currently, *Ostreopsis* produced toxins are not regulated in Europe and thus, not monitored in marine food products. The monitoring is focused on the detection of *Ostreopsis* cells and blooms on beaches to prevent respiratory, otorhinolaryngology and cutaneous impacts on users. The toxicity effects are diverse, and not clearly associated to a particular species.

Who are the stakeholders and what were their needs?

Ostreopsis blooms pose a main risk to beach users. In the Mediterranean region, the summer vacation season coincides with the period of *Ostreopsis* blooms (in general), with potential economic cost in the touristic zones due to beach closures. Since the first health outbreaks potentially attributed to *Ostreopsis* blooms that occurred in Italy, France, Spain and Algeria, scientists were aware that an emerging HAB problem was a common threat in the Mediterranean basin. National and international funds for research facilitated not only scientific activities at local level, but also fostered the coordination of the research among scientists of different Mediterranean countries. Examples of such projects were MediOs in France, Ebitox and OstreoRisk in Spain and the Interreg Med coordinated project M3-HABs (Drouet *et al.*, 2022).

A general framework was established to structure an efficient preventive strategy, with particularities in each country (Funari *et al.*, 2015; Lemée *et al.*, 2012; Vila *et al.*, 2012; Meroni *et al.*, 2018). Smooth communication was established between scientists and the Environmental Agencies to establish the *Ostreopsis* monitoring program. Information about the health symptoms was also provided and determined by public health authorities, and special attention was given to train related professionals in primary healthcare centres and hospitals, and first aid support (International Committee of the Red Cross and lifeguards in the beaches). Local authorities of the affected beaches were informed about the evolution of *Ostreopsis* blooms in the beach and alert signs were installed, when necessary, to inform the general public about the presence of the microalga, the potential symptoms, what to do. When necessary, the beach was closed. Informative brochures were prepared by scientists with the essential information as well. Communication between scientists and the public was established through common citizen science mechanisms.

The collected information during more than 10 years can allow for evaluating key questions concerning risk assessment, including the sampling method as well as the alert threshold values for health symptoms, which still differ among countries. The coordination structure developed under the RAMOGE umbrella constitutes the foundation of a solid approach for the *Ostreopsis* EWS in the Mediterranean.

What was the development status of the country or region in terms of monitoring?

Regular monitoring of *Ostreopsis* blooms is conducted in some areas where almost annual blooms are known to occur.

FIGURE 3.10
The RAMOGE Agreement is a consortium of the French, Monegasque and Italian governments to implement actions for the prevention of pollution in marine environments (www.ramoge.org). This agreement includes cooperative programs to monitor harmful algae, especially *Ostreopsis*.

Source: Accord Ramoger. 2023. Gestion et protection du littoral. Monaco

Until now, most monitoring programs conduct traditional plankton samplings assuming that cells in the water column are preferentially related to the cutaneous and respiratory impacts reported. However, the *Ostreopsis* benthic populations constitute the pool or reservoir of cells that determine the duration and maintenance of the blooms. Planktonic cells and floating aggregates transported by currents facilitate the dispersion of the bloom and the colonization of new sites. For this reason, sampling of macroalgae to estimate the *Ostreopsis* benthic population has been introduced progressively in France, Italy, Monaco and Spain (Catalonia) as well.

What approach and what technology were used to solve the problem?

The most common sampling method for benthic populations is the collection of the dominant macroalgae in the area that appear covered by the mucilaginous biofilm containing *Ostreopsis* cells as described by Moreira and Tester (2016). Cell abundance is referred to as "cells per gram of macroalgae fresh weight". Plankton samples in the same area are also collected. Scientists working in the Mediterranean Sea were soon aware that a major challenge was to find a consistent collection method to standardize cell abundances from benthic substrates. The Benthic Dinoflagellate Integrator (BEDI) protocol is a proposed integrative approach to estimate the cell concentration in the benthic populations and the surrounding water (Mangialajo *et al.*, 2017) while providing a direct measure of the risk associated with inhalation of aerosols. Also, the artificial substrate by Tester *et al.*, 2014) has been examined and adapted in the Mediterranean Sea and the Atlantic coast of France for *Ostreopsis* collection by Jauzein *et al.* (2018) and in the Pacific by Lee *et al.* (2020). The artificial substrate is deployed during 24 h, which integrates the diurnal variability of the cell concentrations of the benthic and planktonic *Ostreopsis* populations as recently studied in detail by Pavaux *et al.*, (2021).

What forecast data were used?

The available data from some Mediterranean countries suggest that human health effects may occur at thresholds values of 2×10^5 cells g^{-1} wet weight of macroalgae and/or 3×10^4 cells/L of seawater (Lemée *et al.*, 2012, Funari *et al.*, 2015, Mangialajo *et al.*, 2017). Symptoms may not occur along the whole duration of the bloom but during certain periods (Vila *et al.*, 2016; Berdalet *et al.*, under revision). Environmental effects depend on the duration and extension of the bloom event. In the field, massive mortalities have been observed at 1.4×10^6 cells g^{-1} fresh weight of macroalgae in New

Zealand (for example, Shears and Ross, 2009) or 250 x 10^6 -3 x 10^9 cells/L of seawater in Italy (Sansoni *et al.*, 2003). Data on threshold cell abundances having toxic effects on different organisms have been mainly obtained from experimental ecotoxicity tests (for example, Giussani *et al.*, 2015, 2017). The responses are variable, depending on experimental conditions and the tested organism. Overall, this information helps the authorities to provide information to the public, and to decide about the necessity to close the access to beaches.

What early warning system was put in place?

Recently, in the frame of the CoCliME project (www.coclime.eu), an easy and reliable protocol was co-designed and implemented by scientists and stakeholders. Scientists elabourated and distributed a benthic sampling kit and provided simple training sessions on sampling. Results (*Ostreopsis* abundance) were shared between scientists and public agencies using coordinated online platforms. Microscopy cell counts were conducted at the research centres but can also be done by trained personnel and citizens if microscopes are available (for example, Surfrider Foundation https://surfrider.eu/en/). This protocol has been successfully tested in Catalonia and France during the summers 2019 and 2020 and is described by Vila *et al.* (2022).

What were the results forecast operation?

The recurrence of the events in some areas and the appearance of blooms of some relevant intensity in others are consolidating the need to maintain and consolidate the monitoring in more beaches in Catalonia in the summer vacation period.

What were the consequences of the early warning system for stakeholders?

The EWS based on benthic sampling conducted by trained personnel (described in Vila *et al.*, 2022) proved to be an effective EWS for authorities to quickly make recommendations on the health risks associated to *Ostreopsis* blooms in Mediterranean beaches. The monitoring data protocol also contributes to the joint effort to build a time-series dataset for elucidation of climate change effects on *Ostreopsis* blooms.

What lessons were learned?

Ostreopsis is becoming an emerging issue in new (not yet monitored) areas. The problems posed by *Ostreopsis* blooms have, for now, a smaller relevance compared to *Gambierdiscus* and *Fukuyoa* blooms and ciguatera poisoning in the tropical regions of the world. For *Ostreopsis*, uncertainties include identification of the toxic compounds and harmful mechanisms involved in human and environmental health problems, and the future trends of the blooms under a general warming scenario. Meanwhile, the benthic nature of *Ostreopsis* and their blooms in temperate latitudes and different habitats offer the possibility to approach the challenges of addressing other potential benthic HABs and providing a template for establishing EWSs.

3.5 SUMMARY

Despite the challenges of sampling complex habitats, Early Warning Systems (EWSs) for Benthic Harmful Algal Blooms (BHABs) are possible. As *Gambierdiscus* cell-based EWS monitoring matures, it must include artificial substrate sampling combined with techniques like high-throughput sequencing (HTS). HTS metabarcoding in tandem with PCR assays has already been demonstrated for BHABs, opening the way to “next generation” biomonitoring to provide a community view followed by species -specific information. Data on increased cell abundance of toxic *Gambierdiscus* species can inform targeted toxin analysis of marine food-webs and seafood products. Using analytical data to confirm local, traditional knowledge of “hot spots” and mapping ciguatera-prone fishing habitats helps EWSs focus the resources and efforts of seafood harvesters. environmental managers and health authorities to safeguard the health of seafood consumers.

A cell-based EWS also applies for *Ostreopsis* blooms reported to cause respiratory irritations in Mediterranean beaches. In this region, EWSs have been developed with the coordination among scientists, stakeholders, the general public and health and marine management authorities. The knowledge gained and successful experiences constitute a basis for preventing potential (not yet clarified) food-borne poisonings by *Ostreopsis* produced toxins. Coordination efforts are conducted from local to transnational levels, because HABs have global dimensions.

For all HAB events, communication, epidemiological reports, unified online databases with environmental data, should be a near term goal.

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4. Toxic pelagic harmful algal blooms impacting shellfish safety

4.1 PELAGIC HARMFUL ALGAL BLOOM ORGANISMS, TOXINS AND IMPACTS

Following the IOC–UNESCO definition of harmful algal blooms (HABs) and the categorization of the impacts of harmful microalgae in the world's oceans proposed by Lassus *et al.* (2016), this chapter will focus on pelagic species that affect shellfish safety by producing toxins and consequently causing various symptoms in humans by consuming poisoned food. Pelagic HABs that have a dormant benthic phase in their life cycle are also included (Table 4.1). The resulting human poisoning syndromes linked to consumption of shellfish are classified into five groups, to describe the primary symptoms or toxins involved:

- amnesic shellfish poisoning (ASP)
- azaspiracid shellfish poisoning (AZP)
- diarrhetic shellfish poisoning (DSP)
- neurotoxic shellfish poisoning (NSP)
- paralytic shellfish poisoning (PSP)

Within each syndrome, there are typically multiple genera and species that produce the associated toxin (Table 4.1). All are dinoflagellates, except ASP which is caused by representatives of two diatom genera.

Food safety is threatened because toxins accumulate in the food chain, mostly in filter -feeding bivalve molluscs (for example, mussels, oysters, cockles, clams, scallops) and crustaceans (for example, crabs, lobsters, shrimps) that are either farmed or grow in the wild. The negative impact on the seafood industry is worldwide (for example, Hallegraeff *et al.*, 2021a). The accumulation of toxins is generally harmless to these shellfish but can lead to serious risks to consumer health (for example, Backer *et al.*, 2003) either by ingestion or by exposure. For example, during *K. brevis* red tides, people may develop respiratory problems or eye irritation after exposure to aerosols from bloom water (https://coastalscience.noaa.gov/habforecasts/gulfofmexico).

There are other toxic pelagic HABs that may be widespread in certain areas (for example, in the Mediterranean Sea) (Zingone *et al.*, 2021), but whose toxins are not considered toxic to humans (EFSA, 2008; Tubaro *et al.*, 2010). This is the case of dinoflagellates, which produce yessotoxins and are therefore monitored as a precautionary measure. In the same category of unproven effects fall cyclic imines, a new class of emerging toxins that have no proven acute toxicity to humans and are therefore not regulated, although some neurotoxicity has been observed in bioassays (Molgó *et al.*, 2017). Similarly, there are no reports of adverse effects in humans associated with pectenotoxins, which are always accompanied by toxins of the okadaic group in several *Dinophysis* species and for this reason have recently been deregulated or recommended for deregulation in Europe (Commission Delegated Regulation (EU) 2021/1374 of 12 April 2021) and New Zealand (Boundy *et al.*, 2020).

Some of the planktonic and/or toxic species are not covered in this chapter, but are described elsewhere in this document, such as:

- Toxic benthic HABs and their impacts are presented in detail in Chapter 3 due to differences in habitat and thus sampling methodology. Included are the ciguatera poisoning (CP) associated with marine fish that become toxic from ciguatoxins produced by benthic dinoflagellates and the benthic diarrhetic shellfish toxin (DST) producers of the genus *Prorocentrum* that can be found in the water column due to their swimming behaviour or attached to aquaculture mussel lines (McKenzie *et al.*, 2021). For clarity, we list both planktonic and benthic species when considering all DSP -producing dinoflagellates (see Table 4.1).

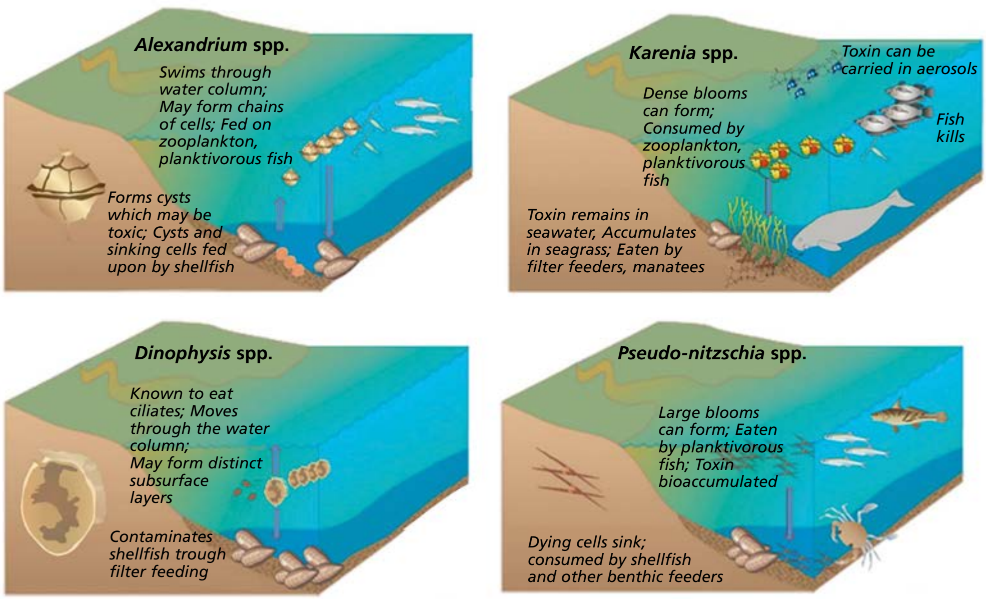
- Bloom -forming and toxic planktonic species that cause mass mortality of fish and invertebrates by mechanical action but without harm to humans and by excretion of ichthyotoxins and other bioactive compounds, respectively, are described in Chapter 5.
- Harmful bloom -forming cyanobacteria that threaten ecosystem functioning and degrade water quality are presented in Chapter 6.

The groups of toxins, responsible organisms, syndromes and other relevant information for the establishment of the early warning system concerning toxic pelagic HABS are presented in Table 4.1. Entries are based on the IOC–UNESCO Taxonomic Reference List of Harmful Microalgae (www.marinespecies.org/HAB) (Moestrup *et al.*, 2009), a comprehensive publication on toxic and harmful microalgae of the world (Lassus *et al.*, 2016) and recent articles. Information on whether species produce resting stages, that is, cysts, is also provided.

4.2 LIFE CYCLE

Here we present some key notes on the life cycle of three of the most intensely studied pelagic HAB genera, *Pseudo-nitzschia*, *Alexandrium* and *Dinophysis*, relevant for its EWS (Figure 4.1).

FIGURE 4.1
Life cycle dynamics of some common pelagic HAB species, including their resting stages. Means of transmission through the food chain include direct ingestion of cells by shellfish and planktivorous fish



Source: Rowles, T., Hall, A., Baker, C.S., Brownell, B., Cipriano, F., Glibert, P., Gulland, F., Kirkpatrick, B., Paerl, H., Schwacke, L., Simeone, C., Stimmelmayr, R., Suydam, R., Trainer, V.L. and Van Dolah, F. 2017. Report of the workshop on Harmful Algal Blooms (HABs) and associated toxins. International Whaling Commission Report SC/67A/REP/09. 22 pp.

4.2.1 *Pseudo-nitzschia*

- Day length can affect sexual reproduction (Hiltz *et al.*, 2000), growth rates, cell yield, toxin production and influence which species of *Pseudo-nitzschia* becomes dominant (Fehling *et al.*, 2005).
- Sexual reproduction in *Pseudo-nitzschia* may be important for domoic acid (DA) production as clonal cultures of *Pseudo-nitzschia* decrease in size over time and lose their ability to produce DA (Bates, 1998). Auxosporulation begins when the lowest size classes reach their maximum cell concentrations, observed for both *P. pungens* and *P. australis*. The highest rate of increase in auxosporulation and production of large new cells for both species occurs within a narrow window of time and coincides with the decline in nutrients after the bloom has peaked (Holtermann *et al.*, 2010). When parental cells are mated, their offspring can be toxic, sometimes even more so than their parents were initially (Bates *et al.*, 1999).
- DA production is usually minimal or non-detectable during exponential growth in batch cultures and increases during the stationary phase as cell division slows because of either silicon (Si) or phosphorus (P) limitation (Bates, 1998; Bates and Trainer, 2006; Lema *et al.*, 2017)

4.2.2 *Dinophysis*

- *Dinophysis* has a polymorphic life cycle (Figure 4.2). The formation of small cells, which can become normalized- cells if not involved in planozygote formation, might represent the key life-cycle transition for the persistence of planktonic populations of *Dinophysis* spp. (Delgado *et al.*, 1996; Reguera and González-Gil, 2001). Cell division in *Dinophysis* peaks in the transition from night to day or a bit later, depending on the species (Reguera *et al.*, 2012).
- *Dinophysis* species are mixotrophic. When prey is no longer available, *Dinophysis* can modulate their division rate and/or size. Cells keep dividing for a while and their growth rate will depend on light availability for gross carbon uptake by photosynthesis (Kim *et al.*, 2008; Riisgaard and Hansen, 2009). After a certain period under starvation, *Dinophysis* growth and division become extremely slow and may stop for months. Maximum mixotrophic growth, 0.91 d-1 is observed with increasing prey, almost five times higher than growth (0.19 d-1) in the absence of prey. *D. acuminata* fails to grow in darkness, despite the presence of prey, suggesting that this species is an obligate mixotroph that requires both light and prey for long-term survival (Kim *et al.*, 2008).

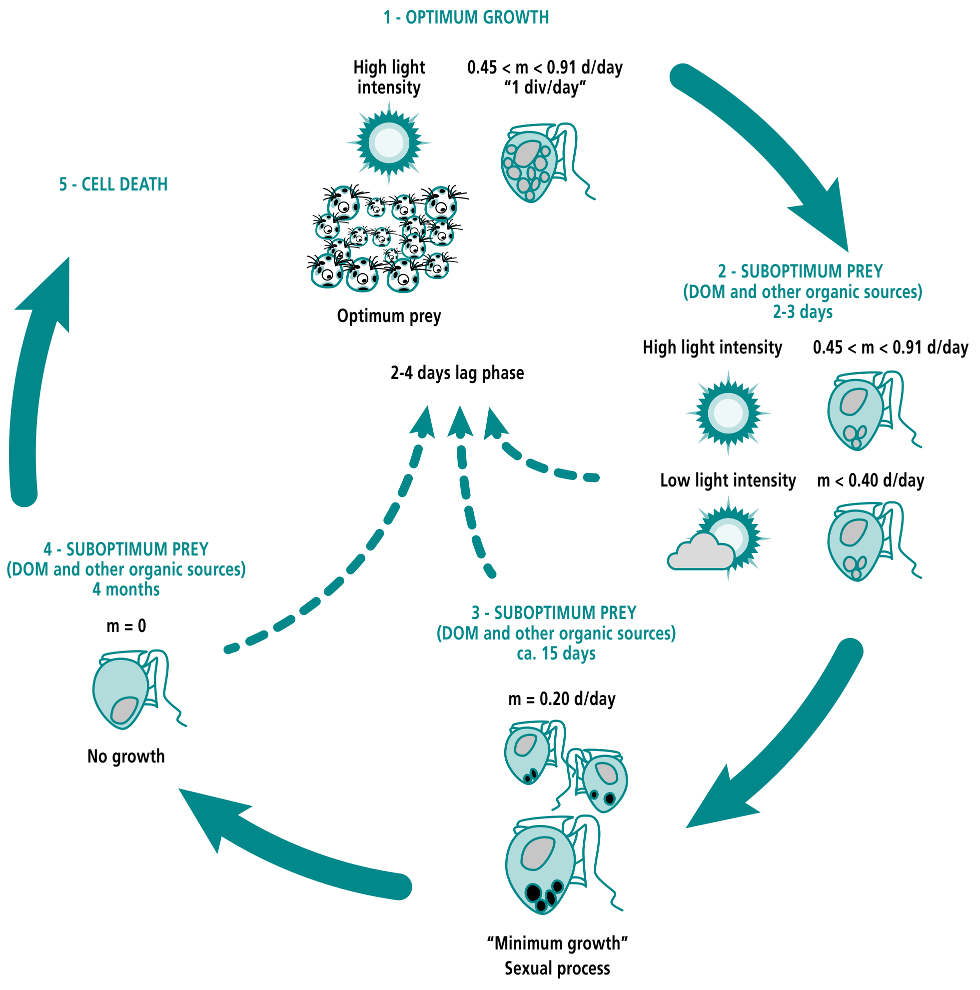
Figure 4.2. Simplified conceptual model describing the growth strategy of mixotrophic *Dinophysis* spp. in response to availability/absence of prey during its growing season. (1) Optimum growth when prey is available and light intensity is high. (2) *Mesodinium* is no longer available and *Dinophysis* division continues for several doublings; division rates will depend on light intensity. (3) If suboptimal conditions remain, *Dinophysis* growth decreases, gametes are formed, and cells accumulate starch deposits as storage products. (4) Long periods of prey limitation may prevent growth completely (mgross = 0) and even lead to cell death. Blue lines indicate time forwards in the absence of prey. Red lines indicate that each stage is reversible provided the population of *Dinophysis* matches a patch of *Mesodinium* (lag phase of 2 to 4 days). (Source: Reguera *et al.*, 2012)

4.2.3 *Alexandrium*

- Many *Alexandrium* species have complex life histories that include sexuality and often, cyst formation, which is characteristic of a meroplanktonic life strategy and offers considerable ecological advantages (Figure 4.3). Morphologically intermediate forms of *Alexandrium* have been observed under different environmental conditions (for example, Anderson *et al.*, 1994), and toxic and non-toxic ribotypes of the same morphologically defined species rarely co-occur (for example, Touzet *et al.*, 2009, Brosnahan *et al.*, (2010).

FIGURE 4.2

Simplified conceptual model describing the growth strategy of mixotrophic *Dinophysis* spp. in response to availability/absence of prey during its growing season. (1) Optimum growth when prey is available and light intensity is high. (2) *Mesodinium* is no longer available and *Dinophysis* division continues for several doublings; division rates will depend on light intensity. (3) If suboptimal conditions remain, *Dinophysis* growth decreases, gametes are formed, and cells accumulate starch deposits as storage products. (4) Long periods of prey limitation may prevent growth completely (mgross = 0) and even lead to cell death. Blue lines indicate time forwards in the absence of prey. Dashed arrows indicate that each stage is reversible provided the population of Dinophysis matches a patch of *Mesodinium* (lag phase of 2 to 4 days).

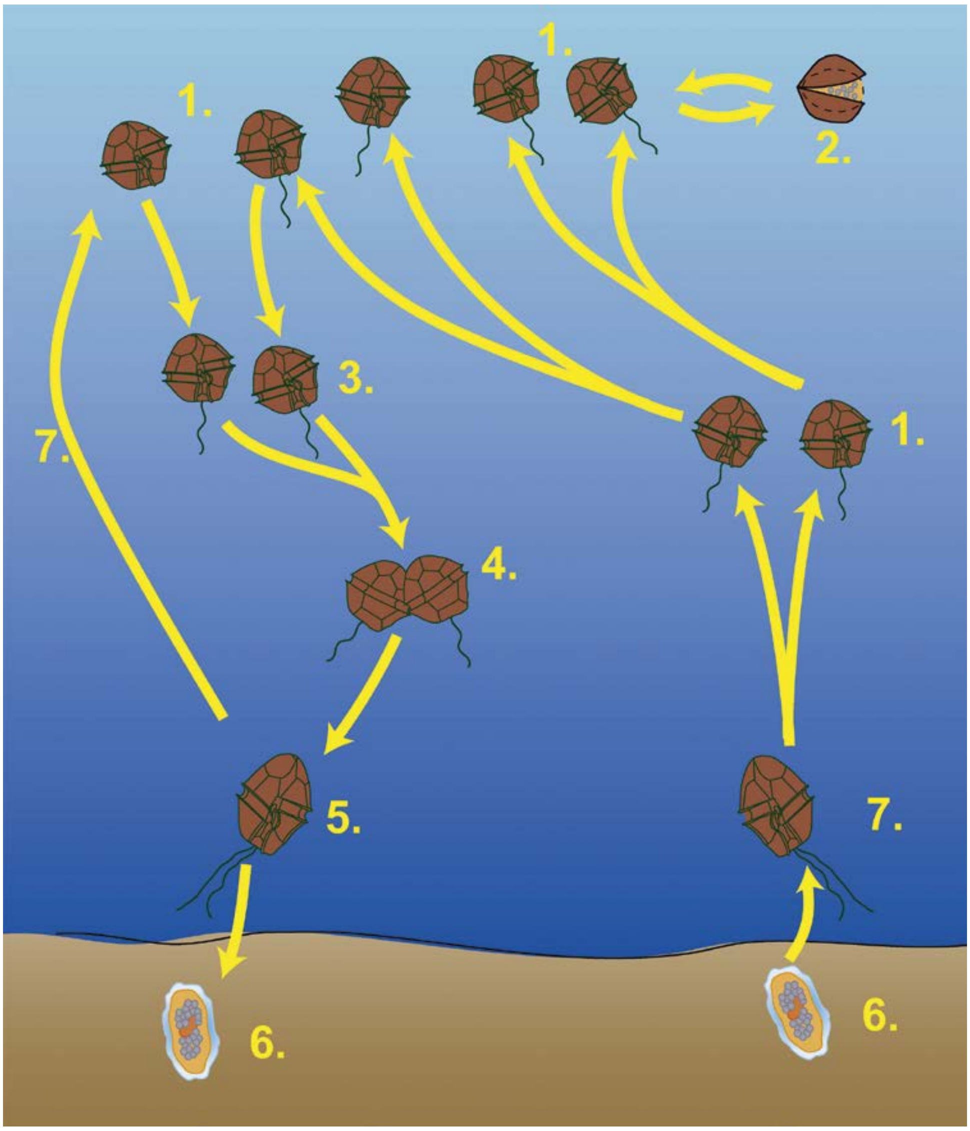


Source: Modified from Reguera, B., Velo-Suárez, L., Raine, R. and Park, M. 2012. Harmful *Dinophysis* species: a review. *Harmful Algae*, 14: 87-106.

- Chain formation is a definable species characteristic that also represents an example of life stage transition within the vegetative pelagic phase; the capability to form long chains is reported for several species such as *A. catenella*, *A. affine*, *A. fraterculus*, *A. cohorticula*, and *A. tamiyavanichii*. Chain formation in *A. catenella* may be stimulated by turbulence (Sullivan *et al.*, 2003), and chain length may decrease in culture, suggesting that this feature represents an adaptation to high turbulence upwelling systems.
- Another stage transition within the vegetative phase is represented by the formation of pellicle cysts, which are nonmotile cells surrounded by a thin wall (Anderson and Wall, 1978; pellicle cyst terminology is reviewed in Bravo *et al.* (2010).

FIGURE 4.3

Schematic representation of the life cycle of heterothallic *Alexandrium* species. Species have a haplontic life cycle, that is, the motile vegetative cells (1) are haploid. Under specific conditions, usually related to stress, some vegetative cells can transform into a non-motile pellicle cyst (2) that can rapidly switch back to the motile stage when conditions improve. The sexual phase starts with the formation of gametes (3), which conjugate (4) and form a diploid planozygote (5). Depending on environmental conditions, the planozygote can transform into a resting cyst (hypnozygote) (6) or, for some species, can undergo meiosis and produce a vegetative cell (1). Cysts can spend variable periods of time in the sediments and, upon germination, release a motile cell termed a planomeiocyte (7) which divides to produce vegetative cells (1).



Source: Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E. and Montresor, M. 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae*, 14: 10-35. Doi: 10.1016/j.hal.2011.10.012.

- Pellicle cysts can be formed as a reaction to environmental stress conditions such as turbulence, the presence of parasites, or passage through the gut of grazers. Pellicle cysts have no mandatory maturation period and can revert to the vegetative planktonic motile stage once stress conditions are over (Anderson *et al.*, 2012).
- Resting cysts have been described for many *Alexandrium* species. Cysts of

A. catenella have been found within a surface water temperature range of -0.6 °C to 26.8 °C with the highest relative abundances in regions between 5 °C and 15 °C. Members of the *A. tamarensis* species complex can be regarded as characteristic of temperate–subtropical regions in brackish to fully marine and oligotrophic to eutrophic environments (Marret and Zonneveld, 2003).

TABLE 4.1
Causative toxic pelagic species affecting shellfish safety and effects on human poisoning

Causative organism	Toxin	Clinical symptoms	Syndrome	Impact	Resting stages that promote survival
Pseudo-nitzschia australis, P. abrensis, P. batesiana, P. bipertita, P. brasiliana, P. caciantha, P. calliantha, P. cuspidata, P. delicatissima, P. fraudulenta, P. fukuyoi, P. galaxiae, P. granii, P. hasleana, P.kodamae, P. lundholmiae, P. multiseries, P. multistriata, P. obtusa, P. plurisecta, P. pseudodelicatissima, P. pungens, P. seriata, P. simulans, P. subcurvata, P. subfraudulenta, P. subpacifica, P. turgidula Nitzschia bizertenzis[a], *N. navis-varingica*[a]	Domoic acid (DA)	Neurological	ASP	Food safety, human, marine animal health	Some *Pseudo-nitzschia* species produce auxospores
Amphidoma languida, Azadinium poporum, A. spinosum, A. dexteroporum	Azaspiracids (AZA)	Gastrointestinal	AZP	Food safety	Unconfirmed cyst formation for *A. poporum*
Dinophysis acuminata[b], *D. acuta*[b], *D. caudata*[b], *D. fortiib, D. infundibulum*[b], *D. miles, D. norvegica*[b], *D. ovum*[b], *D. sacculus*[b], *D. tripos*[b], *Phalacroma rotundatum, P. rapa, P. mitra*	Okadaic acid (OA), *Dinophysis* toxins (DTX), pectenotoxins (PTX)	Gastrointestinal	DSP	Food safety	No cyst stage has been identified
Prorocentrum caipirignum, P. faustiae, P. hoffmannianum, P. leve , P. concavum, P. lima, P. rhathymum, P. texanum	Okadaic acid, DTX1, DTX2, FAT and prorocentrolide producer	Gastrointestinal	DSP[c]	Food safety	Benthic species (see chapter BHAB) with diel vertical migrations (pelagic behaviour) Resting stages in *P. lima*
Karenia brevis, K. papilionacea	Brevetoxins (BTX)[d]	Neurological, respiratory irritations	NSP	Food safety, food security (fish kills), human health (aerosols), marine animal health	No cyst stage has been identified

TABLE 4.1

Causative toxic pelagic species affecting shellfish safety and effects on human poisoning (cont.)

Causative organism	Toxin	Clinical symptoms	Syndrome	Impact	Resting stages that promote survival
Alexandrium affine, A. ostenfeldii, A. acatenella, A. catenella, A. cohorticula, A. pacificum, A. tamiyavanichii, A. andersonii, A. tamarense (group III)[e], *A. leei, A. minutum, Centrodinium punctatum, Gymnodinium catenatum, Pyrodinium bahamense*	Saxitoxins (STX)	Neurological and gastrointestinal	PSP	Food safety, food security, marine animal health	Resting cysts (except for *A. acatenella*) Pellicle cysts
Lingulodinium polyedra, Protoceratium reticulatum[f], *Gonyaulax spinifera, G. taylori*	Yessotoxins (YTX), adriatoxin	n.e.p.	n.e.p.		Resting cysts *Pellicle cysts*
Prorocentrum borbonicum[1], *P. emarginatum*[2], *P. mexicanum*[3], *P. rhathymum, P. cassubicum*[4]	[1] neurotoxic [2] low hemolytic and fibroblast activity [3] producing hemolytic toxins of unknown nature [4] Tindall *et al.*, (1989) reported two unnamed toxins in a culture	Species not listed as toxic to humans but found to produce toxins when using bioassays, and therefore likely to represent a potential danger	n.e.p		Benthic species (see chapter BHAB) with diel vertical migrations (pelagic behaviour)
Alexandrium ostenfeldii, Karenia selliformis, Vulcanodinium rugosum	Cyclic imines (CIs): gymnodimines, pinnatoxins, portimine, spirolides	To date, no effect of CIs on humans has been demonstrated, although some may cause neurotoxicity in mice.	n.e.p.	Food safety[g]	Resting cysts (*A. ostenfeldii*)

[a] The original report (Bates, 2000) of the toxicity of this species has not yet been confirmed (Bates *et al.*, 2018)
[b] Species producing pectenotoxins
[c] Benthic species
[d] Potential producers of BTX or BTX-like compounds (for example, brevisculatic acid) are some other *Karenia* species and raphidophytes (in Brand *et al.*, 2012).
[e] Group III of the complex *A. tamarense* (John *et al.*, 2014)
[f] Species producing adriatoxin
[g] CIs are not regulated, but nevertheless their potential impact on food safety should be monitored and their ecophysiological properties investigated (Molgó et al, 2017).
n.e.p. = no effect proven in humans (EFSA, 2008; Lassus *et al.*, 2016)
Source: Elaborated by the authors.

4.3 PELAGIC HARMFUL ALGAL BLOOM DYNAMICS AND FORCING FACTORS

Toxic pelagic HABs are observed in distinct types of marine ecosystems including upwelling systems (Pitcher *et al.*, 2017), monsoon systems (Yñiguez *et al.*, 2018), fjords, coastal embayments and stratified systems (Berdalet *et al.*, 2017) and eutrophic systems (Glibert and Burford, 2017). Within them, the occurrence of pelagic HABs is controlled by a wide range of combined nutrient availability and physical dynamics that allow individual species to proliferate (GlobalHAB, 2017). The abiotic conditions differ significantly between open ocean systems and nearshore/coastal, resulting in differences in both the types of phytoplankton species and the timing of outbreaks. Thus, blooms are diverse and, in some cases, not predictable. Seasonality is not a constant feature; while most blooms occur in the spring and summer they may also appear in fall and winter. The suite of physiological, morphological and life cycle features (for example, bottom-dwelling resting stages) of each species determines its distribution in space and time. How environmental factors impact toxin production is the subject of ongoing research, but nutrient (nitrogen [N], phosphorus [P]) supply rates, light, temperature, oxidative stressors, interactions with other biota (bacteria, viruses, and animal grazers), and the combined effects of these factors are all involved (Glibert and Burford, 2017). Some algal species produce sufficient toxin to pose a threat at low abundances of some hundreds of cells per litre, while other species must occur at concentrations of millions of cells per litre to cause any harm.

Coastal HABs are diverse, highly localized, and can be either recurrent and emerge in a seemingly arbitrary manner without a defined pattern (for example, blooms of *K. brevis* in the Gulf of Mexico) (https://coastalscience.noaa.gov/hab-forecasts/gulf-of-mexico). They may be of short duration (2 or 3 weeks) or prolonged (several months) depending on multiple factors which, individually or collectively, influence bloom dynamics (Garcés and Camp, 2012). Coastal blooms often have a seasonal phenology responding to physical and environmental forcing. Both alongshore and cross -shelf transport, at large and local scales, have been implicated in the generation of toxic events along the coastline. For instance, the increase in cell density at an aquaculture site may be more rapid than would be assumed from *in situ* growth alone if advective physical factors such as convergence, onshore winds, and vertical migration (Davidson *et al.*, 2016) play a role concentrating cells from offshore at the site. Local hydrographic regimes, especially in coastal upwelling areas (such as the Atlantic Ocean and Benguela Current) (Fawcett *et al.*, 2007) and upwelling systems must be considered for the promotion of HABs into adjacent semi-confined systems as embayments, estuaries and coastal lagoons. Typically, these are sites of cultured or wild harvest shellfish and other aquaculture activities that can experience prolonged shellfish harvesting- bans. Bays subject to upwelling are very productive due to ingress of cool nutrient rich water. Often a strong cross -shelf gradient in plankton populations can exist with a narrow coastal band of dinoflagellate -dominated water lying inshore of the typically diatom -rich upwelled water (GEOHAB, 2005). In other embayments, such as Sorsogon Bay in the northeastern region of the Philippines, HABs are highly influenced by runoff and stratification (Yñiguez *et al.*, 2018).

Dormant cells are well recognized- stages of the life cycles of HAB species, particularly for pelagic species whose life cycle is meroplanktonic as well (that is, both planktonic and benthic resting stages, or cysts, in a life cycle for example, *Alexandrium* spp.) (Table 4.1). The duration of the dormant/resting phase is often much greater than that of the multiplicative vegetative one. The dormant cells are non-motile hence, during the resting period, dispersion and concentration are determined by the same forcing functions that control the dynamics of passive particles in the water column. These life cycle stages provide a recurrent seed source of inoculum for planktonic populations and this characteristic may be a critical factor in determining not only the geographic distribution of species, but also how it may proliferate when future conditions become favourable. Due to these features, the dormant stages promote survival, contributing to the persistence and dispersion of harmful species. These seed banks or cyst beds have a high biodiversity, hosting more and/or distinct species from those in the overlying water at any given time. Knowledge of the geographic distribution and density of cyst beds or initiation sites (Trainer *et al.*, 2020; Hickey *et al.*, 2013; Anderson *et al.*, 2014) of Harmful Algae can help to identify risk areas under different ecological and hydrographical scenarios, providing useful information for the future management of coastal areas.

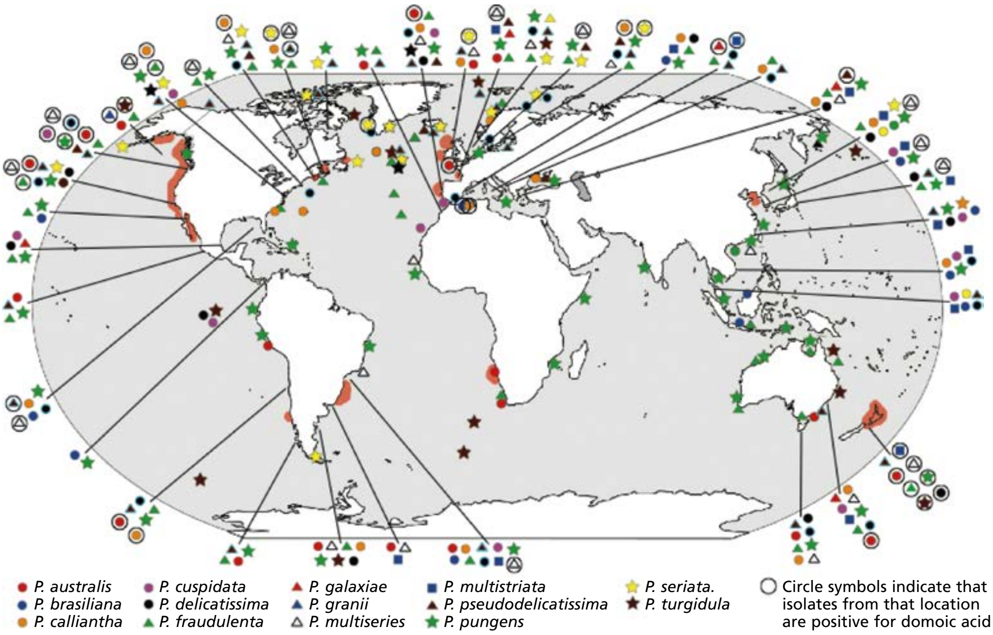
Here we present relevant information for the EWS, regarding the spatial distribution, seasonality and regulating factors of three of the most intensely studied pelagic HAB genera, *Pseudo-nitzschia*, *Alexandrium* and *Dinophysis*.

4.3.1 *Pseudo-nitzschia* dynamics and forcing factors

Pseudo-nitzschia is a globally distributed diatom genus (Lelong *et al.*, 2012) (Figure 4.4). The species associated with DA production (Table 4.1) are frequently present, albeit in low numbers, in most water samples (for example, Walz *et al.*, 1994), on average contributing less than 17 percent of the total carbon biomass (for example, Trainer *et al.*, 2009a).

FIGURE 4.4

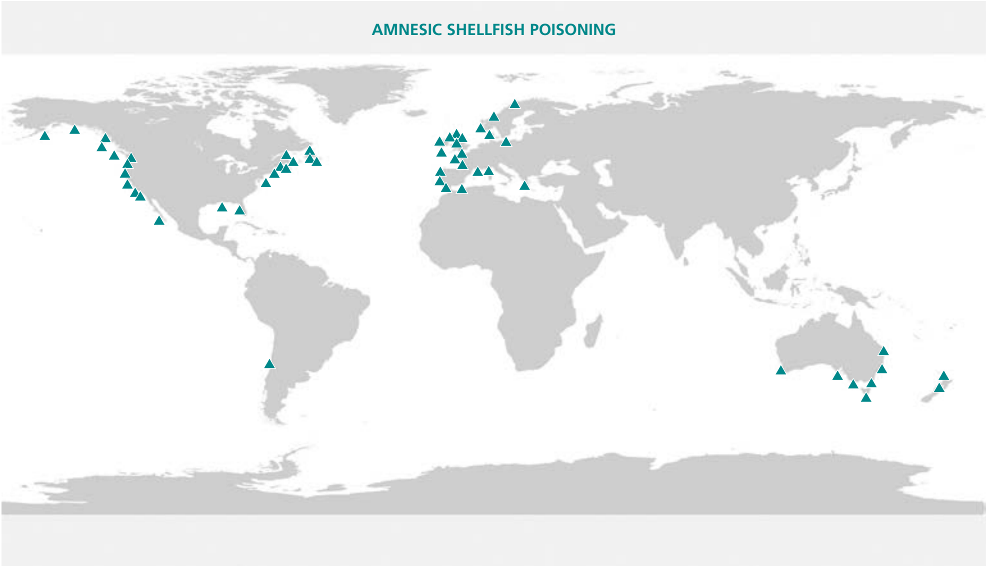
Toxigenic species of *Pseudo-nitzschia*. Symbols of those species that have been demonstrated to produce domoic acid (DA) in culture are circled and shown at the locations from which they were isolated. Areas along coastlines marked with red are locations where closures of shellfish harvesting due to elevated levels of DA (>20 mg DA g-1 wet weight of shellfish tissue) or animal mortalities have occurred. Symbols outlined in blue indicate a species description that was made before major taxonomic revisions were implemented for *P. delicatissima* and *P. pseudodelicatissima.*



Source: Trainer, V.L., S.S. Bates, N. Lundholm, A.E. Thessen, W.P. Cochlan, N.G. Adams and C.G. Trick. 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae*, 14: 271-300.

The genus can be found in coastal or mid-oceanic waters (Trainer *et al.*, 2008, Trainer *et al.*, 2009a, Trainer *et al.*, 2009b), as well as in cold (polar) (Orlova and Shevchenko, 2002; Almandoz *et al.*, 2008; McKenzie *et al.*, 2021), temperate (Amato *et al.*, 2005) or tropical/subtropical waters (Hernández-Becerril, 1998; Hasle, 2002). With a few exceptions, most blooms and the impacts of DA are described primarily in eastern boundary upwelling systems (Trainer *et al.*, 2008, 2010) as on the west coast of the United States of America. Major blooms can also occur in other systems though, such as those in the Gulf of Maine (Clark *et al.*, 2019). *Pseudo-nitzschia* abundances and DA concentrations are often associated with low temperature, high salinity, and high nutrient conditions typical of upwelling (Villac, 1996; Trainer *et al.*, 2000, 2002) (Figure 4.5). Similarly, upwelling regions off the coast of Portugal record high numbers of *Pseudo-nitzschia* cells, which are used as upwelling indicators during spring and summer (Loureiro *et al.*, 2005; Palma *et al.*, 2010). On the other hand, several blooms have also been reported in many regions where riverine inputs have stimulated toxic events and are characterized by lower salinities and higher temperatures than upwelling zones (Horner and Postel, 1993; Dortch *et al.*, 1997; Trainer *et al.*, 1998; Scholin *et al.*, 2000; Spatharis *et al.*, 2007; Kudela *et al.*, 2008). *Pseudo-nitzschia* and low levels of DA are found in other parts of the world, for example, the Mediterranean region, the Russian Federation, Japan, and Viet Nam, although damaging blooms have not been noted.

FIGURE 4.5
World distribution of reported presence of domoic acid in shellfish associated with the occurrence of *Pseudo-nitzschia* spp.; last updated in 2016



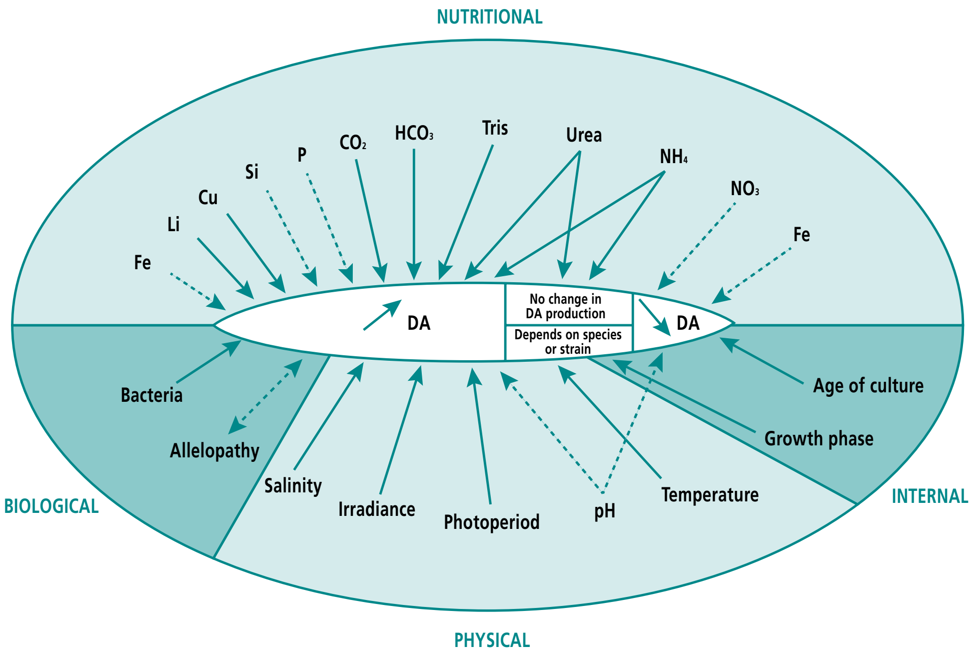
Source: Change to: U.S. National Office for Harmful Algal Blooms. 2023. Global Distribution of HABs.Distribution – World – Harmful Algal Blooms (whoi.edu).

Environmental cues causing toxic blooms of *Pseudo-nitzschia* are complex, and can be unique to the bays, coastal, or open ocean regions where they occur (Trainer *et al.*, 2012). Species may be located throughout the water column or concentrated in thin layers that may be missed by conventional monitoring methods (Rines *et al.*, 2002). In restricted bays, intense, high-density, visible blooms can form, whereas in open offshore waters, *Pseudo-nitzschia* species more often form less dense blooms that are not visible to the naked eye. Many species may coexist, but different growth and loss rates can lead to complex bloom dynamics and seasonal succession.

The species have a unique capability of surviving extreme ocean conditions and each species can be linked to different physicochemical factors (Figure 4.6):

- nutritional (for example, increased nutrients from several sources, including upwelling or mixing events and riverine inputs, Trainer *et al.*, 2012); variability in the ratio of nitrogen to silicate (Clark *et al.*, 2019; Ryan *et al.*, 2017); nutrient or trace metal stress (Wells *et al.*, 2005), changes in the ambient concentrations of macronutrients (Trainer *et al.*, 2009b);
- biological (for example, presence of bacteria), physical (for example, low air pressure and rainfall preceding the blooms; increase of daylight or increase in temperature); and
- internal (for example, growth phase).

FIGURE 4.6
Synthesis of all the factors (nutritional, internal, physical, or biological) studied and their impacts on domoic acid (DA) production (increase or decrease). Black arrow indicates effect of increasing the parameter; black dotted arrow indicates effect of decreasing the parameter; grey dashed arrow indicates conflicting results found; grey arrow indicates result depends on species or strain, or no change observed in DA production



Source: Lelong, A., Hégaret, H., Soudant, P., Bates, S.S. 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms *Phycologia*, 51: 168-216.

Much of the seasonal variability in *Pseudo-nitzschia* abundance can be explained by the seasonal and interannual frequency of physical and hydrographic processes, namely regular shifts in wind, irradiance, temperature, and river flow. Local meteorological phenomena, such as winds and heavy rainfall, can stimulate *Pseudo-nitzschia* blooms. Wind events can be especially important for transporting toxic blooms inland from upwelling sites offshore (Trainer *et al.*, 2000, 2002) or providing mixing necessary for currents to bring nutrients into the photic zone (Lund-Hansen and Vang, 2004; Lelong et al 2012). Heavy rainfall after a drought can cause a dramatic increase in toxic *Pseudo-nitzschia* abundances in river outflow, such as in eastern Canada in 1987 (Bates *et al.*, 1998).

Many *Pseudo-nitzschia* blooms occur in the spring and fall, when irradiance is relatively low (Parsons *et al.*, 1998; Mercado *et al.*, 2005). However, low light may contribute to the demise of autumn blooms (Bates *et al.*, 1998). Blooms mainly occur as follows: between January and May in European waters, rarely during summer (Hasle *et al.*, 1996); in the fall in eastern North America (Bates *et al.*, 1998); in early summer (Trainer *et al.*, 2002) or early fall in Washington State of the United States of America (Trainer *et al.*, 2010); or late spring in southern California, United States of America (Anderson *et al.*, 2006, 2009) and the Pacific Mexican coast (García-Mendoza *et al.*, 2009).

In culture, *Pseudo-nitzschia* spp. can grow at salinities as low as 6 and as high as 48, and at temperatures as low as 5 °C and as high as 30 °C, with a broad range for optimum growth (Miller and Kamykowski, 1986; Jackson *et al.*, 1992; Lundholm *et al.*, 1997; Cho *et al.*, 2001; Thessen *et al.*, 2005). However, distinct species in natural populations can demonstrate distinct correlations with environmental characteristics, which suggests seasonal succession of species or regional specificity (Fryxell *et al.*, 1997; Clark *et al.*, 2019).

Both upwelling and riverine nutrient sources stimulate *Pseudo-nitzschia* blooms; concentrations of 8–22 mM NO_3^-, 2.4–35 mM Si and 0.2–2 mM PO_4^{3-}, but in different temperature and salinity regimes were recorded by several authors (Dortch *et al.*, 1997; Scholin *et al.*, 2000; Trainer *et al.*, 2000; Loureiro *et al.*, 2005). A distinction between nutrients in upwelling and river plumes is that riverine inputs are likely the result of anthropogenic nutrient loading, including agricultural runoff and sewage. There is increasing evidence that the N substrate fuelling growth may influence both the exponential growth rate and the DA production rate achieved by various species of *Pseudo-nitzschia*, prior to either Si or P induction of the stationary phase (Trainer *et al.*, 2012). The toxic *P. australis* has a high affinity for nitrate and ammonium (Cochlan *et al.*, 2008), providing it with a competitive advantage for acquiring N under N -depleted conditions, but especially following a N surge during upwelling when its maximal rate of nitrate uptake exceeds those of virtually all the other phytoplankton species commonly found in upwelling systems (Kudela *et al.*, 2010).

New triggers or enhancers of DA production have been found (Lelong *et al.*, 2012), in addition to the already known triggers caused by silicon or phosphorus limitation:

- Iron deficiency or copper excess are believed to enhance DA production and release from the cells, because of the ability of DA to chelate these trace metals;
- High salinities (30 ° to 40 ° psu) enhanced DA production by *P. multiseries*, the only species studied so far;
- The role of inorganic carbon, whose concentrations are controlled by CO_2 addition or removal, remains unclear because of conflicting evidence. DA production was reported to be enhanced by both high and low pH, and was also shown to be limited, or not, by total inorganic carbon concentration; and
- Organic sources of nitrogen, that is, glutamine and urea, were shown to enhance DA production, relative to inorganic nitrogen sources, by several species of *Pseudo-nitzschia*, although there are many inter- and intraspecies variations.

Larger scale changes in weather, such as the El Niño Southern Oscillation (ENSO), can affect *Pseudo-nitzschia* abundances by controlling upwelling near the west coast. During weak ENSO years, upwelling is high and, therefore, so are *Pseudo-nitzschia* abundances (Fryxell *et al.*, 1997). During strong ENSO years, *Pseudo-nitzschia* can still bloom by taking advantage of other favorable events, such as increased runoff after rainfall. Both 1991 and 1998, years with large toxic events on the west coast of the United States of America, were strong ENSO years (Trainer *et al.*, 2012).

4.3.2 *Dinophysis* dynamics and forcing factors

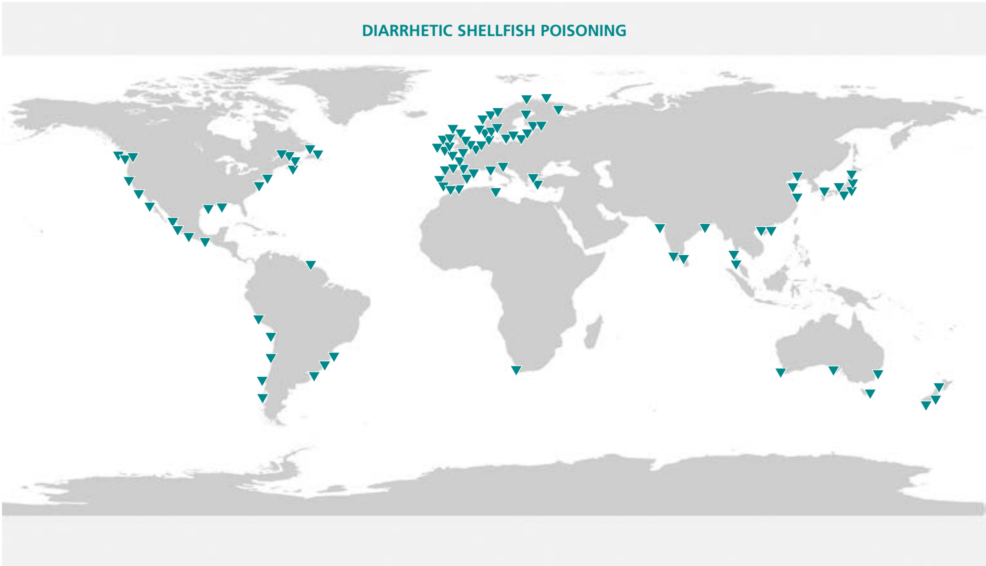
Dinophysis species are distributed from tropical to temperate and boreal waters (Figure 4.7). Latitude, water column and food web dynamics influence the biogeographic distribution and seasonality of each species/strain.

Some *Dinophysis* spp., such as *D. miles* (Marasigan *et al.*, 2001; Taylor *et al.*, 2008; Gull and Saifullah, 2010) and *D. norvegica* (Carpenter *et al.*, 1995; Subba Rao *et al.*, 1993), have restricted geographical ranges and are only detected in tropical and boreal waters, respectively. Others, such as *D. caudata* and *D. tripos*, bloom in tropical and subtropical waters but can reach much higher latitudes, such as northern Norway, on rare occasions (Johnsen and Lømsland, 2010). The "*D. acuminata* complex", including morphospecies described as *D. acuminata*, *D. sacculus* and *D. ovum*, is the most widespread group of *Dinophysis* spp., with strains all over the world that thrive in coastal waters with freshwater inputs and exhibit long growing seasons (spring to autumn) under a broad range of environmental conditions.

The most common assemblages of *Dinophysis* spp. associated with DSP events, by latitude, are:

- boreal seas: *D. acuminata*, *D. acuta*, *D. norvegica*;
- temperate seas: *D. acuminata*/*D. sacculus*/*D. ovum*, *D. acuta*, *D. fortii*, *D. caudata*; and
- tropical seas: *D. caudata*, *D. miles*.

FIGURE 4.7
World distribution of reported presence of okadates and/or pectenotoxins in shellfish associated with the occurrence of *Dinophysis* spp; last updated in 2016



Source: U.S. National Office for Harmful Algal Blooms. 2023. Global Distribution of HABs. Distribution – World – Harmful Algal Blooms (whoi.edu)

A common feature observed in most seasonal samplings of *Dinophysis* populations is that numbers start to increase when there is water column stability. *In situ* growth has been associated with stable stratified conditions (Reguera *et al.*, 2003). Once a population of *Dinophysis* is established, it exhibits peaks and troughs closely related to local hydrodynamic features (upwelling–downwelling cycles, tides) favouring dispersion or aggregation and/or retention. Whether the onset of thermal stratification in the spring triggers aggregation of dispersed overwintering cells around density gradients or these cells remain in offshore small scale retentive structures (Xie *et al.*, 2007) or associated with near sediment layers remains a crucial question.

Local hydrographic regimes, especially in coastal upwelling areas (such as the Atlantic Ocean and Benguela Current [Fawcett *et al.*, 2007], upwelling systems must be considered for the promotion of *Dinophysis* blooms into adjacent bays. During summer, bays subject to upwelling are very productive due to ingress of cool nutrient-rich water. Often a strong cross-shelf gradient in plankton populations can exist with a narrow coastal band of dinoflagellate-dominated water lying inshore of the typically diatom-rich upwelled water (GEOHAB, 2005). Relaxation of upwelling favourable winds and the presence of thermohaline stratification can promote the development of *Dinophysis* populations and further their accumulation shoreward (GEOHAB, 2005; Reguera *et al.*, 1995; Velo-Suárez *et al.*, 2008). Wind reversals result in advection of shelf waters importing larger forms of dinoflagellates, including *Dinophysis* (Sordo *et al.*, 2001; Pizarro *et al.*, 2008). On the continental shelf, the most harmful events arise from the transport of *Dinophysis* cells into bays used for shellfish production. Both alongshore and cross-shelf transport have been implicated in the generation of toxic events. This is true for events which have occurred along the

Atlantic seaboard of Europe such as western France (Delmas *et al.*, 1992, Batifoulier *et al.*,2013), southwest Ireland (Raine *et al.*, 2010a, b), northwest Spain (Escalera *et al.*, 2010; Reguera *et al.*, 1995; Sordo *et al.*, 2001), and is also the case for blooms of *D. ovum* in Texas, United States of America (Campbell *et al.*, 2010). Tide induced- currents have been also shown to have a significant effect in the distribution of *Dinophysis* populations in bays and estuaries. Fast flowing, near surface narrow flows are also found above sharp horizontal gradients in temperature and/or salinity at the seabed. These flows exist in summer along all the coasts of northwest Europe adjacent to thermally stratified regions of the shelf.

Species typically occur at low cell densities (10–40 cells/L) that can escape detection by standard quantitative methods, however some are known to reach densities of 10^2–10^5 cells L--1 in coastal waters, under favourable conditions (high light and unlimited prey). The "initiation" of a *Dinophysis* bloom is understood as the time when cells start being detected by quantitative methods (>10–10^2 cells L--1). Observations made from the Iberian shelf (Moita *et al.*, 2006, 2016), northwestern Europe (Marcaillou *et al.*, 2001) and South America (Proença *et al.*, 2007) indicate that the upper limit of the cell density of *Dinophysis* communities is of the order 50 000–150 000 cells/L and thus a population of this density can be considered a developed population. An increasing number of observations show that over the near continental shelf, this organism can occur in high density thin layers of the order 10^4–10^5 cells/L (Moita *et al.*, 2006; Velo-Suárez *et al.*, 2008; Farrell *et al.*, 2010), at any depth in the water column. If shallow enough (typically <30 m), these thin layers can be dispersed through the surface mixed zone of the water column following mixing events such as summer storms.

Conditions favouring development of different strains of each species are site specific, but some trends and common features may be found (Reguera *et al.*, 2012):

- Large -sized forms with highly developed cingular and sulcal lists and hypothecal processes, such as *D. caudata*, *D. tripos* and *D. miles* bloom in less dense- tropical waters and the first two rarely reach high densities (>10^3 cells/L) in higher latitudes;
- Within the *D. acuminata* complex, noxious *D. ovum* blooms occur at the warmer extreme of its latitudinal range (Iberian and Greek waters in Europe, Gulf of Mexico in the United States of America);
- In temperate seas, the *D. acuminata* complex appears first in spring followed by *D. norvegica* (only in cold-temperate seas) as in Norway, *D. acuminata* and *D. norvegica* have mainly been abundant from March to December, whereas *D. acuta* has typically occurred in late summer and autumn (August-December). In Scandinavian waters *Dinophysis* spp. seasonality has been changing over the years, with increasing overlap in time of the different species with highest densities during summer and marked reductions during late autumn, going from two peak periods to only one and a consequent dramatic reduction in the accumulation of DST toxins in mussels (Naustvoll and Dahl, 2012);
- *D. sacculus*, reported mainly in the Mediterranean Sea, blooms in estuarine waters with important freshwater inputs such as the Northern Galician Rías (Reguera *et al.*, 1993) and in brackish lagoons in the Tyrrhenian Sea (Giacobbe *et al.*, 2000);
- *D. acuta* (sometimes accompanied by *D. caudata* and/or *D. tripos*) is a late summer to autumn species that thrives in thermally stratified temperate waters (Dahl and Johannessen, 2001; Escalera *et al.*, 2006; Hällfors *et al.*, 2011; Palma *et al.*, 1998). Within Atlantic Europe, this species is practically absent in the colder, more turbulent waters of the Southern Bight and Central North Sea, and in the Cantabrian Sea-Bay of Biscay, but may form dense blooms in northwest (within a narrow range of salinity and temperature) (Reguera *et al.*, 1995; Moita *et al.*, 2006, 2016) and southwest (Jaén *et al.*, 2009) Iberian waters, southwest Ireland (Raine *et al.*, 2010a, 2010b), in Swedish and Norwegian fjords (Lindahl *et al.*,

2007; Karlson *et al.*, 2021), New Zealand (MacKenzie, 2019) and Chile (Lembeye *et al.*, 1993; Díaz *et al.*, 2011).

In places where the pair *D. acuminata*/*D. acuta* is the main agent of DSP outbreaks, *D. acuta* usually appears in late summer months when thermoclines are deeper (Reguera *et al.*, 1993; Palma *et al.*, 1998). Therefore, although the initiation of the growth season for both species is associated with stratification, they seem to require different patterns in water column structure.

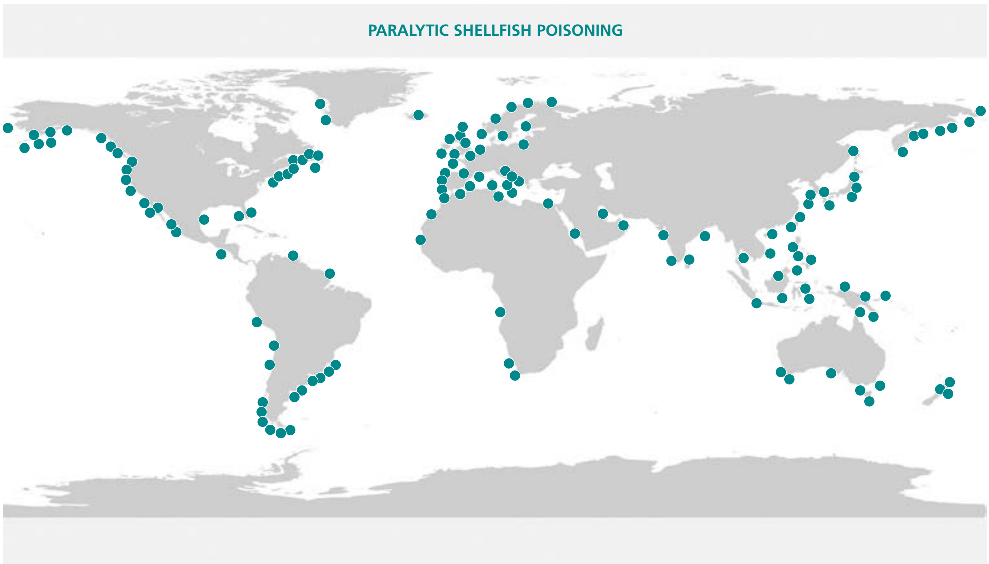
There are occasional reports of discoloured water due to *Dinophysis* blooms where cell densities have been over an order of magnitude greater, from Chile (Guzmán and Campodonico, 1975), Scotland (MacDonald, 1994), Canada (Subba Rao *et al.*, 1993), Norway (Dahl *et al.*, 1996), India (Santhanam and Srinivasan, 1996) and South Africa (Pitcher *et al.*, 2011). In most of these cases, the accumulations of cells are driven by a combination of physical accumulation processes (that is, wind and convergences) and vertical cell migration instead of periods of rapid cell growth.

4.3.3 *Alexandrium* dynamics and forcing factors

This genus is globally distributed (Figure 4.8) and can be found in a vast range of temperatures, salinity, and nutrient conditions in coastal, shelf and slope waters of subarctic, temperate, and tropical regions of the Northern and Southern Hemispheres (Taylor *et al.*, 1995; Lilly *et al.*, 2007, Band-Schmidt *et al.*, 2019). The ability of *Alexandrium* to colonize multiple habitats and to persist over large regions through time is testimony to the adaptability and resilience of this group of species. Blooms of several *Alexandrium* species have been linked to particular water masses. Both in small-scale blooms in embayments and in widespread coastal blooms, physical/biological coupling is a critical feature of population accumulation, growth, and dispersal.

FIGURE 4.8

World distribution of reported presence of saxitoxins in shellfish associated with the occurrence of *Alexandrium* spp., *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum*; last updated in 2016



Source: U.S. National Office for Harmful Algal Blooms. 2023. Global Distribution of HABs. Distribution – World – Harmful Algal Blooms (whoi.edu)

The *A. tamarense* species complex appears to be the most widely dispersed within the genus, and occurs in many locations worldwide, covering all ocean basins and many regional seas (Lilly *et al.*, 2007; Brosnahan *et al.*, 2020). Members of this species complex are absent from the equatorial tropics (Anderson *et al.*, 2012, *Alexandrium tamarense* [cabi.org] www.cabi.org/isc/datasheet/112110#todistributionDatabaseTable). The distribution of toxic and non-toxic strains of the same species, or of closely related species, generally, do not overlap spatially or temporally (Brosnahan *et al.*, 2010), as is the case for *A. minutum* in Ireland, where toxic forms are found in the south, and non-toxic strains in the west (Touzet *et al.*, 2008). Two known exceptions are the Shetland Islands in Scotland, United Kingdom (Touzet *et al.*, 2010), and Belfast Lough in Northern Ireland (Brosnahan *et al.*, 2010) where toxic and non-toxic species within the *A. tamarense* complex have been documented in both locations.

Alexandrium species are not known for rapid or "explosive" growth rates. Populations can form dense, near-surface aggregations during daylight hours and subsurface layers at night where higher nutrients are available (Anderson *et al.*, 2021). Similar strategies are critical to blooms of *Pyrodinium bahamense* in tropical and subtropical waters. This important paralytic shellfish toxin (PST)-producing species is addressed and detailed in a case study on the EWS of the Philippines, presented in Section 4.9.1 at the end of the chapter. At large spatial scales (>100 km), population growth is typically not reflected in monospecific blooms but in moderate biomass levels and co-occurrence with other species. Also, blooms are not particularly long lasting (days to a few months) and seem restricted in time by life -cycle transitions.

The considerable number of *Alexandrium* species makes it difficult to generalize about environmental controls of bloom dynamics, particularly the nutrient niche- of *Alexandrium*, and the nutrient dependent- mechanisms that select for individual genera and among species that will bloom (Anderson *et al.*, 2012). The genus can grow in nutrient rich- (Townsend *et al.*, 2005; Spatharis *et al.*, 2007) relatively pristine waters (Anderson *et al.*, 2002) but also in nutrient -poor waters (for example, Collos *et al.*, 2009). Nutritional strategies are diverse, so simple relationships with classical nutrients should not be expected. It is an opportunistic genus with the ability to utilize a range of inorganic and organic nutrient sources, feeding by ingestion of other organisms. *Alexandrium* species can migrate vertically in the water column depending on light intensity, time of day and water movement to inhabit optimum growing conditions.

The cyst stage is clearly important in the population dynamics of many *Alexandrium* species, but the nature of this linkage varies among habitats (Anderson *et al.*, 2012). Once vegetative cells enter the water column following cyst germination, their net growth and transport are heavily affected by circulation (large- and small scale-), nutrients (including from terrestrial sources), thermal stratification and other chemical (for example, as the unique chemistry of freshwater plumes) or physical (for example, fronts) factors. In shallow embayments, cysts and motile cell blooms are tightly coupled; whereas, in large temperate estuaries and open coastal waters, the linkage is more difficult to define and quantify. In both habitats, most of the cysts in the sediments do not germinate due to bioturbation, burial, and inhibition of germination by anoxia (Anderson *et al.*, 2012, 2021). Cyst germination provides the inoculum for blooms, and the transformation back to the resting state can remove substantial numbers of cells from the bloom population and act as a major factor in bloom termination (Anderson *et al.*, 2012). The timing of cyst germination (excystment) and the ultimate formation of new cysts (encystment) is regulated by both internal and external factors, leading to highly episodic or seasonal outbreaks (Fischer *et al.*, 2018; Band-Schmidt *et al.*, 2019). Cysts are also important for population dispersal and can even be sources of toxin to shellfish and other benthic animals. Estimates of the inoculum size from excystment are small, about tens to hundreds of cells per litre, suggesting that major blooms require multiple, sustained vegetative divisions that, in turn, depend on environmental conditions affecting motile cells. Nevertheless, the size of an excystment inoculum can have a bearing on the magnitude of a bloom, especially if that bloom is limited temporally due to seasonal temperatures or to some form of periodic regulation. Bloom termination is clearly linked to life cycle transitions, although the relative importance of encystment relative to grazing or other loss factors has not been sufficiently investigated (Anderson *et al.*, 2021).

4.4 OBSERVATIONS OF HARMFUL ALGAL BLOOM CELLS AND TOXINS

Among the most informative and key targets for observations underpinning an EWS for pelagic HABs are the cells of the causative organism(s) and, in the case of toxigenic species, the toxin(s) they produce.

Monitoring changes in cell concentration during the very earliest stages of a bloom event can reveal critical information about the growth and development of a population and thus be important, along with assessments of bloom trajectory, in predicting the timing and location of potential impacts. Defining thresholds (Table 4.2), individual values or ranges of cell concentrations in water for an impending bloom formation, or for the point at which the concentration of cells has a toxic impact on humans through contamination of shellfish exceeding a regulatory limit for human consumption, is a recognizable harvesting management tool to assist federal, state, and local officials, and managers of public or community water systems to protect public health and harvesting practices. Setting limits is not mandatory; some countries have adopted reference values for the most concerning species in their region. As an example, for the *Dinophysis* genus (DST producer) 100–200 cells/L can trigger an alert for bloom formation. For the genus *Alexandrium* or the species *Gymnodinium catenatum* (PST producers), concentrations of 500 cells/L are a warning in some regions, or for the *Pseudo-nitzschia* genus (AST producer), 200 000 cells/L are usually a reference, but thresholds can vary as blooms are normally multi-specific and species composition and toxicity vary worldwide. Defining thresholds entails knowing the intrinsic toxicity of the local HAB species, as well as what causes a toxic event to occur and what affects the timing, spatial extent, and intensity of such an episode. Actions from the competent authorities may include increasing the frequency and intensity of sampling for toxin producing- phytoplankton and toxins in bivalves, implementation of tracking and tracing measures, or precautionary closures. Thresholds differ in various parts of the world. The potential for bivalve toxification is different between distinct groups of toxins -producing phytoplankton and differs between bivalve species. Consequently, a harmonized assessment of the risk requires algal abundance thresholds that are species- or genus -specific.

Monitoring changes in the toxicity of cells (that is, cellular toxicity or toxin quota) is essential for timely assessment of the potential risk of a bloom contaminating local fishery (shellfish, finfish) resources and mitigating its impacts, since toxin levels associated with certain potentially toxic HAB organisms can be highly variable (for example, *Pseudo-nitzschia* spp.; Bates *et al.*, 2018; Smith *et al.*, 2018), ranging from minimally or even non-toxic to highly toxic (occasionally during the course of a single bloom event). In other words, elevated cell concentrations do not necessarily imply similarly high toxin levels, and thus strategic and informed management actions must consider timely observations of both cells and toxins (in algal and vector species) to deal effectively with the potential uncoupling of algal growth and toxicity. Notably, there are some toxigenic organisms (for example, *Alexandrium* spp., Dyhrman *et al.*, 2010) in which changes in toxin levels most often (but not always) tend to mirror trends in cell concentration and thus determinations of cell abundance can be used to infer associated fluctuations in particulate toxin levels and thus assess the potential for resource contamination.

Monitoring of toxins in strategic vector species (that is, molluscan shellfish, crustaceans, finfish) has also been adopted in certain regions as the primary focus of an EWS. Such an approach is characterized by an increasing scale of spatio-temporal sampling resolution, whereby low-level monitoring or screening efforts (that is, intermittently at a small subset of routine sites) are initiated prior to the normal start of the bloom season and intensify progressively over time or as dictated by the return of positive results indicative of increasing resource contamination. *Mytilus edulis* is extensively used as an indicator species because of its abundance and ability to filter feed rapidly in colder waters than other shellfish, which leads these mussels to become toxic before other species (Bean *et al.*, 2005). Toxins accumulated by *M. edulis* are also depurated rapidly upon removal of the algal toxin source. Like approaches targeting HAB cell and toxin observations, a variety of methods are used for screening and measurement of toxins in fishery resources and examples of these approaches are outlined in Chapter 3, Sections 3.1.5 and 3.3.4.

A statistical analysis was conducted on a global dataset extracted from the Harmful Algae Event Database (HAEDAT) and Ocean Biodiversity Information System (OBIS) for the period 1985 to 2018 to investigate global trends in the occurrence, toxicity and risk posed by harmful algal blooms to natural systems, human health, and coastal economies (Figure 4.9). The results suggest that the intensity and frequency of HABs vary at regional and local scale and at species level, with increasing or decreasing trends and sudden occasional outbursts, but with no general uniform trend that can be discerned from that of increased observational efforts. Despite increased monitoring activities, impacts on human activities such as aquaculture, fishery, use of natural marine resources and tourism keep on posing economic activities at risk in many regions (Hallegraeff *et al.*, 2021b).

Here we present some relevant data considerations regarding the observation of cells and toxins of three of the most intensely studied pelagic HAB genera, *Pseudo-nitzschia*, *Alexandrium* and *Dinophysis*.

4.4.1 *Pseudo-nitzschia* observations of cells and toxins

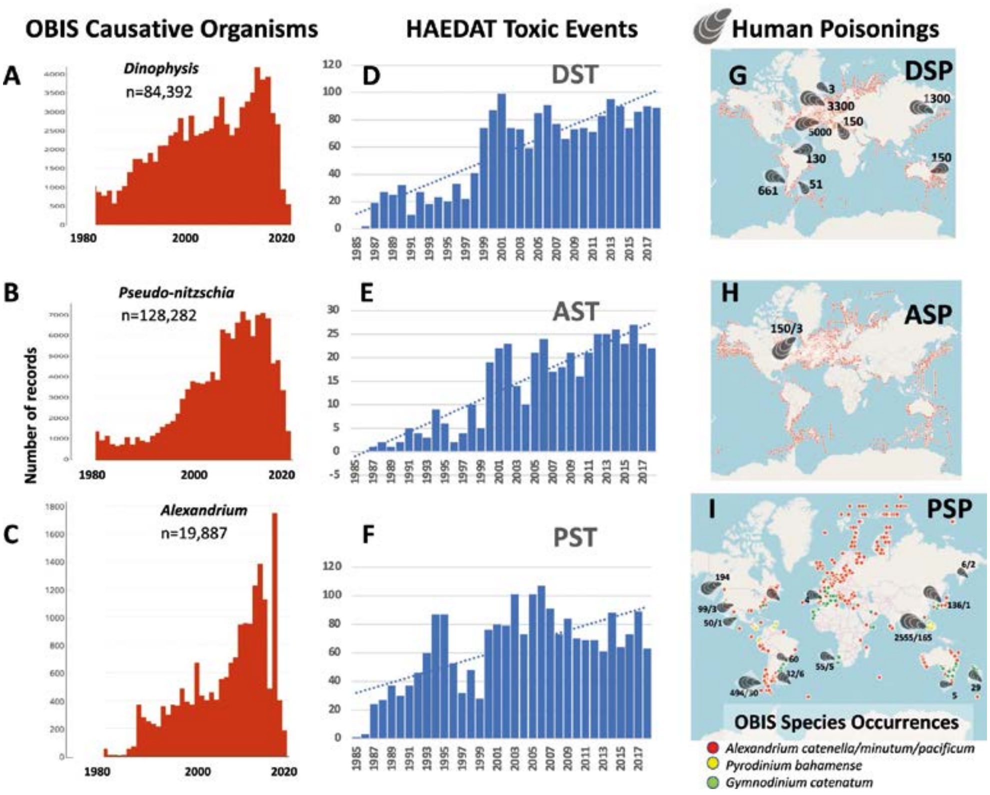
All species display the same general morphology. Precise determination of *Pseudo-nitzschia* species' identity by light microscopy (light, epifluorescence) is difficult and, therefore, it is becoming increasingly important to supplement this with molecular tools. Although molecular methods are a most valuable addition to microscopy, the latter is the official method to control cells in the water at shellfish production sites (EN 15204:2006: Water quality — Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique).

When analysing a field sample using the light microscope, a categorization can be made by separating *Pseudo-nitzschia* species into morphologically distinct groups to facilitate recognition during routine enumeration of phytoplankton. This separation can take into consideration the transapical and apical axes as well as cell shape and the degree of cell overlap. Aggregations can be made by target species of concern for example:

- *seriata* group, containing wider species (> 3 µm width) and *delicatissima* group (< 3 µm width) (Hasle and Syvertsen, 1997);
- *multiseries/pungens* (symmetrically wide, long shape), *australis/fraudulenta/heimii* (asymmetrically wide, shorter) and *pseudodelicatissima/delicatissima* (much smaller) (Trainer and Suddleson, 2005); and
- *P. delicatissima/P. pseudodelicatissima* complex (short [<60 µm long] and thin [< 2.3 µm wide] cells); "*P. seriata*" group (wide cells [>4.0 µm], symmetrical or asymmetrical depending on species) include *P. fraudulenta*, *P. subpacifica*, *P. seriata* and *P. heimii* and the "*P. pungens*" group (transapical width [2.5–4.0 µm)] include *P. pungens*, *P. turgidula* and *P. multiseries*. *P. americana* can most easily be distinguished by its features, solitary, small cells (length < 26 µm, width < 2.0 µm) with rounded apices compared to other species, can stand by itself (for example, Hargraves and Maranda, 2002, Fernandes *et al.*, 2014).

FIGURE 4.9

Occurrence, toxicity and risk posed by *Dinophysis* spp., *Pseudo-nitzschia* spp. and *Alexandrium* spp./*P. bahamense*/*G. catenatum* and associated toxin syndromes Diarrhetic, Amnesic and Paralytic Shellfish Poisoning., respectively. (A–C) Total number of global observations from OBIS.; (D–F) The number of records of HAEDAT Toxic Events of DST, AST and PST.; (G–I) Global distribution maps (as red dots, from OBIS) as of 2018 of Dinophysis spp. (DSP), *Pseudo-nitzschia* spp. (ASP) and *Alexandrium*, *Pyrodinium*, *Gymnodinium catenatum* (PSP). The locations of toxic events resulting in human poisonings are indicated by the size of the shellfish icons. The first number shows the number of poisonings, the second number indicates fatalities. For ASP, 150/3 signals 150 clinical cases with three fatalities. No human fatalities have ever occurred from DSP



Source: Hallegraeff, G.M., Anderson, D.M., Belin, C. *et al.* 2021a. Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. *Communications Earth Environmental* 2: 117. https://doi.org/10.1038/s43247-021- 00178-8

A definitive identification can only be made by examining the frustule (diatoms´ exoskeleton) morphometrics with a transmission electron microscope (TEM), or a scanning electron microscope (SEM) (http://nordicmicroalgae.org/taxon/*Pseudo-nitzschia*). The cells are first cleaned with concentrated acids to remove the organic cell content as well as the outer organic layer. This exposes the siliceous valves, which have intricated structural elements, including poroids, fibulae, interstriae and, if present, a central interspace. The number and spacing of these genetically fixed ornaments, as well as the shape, length, and width of the cell, are used to identify each species (Hasle and Syvertsen, 1997).

Within the last decade, there have been advances in the development of molecular tools for identifying *Pseudo-nitzschia* species within mixed assemblages and for determining population structure for species of *Pseudo-nitzschia*. Many are based on designing molecular probes targeting ribosomal RNA sequences that are unique to each species. However, the challenge has been that a probe developed for a *Pseudo-nitzschia* strain from one geographic region is not always able to detect the same species in another part of the world. Thus, it is sometimes necessary to tailor the probes for the diatom strains at the location studied (Trainer *et al.*, 2008). An automated ribosomal intergenic spacer analysis (ARISA) was developed by Hubbard *et al.*, (2008) for the rapid identification of *Pseudo-nitzschia* species in environmental samples. This approach does not give the concentration of a species within an assemblage but, rather, the relative abundance of that species and others within *Pseudo-nitzschia*. Another approach with microsatellite markers was developed for *P. pungens* (Evans and Hayes, 2004), *P. multiseries* (Evans *et al.*, 2004), *P. multistriata* (Tesson *et al.*, 2011), and *P. australis* (Clarke *et al.*, 2019). Sandwich hybridization assay (SHA) (Bowers *et al.*, 2017), Fluorescence *in situ* hybridization (FISH) and Quantitative real time PCR (qPCR) are other molecular detection methods available to determine cell concentration (Karlson *et al.*, 2010; Medlin and Orozco,2017). An extensive list of Laboratory-based Test Methods and Analytical tools is available in Chapter 2, Table2.4.

Of the more than 50 *Pseudo-nitzschia* species identified, over 25 are known to produce ASTs at varying concentrations, dependent upon the species and the environmental conditions (reviewed in Lelong *et al.*, 2012; Trainer *et al.*, 2012; Bates *et al.*, 2018).

4.4.2 *Dinophysis* observations of cells and toxins

The taxonomic identification of *Dinophysis* spp. is principally based on the size, shape and ornamentation of their large hypothecal plates which give the cell its contour and the shape of the left sulcal lists with their three supporting ribs (Larsen and Moestrup, 1992).

Light microscopy is the most straightforward tool to observe the presence of this genus in the water. However, each species of *Dinophysis*, in each biogeographic region, may exhibit varied sizes and shapes between the large vegetative specimens and small gamete-like cells, resulting from their polymorphic life cycles with different cell-cycle phases and feeding behaviours (Reguera and González-Gil, 2001; Reguera *et al.*, 2003). Morphological variability can cause uncertainty in identification, particularly when two close species of *Dinophysis* co-occur, such as the pairs *D. acuminata*–*D. sacculus* (Zingone *et al.*, 1998) and *D. caudata*–*D. tripos* (Reguera *et al.*, 2007). The term "*D. acuminata* complex", coined by several authors (Lassus and Bardouil, 1991; Bravo *et al.*, 1995; Koukaras and Nikolaidis, 2004) is a good example of how to label a group of co-occurring species, sometimes difficult to discriminate with conventional microscopy. A large array of morphologically distinct morphotypes of *Dinophysis* have been labelled as *D. acuminata*, *D.* cf. *acuminata*, *D. ovum*, D. cf. *ovum* based on large hypothecal plates that are dorsally convex and an oval/suboval shape in lateral view. These distinct morphospecies cannot yet be consistently differentiated using molecular methods (Wolny *et al.*, 2020; Sechet *et al.*, 2021).

Thirteen species (Table 4.1) produce DSTs and pectenotoxins and are considered the causative agents of DSP (Reguera and Pizarro, 2008, and references therein). DSP outbreaks have been associated with cell densities of *Dinophysis* as low as 100–200 cells/L (Yasumoto *et al.*, 1985), no water colouration is normally observed. The outbreaks with the highest impact on public health and shellfish contamination were those where the causative *Dinophysis* have moderate to elevated levels (>1 pg OA equiv. cell-1) of okadates as the dominant toxins in their profile (Reguera *et al.*, 2012).

Toxin profiles are different among *Dinophysis* species and within strains; latitudinal variations are also observed and can help early detection in water. The predominance of PTX2 in *D. fortii* and *D. acuminata* strains from the western Japanese coast would explain why DSP events there are so mild in contrast to those in the north and northeast, where DTX1 is a critical component of the toxin profile of *Dinophysis* spp. (Suzuki and Mitsuya, 2001). PTX2 was also found to be predominant in *D. acuminata* cultures from the northeast United States of America (Hackett *et al.*, 2009). There are doubts about the toxigenic nature of the heterotroph *Dinophysis rotundata* (= *Phalacroma rotundatum*) that may act as a vector of toxins taken up from ciliate preys that had previously fed on co-occurring toxic *Dinophysis* spp. (González-Gil *et al.*, 2011). No reports of DSP outbreaks are associated either with *D. rotundata* or with *D. tripos* when these species were the only potentially toxic *Dinophysis* spp. present in the microplankton community (Caroppo *et al.*, 1999; Pazos *et al.*, 2010).

4.4.3. *Alexandrium* observations of cells and toxins

Cells are relatively featureless when observed by light microscopy, but minor morphological characters become visible after staining and dissection of thecal plates and/or after examination by scanning electron microscopy. The subtle morphological characteristics are used for classification, but many are not easily resolved during monitoring or research programs.

Within the *Alexandrium* genus, it might be possible to identify "species-complexes" that share some morphological characters: *A. tamarense* species complex (includes *Alexandrium catenella* [Group I]; *Alexandrium mediterraneum* [Group II]; *Alexandrium tamarense* [Group III]; *Alexandrium pacificum* [Group IV]; *Alexandrium australiense* [Group V]) and *A. minutum* species complex (includes *A. lusitanicum*, *A. angustitabulatum*, *A. minutum*, *A. andersonii*, *A. tamutum*, and *A. insuetum*). The main difference between this group and the *tamarense* species complex is that the *A. minutum* group species are much smaller, and the development of toxicity is more variable, even between different strains.

As exemplified by the *A. tamarense* species complex, chain-forming ability, thecal tabulation and cell shape are considered by some to be not reliable taxonomic markers (John *et al.*, 2003). Morphologically intermediate forms have been observed under different environmental conditions both in culture and in the field (for example, Anderson *et al.*, 1994), and toxic and non-toxic ribotypes of the same morphologically defined species sometimes, though rarely, co-occur (for example, Touzet *et al.*, 2009; Brosnahan *et al.*, 2010).

A common molecular approach taken with *Alexandrium* species involves the development of species- or intra-specific molecular "probes" that can label cells of interest so they can be detected visually, electronically, or chemically. Progress has been rapid, and probes and assays of multiple types are already available for many species and distinct ribotypes (that is, potential cryptic species). Although cell-surface antibodies have been used, the most promising approach involves short pieces of synthetic DNA (probes or primers) that bind to complementary portions of target molecules in the corresponding HAB species. These molecular targets, typically ribosomal RNA (rRNA), can be visualized and/or quantified by a variety of techniques such as fluorescent *in situ* hybridization (FISH), sandwich hybridization assays (SHA), and a variety of PCR-based assays that are becoming routinely employed in some monitoring programs (Anderson *et al.*, 2012) (see Chapter 2, Table 2.4). Most used field deployable- technologies and methods to detect *Alexandrium* cell concentration include flow-through image technologies such as the Environmental Sample Processor (ESP), FlowCam or the IFCB and sensors with molecular probes (see Chapter 2, Table 2.4 for further details on tools and technologies available for *Alexandrium* and PST determinations).

Of the more than 30 morphologically defined species in this genus, at least half are known to be toxic or have otherwise harmful effects (Table 4.1). One unique feature of this genus is that three different families of known toxins are produced among species within it – saxitoxins, spirolides and goniodomins. This toxigenic diversity is not found in any other HAB genus (Anderson et al, 2012). The most significant of these toxins in terms of impacts is the saxitoxins, responsible for outbreaks of PSP, the most widespread of the HAB -related shellfish poisoning syndromes. The impacts of PSP outbreaks include human intoxications and death from contaminated shellfish or fish, loss of wild and cultured seafood resources, impairment of tourism and recreational activities, alterations of marine trophic structure, and death of marine mammals, fish, and seabirds. PSP toxin profiles vary widely within and among *Alexandrium* species, and general characteristics can usually serve to distinguish other dinoflagellate genera (*Pyrodinium* and *Gymnodinium*) and cyanobacteria, or as accumulated in shellfish. Within *Alexandrium*, it is sometimes but not always possible to identify species specific- toxin markers. For example, members of the *A. minutum* group (including *A. ibericum*, *A. lusitanicum*, *A. angustitabulatum*) tend to produce primarily or exclusively gonyautoxins (GTX1–GTX4) (Cembella *et al.*, 1987). Among species of the *A. tamarense* complex, however, toxin profiles are too diverse to be diagnostic for species discrimination.

Average cellular toxin contents of toxigenic *Alexandrium* isolates vary considerably (up to an order of magnitude) among different growth phases and environmental regimes in batch cultures, with maxima usually found in exponential phase and under P-limitation (for example, Anderson *et al.*, 1990). Cell PSP toxin content is not reliable as a species-, ribotype-, or population characteristic- and must be interpreted cautiously (Anderson *et al.*, 2012).

4.5 DATA CONSIDERATIONS

Preparation of EWS for pelagic HABs requires region -specific expertise. Three case studies (the Phillipines, the United States of America, and Spain/Mediterranean region) are presented at the end of the chapter (Section 4.9) detailing all steps and actions regarding the implementation of an EWS, and in Chapter 5 (Figure 5.7) there is a schematic overview of the emerging monitoring and early warning system under development for the Norwegian waters. Following are the key parameters needed to establish effective and accurate EWS for pelagic HABs:

- *In situ* physical and biological observations (wind direction and speed, water temperature, salinity, chlorophyll, dissolved oxygen) and *in situ* HAB-specific biological sampling and observations at the coast (HAB cells and toxins in water and shellfish samples). Samples need to be collected from known HAB initiation sites. These data are collected by coastal community members, including Indigenous Peoples.
- *In situ* HAB -specific sampling (with a bucket for surface samples, a Niskin bottle for deeper water samples, a hose for an integrate sample of the water column and a phytoplankton net to concentrate cells) and physical and biological observations via ships and buoys at offshore locations (HAB cells, toxins, water column temperature, salinity, and nutrients). With two potential source locations of HABs, offshore samples need to be collected at these sites during bloom season.
- Transport and particle tracks from models, such as circulation–biogeochemistry hindcast/forecast models, incorporating carbon chemistry and including particle transport. Modelling or historical hydrographic and phytoplankton data provide supporting information on high -risk seasons and long -term changes in toxicity and distribution.
- Ocean surface currents provided by high frequency radar (HFR) data. Upwelling and other indices are included to provide a view of longer-term conditions and the presence or absence of El Niño conditions.
- Nowcast and forecast weather conditions, especially wind direction and speed from Weather Research and Forecasting models (see www.mmm.ucar.edu/weather-research-and-forecasting-model).
- Continued input and improvement based on the needs of stakeholders, including managers of shellfish resources and local health departments.

Sampling plans must be regular and comply with standard quantitative methods to determine the abundance and natural variability of toxin producing phytoplankton in the water column. Plans need to be feasible, unbiased, suitable for a range of oceanographic conditions, types of shellfish harvesting, and practical for technical and logistical requirements. It is important to define the minimum but adequate monitoring requirements to achieve the EWS goals. What are the core variables linked to the adverse outcome? All the variables needed to complete the interpretation of phytoplankton abundance data should be included, so that the requirements of the legislation regarding risk evaluation and early warning can be fulfilled. A complete list of relevant information available to support the implementation and monitoring of toxic pelagic HABs is detailed in Appendix 1, Existing Resources for Biotoxin Monitoring, Management and Regulation of this *Technical Guidance*. Of particular interest for daily use with toxic pelagic HABS are:

- Guide for designing and implementing a plan to monitor toxin producing microalgae (*IOC Manuals and Guides* No. 59);
- Microscopic and molecular methods for quantitative phytoplankton analysis (*IOC Manuals and Guides* No. 55);
- Monitoring of Toxinproducing Phytoplankton in Bivalve Mollusc Harvesting Areas. Guide to Good Practice: Technical Application (EURLMB);
- IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae website;
- Nordic Microalgae website that also provides a Plankton Toolbox;
- PLANKTON*NET Data Provider; and
- HABs in the Western Pacific Region website.

A list of technologies, tools and methods is available in Chapter 2, Section 2.5. An increasing number of field deployable technologies are available for cell and toxin tracking, from SPATT resins (Solid Phase Adsorption Toxin Tracking) and antibody probes to flow-through imaging technologies and methods. An example of a remote sensing technology is the automated molecular detection and quantification of *Pseudo-nitzschia* cells and DA (by molecular, SHA, and antibody probes Enzyme-linked immunosorbent assay [ELISA]) using the ESP (Scholin *et al.*, 1999; Greenfield *et al.*, 2006, 2008; Doucette *et al.*, 2009; Bowers *et al.*, 2016, Bowers *et al.*, 2017; Moore *et al.*, 2021)

Data processing and analysis is required to be in short notice to anticipate and act in a short period of time (24–48 h, should not exceed 72 h). For immediate actions, the forecast should provide 3–5 d lead time.

The warnings need to be disseminated in a readily accessible format understandable by decision makers charged with protecting the health and safety of seafood consumers as well as safeguarding the economic interests of seafood providers (for example, commercial and recreational fishers, aquaculture interests, etc.).

4.6 NOWCAST, FORECAST AND MODELING

For an EWS for toxic pelagic HABs to be effective, actionable observational data for cell and toxin concentrations should be delivered to resource managers at weekly scales, as pelagic HABs may be sudden and evolve rapidly over time and may be triggered by multiple correlated variables. A wide variety of approaches to modelling and forecasting the development and toxicity of HABs can be found in Anderson *et al.*, (2015), Davidson *et al.*, (2016) and Ralston and Moore (2020). An extensive list of monitoring options for cells and toxins is also available in Chapter 2, Section 2.5 of this Technical Guidance.

For toxic species that occur in sufficient abundance, satellite imagery can be used to assess bloom extent and to track bloom movement in near-real time (Amin *et al.*, 2009; Shutler *et al.*, 201; Maguire *et al.*, 2016; Wolny *et al.*, 2020). In the case of the extensive blooms containing the toxic *Karenia brevis* in the Gulf of Mexico, for example, satellite imagery has been used in combination with field and meteorological data and numerical models to provide near-real-time information on the location of toxic blooms and to assess their potential to be advected into near-shore regions (Hu *et al.*, 2016). In contrast, *A. catenella* occurs at relatively low abundance (<1000 cell/L) and comprises a small proportion of the total phytoplankton signal, precluding satellite-based predictive approaches. Satellite monitoring of algal pigments such as chlorophyll can be useful to detect high-biomass HAB species such as the ASP toxin producing- diatom species from the genus *Pseudo-nitzschia*. However, for a bloom of *Pseudo-nitzschia* to be colour detected, a large concentration of cells is already expected to be in the water, which for some species may be too late for a warning for potential shellfish toxification. Satellites can provide useful information on seawater temperature indicative of water masses and running processes that can favour a specific species. This is information to be considered in the overall risk assessment to be carried out for a given production area.

HAB models can be broadly classified into those that apply statistical (or empirical) techniques, process-based formulations, or merge multiple approaches (i.e., hybrid models) (Ralston and Moore, 2020). Each provides different time and spatial resolutions and time scales of simulation. Statistical models (Table 4.2) are most used for near-term- HAB forecasting and resource management while process-based-models are more complex, difficult to parameterize, and require extensive calibration, but can mechanistically project HAB response under changing forcing conditions.

Statistical models use observations to relate key forcing variables (for example, a nutrient concentration, temperature, upwelling wind index or time of year) to relevant measures of HABs (for example, the timing of HAB events or the abundance, toxicity, and spatial distributions of HAB species). A wide range of forcing variables are typically considered during model development, and its choice is often guided by our understanding of the underlying physical and biological processes. Statistical models require extensive observations to develop robust relationships between forcing variables and HAB response (for example, *Pseudo-nitzschia* and *Dinophysis* blooms off the Iberian Peninsula and Ireland (Raine *et al.*, 2010b; Cusack *et al.*, 2015; Díaz *et al.*, 2016); *Karenia* in the Gulf of Mexico (Stumpf *et al.*, 2009), and multiple HABs on the Northwest European Shelf and in Chesapeake Bay (Anderson *et al.*, 2010; Brown *et al.*, 2013). A broad variety of statistical approaches have been used to model HABs in the present climate, ranging from simple linear regressions to more complex analyses using artificial neural networks, fuzzy logic, or Bayesian inference (Ralston and Moore, 2020). Neural networks have shown some potential for forecasting at weekly timescales (Velo-Suárez and Gutiérrez-Estrada, 2007), and deep learning algorithms have revolutionized machine learning in recent years (Liu *et al.*, 2017, Chollet and Allaire, 2018, Grasso *et al.*, 2019).

Process -based (or mechanistic) models use mathematical equations to explicitly simulate key physical and biological processes that govern HABs dynamics (Ralston and Moore, 2020). Their development requires detailed knowledge of critical life history characteristics (typically derived from simplified laboratory studies of isolated strains) and the factors that modulate them as well as transport pathways. They require substantial amounts of data to represent the many processes in the system. Models based only on biological processes have utility in systems where transport processes are negligible. For example, in Nauset Estuary on Cape Cod (Massachusetts, United States of America), a small embayment with limited exchange and long residence times, interannual variability in timing of *A. catenella* blooms was reproduced with simple model based- temperature dependent- growth rates (Ralston *et al.*, 2014). In contrast, for many HABs, physical transport provides the dominant control on bloom distribution. For these cases, a common approach is to use velocity fields from a circulation model to advect passive particles that are representative of the HAB

(Velo-Suárez *et al.*, 2010, Ruiz-Villarreal *et al.*, 2016, Pinto *et al.*, 2016, Moita *et al.*, 2016). Individual -based models (IBMs), like passive particle tracking, can be run within a circulation model or offline using model output to represent advection by currents, but IBMs also can incorporate biological processes specific to the organism of interest. For example, an IBM with growth dependent on temperature, mortality dependent on shear and population density, and phototaxic vertical migration was used to hindcast *Karenia mikimotoi* blooms along coastal Scotland (Gillibrand *et al.*, 2016). Results showed a strong dependence on bloom source region and uncertainty in the biological rate parameters, making the model less practical for forecasts. In the Gulf of Mexico, an IBM of *Karenia brevis* that included vertical migration based on internal nutrient ratios was used to identify potential source regions by running simulations backwards in time (Henrichs *et al.*, 2015). Another example is the three-dimensional- agent-based-model constructed to study the spatial and temporal aspects of the bloom dynamics of *Pyrodinium bahamense* var. *compressum* in relation to the toxic shellfish events in the Philippines (Yñiguez *et al.*, 2018). Using agent-based modelling, details of the life cycle of *Pyrodinium* (*P. bahamense* var. *compressum*) and shellfish feeding and physiology that impact toxic bloom formation and decline were captured as the direct correlation between increasing cell numbers and rainfall and how *Pyrodinium* decline was affected by shellfish grazing and cell mortality. The model simulates the progression and decline of toxicity levels in both toxic plankton and shellfish throughout all growth phases of a bloom. Using the simulation, insights were inferred on assimilated toxin levels in shellfish and how long the shellfish remained toxic after the bloom event, relevant for the management of shellfish harvest closures and health risks (see Section 4.9.for the case study of EWS in the Philippines).

Coupled biological and physical models have been developed to forecast the development of phytoplankton blooms that include toxic species and to usefully predict the advection of HAB-containing waters into shellfish harvesting regions (for example, Escalera *et al.*, 2010, McGillicuddy *et al.*, 2011). Hybrid approaches normally use physical models to predict transport processes along with empirical models to integrate biological response. A hybrid approach using satellite SST and ocean colour along with particle tracking was used to explain accumulations of *Karenia* spp. in the eastern Gulf of Mexico (Stumpf *et al.*, 2008), although bloom forecasts are based primarily on satellite data (Stumpf *et al.*, 2009).). The utility of satellite data in hybrid models depends on the HAB, as for example in Europe it was found to be useful for early warning of *Karenia mikimotoi* and *Lepidodinium chlorophorum* but not *Dinophysis* (Maguire *et al.*, 2016).

Here we present key notes on nowcast, forecast and modeling of the three most intensively studied pelagic HAB genera, *Pseudo-nitzschia*, *Dinophysis* and *Alexandrium*:

4.6.1 *Pseudo-nitzschia* nowcast, forecast and modeling

In most coastal regions of the world, closures of shellfish harvesting based on monitoring for DA are reactionary. Shellfish are routinely tested for toxins and harvest closures are issued only when the regulatory threshold is exceeded (Reg. EC 853/2004). This system has succeeded in protecting human health, but has often led to conservative, coast-wide closures of shellfish harvesting areas, which negatively impacts the shellfish industry and the economy. Sentinel shellfish, typically mussels in cages, may not always provide the best warning of DA events. Alternatives to mussels, including crustaceans that retain toxins (Powell *et al.*, 2002), or solid phase adsorption toxin tracking (SPATT) technology (Lane *et al.*, 2010) can provide a more effective "history" of DA in the phytoplankton assemblage or in shellfish (Trainer *et al.*, 2012).

TABLE 4.2

Table summarizing the HAB modeling studies for toxic pelagic species. Models are categorized based on whether they focus on present-day (hindcasts, event-based, near-term forecasts) or future climate conditions (using climate model projections) and the modeling approach (statistical, process-based or a hybrid). Information on the HAB organism being modeled, geographic region, and a brief description of the model formulation and timescale are listed.

HAB organism	Model type	Region	Brief description	Source	Present vs future climate
Alexandrium	Process-based	Gulf of Maine	Ecosystem + circulation; seasonal to interannual	Stock *et al.*, 2005; Li *et al.*, 2009	Present
Alexandrium	Statistical	Puget Sound	Regression + trend analysis; interannual	Moore *et al.*, 2009	Present
Alexandrium	Process-based	Cape Cod	Local growth; seasonal to interannual	Ralston *et al.*, 2014	Present
Alexandrium	Process-based	Cape Cod	Ecosystem + circulation; seasonal to interannual	Ralston *et al.*, 2015	Present
Alexandrium	Statistical	Puget Sound	Regression + trend analysis; growth window	Moore *et al.*, 2011	Future
Alexandrium	Process-based	Puget Sound	Regional physical models; growth windows (temperature, salinity)	Moore *et al.*, 2015	Future
Alexandrium minutum, FishKills	Statistical	Philippines	Random forest machine learning algorithm	Yñiguez and Ottong, 2020	Present
Alexandrium, Dinophysis	Process-based	NE and NW Atlantic, NE Pacific, Alaska	Growth rates (temperature)	Gobler *et al.*, 2017	Future
Alexandrium, Vibrio	Process-based	Chesapeake, Puget Sound, Alaska	Growth rates/windows (temperature)	Jacobs *et al.*, 2015	Future
Dinophysis	Statistical	Portugal	General additive model; interannual	Diaz et al, 2016	Present
Dinophysis	Statistical	SW Ireland	Based on wind index, near-term forecast	Raine *et al.*, 2010	Present
Dinophysis	Process-based	NW Spain	Particle tracking; seasonal to interannual	Ruiz-Villarreal *et al.*, 2016	Present
Dinophysis	Process-based	NW Spain	Particle tracking; event	Velo-Suarez *et al.*, 2010	Present
Karenia	Hybrid	Gulf of Mexico	Observations + particle tracking; near-term forecast	Stumpf *et al.*, 2008, 2009	Present
Karenia brevis	Process-based	Gulf of Mexico	IBM with behavior; interannual	Henrichs *et al.*, 2015	Present
Karenia mikimotoi	Process-based	Scotland	IBM with growth; event	Gillibrand *et al.*, 2016	Present
Karlodinium, Prorcentrum, Microcystis	Statistical	Chesapeake Bay	Neural network and logistic regression; spatial + temporal; interannual	Brown *et al.*, 2013	Present
Prorocentrum, Karenia	Process-based	NW European shelf, NE Asia, SE Asia	Regional physical + ecosystem models; habitat suitability (temperature, salinity, nutrients)	Glibert *et al.*, 2014	Future
Pseudo-nitzschia	Statistical	California	Regression; spatial + temporal; seasonal	Anderson *et al.*, 2009	Present

TABLE 4.2

Table summarizing the HAB modeling studies for toxic pelagic species. Models are categorized based on whether they focus on present-day (hindcasts, event-based, near-term forecasts) or future climate conditions (using climate model projections) and the modeling approach (statistical, process-based or a hybrid). Information on the HAB organism being modeled, geographic region, and a brief description of the model formulation and timescale are listed (cont).

HAB organism	Model type	Region	Brief description	Source	Present vs future climate
Pseudo-nitzschia	Statistical	Chesapeake Bay	Generalized linear regression; spatial + temporal; interannual	Anderson *et al.*, 2010	Present
Pseudo-nitzschia	Statistical	SW Ireland	Zero-inflated negative binomial regression; interannual	Cusack *et al.*, 2015	Present
Pseudo-nitzschia	Hybrid	SW Ireland	Observations + particle tracking; near-term	Cusack *et al.*, 2016	Present
Pseudo-nitzschia	Hybrid	Pacific NW	Particle tracking + ecosystem; seasonal to interannual	Giddings *et al.*, 2014	Present
Pseudo-nitzschia	Statistical	NW Spain	Machine learning; spatial + temporal; interannual	González Vilas *et al.*, 2014	Present
Pseudo-nitzschia	Statistical	California	Logistic regression; interannual	Lane *et al.*, 2009	Present
Pyrodinium	Process-based	Philippines	IBM population+circulation - Modularly composed of a watershed nutrient and diffusion model and an integrated hydrodynamic-Pyrodinium-shellfish model	Yñiguez *et al.*, 2018	Present
Pseudo-nitzschia, Alexandrium, Dinophysis	Statistical	NW European shelf	Habitat suitability (temperature, salinity, bathymetry); maximum entropy approach	Townhill *et al.*, 2018	Future

Source: Elaborated by the authors.

Developing forecasting capability for the transport and impact of toxic *Pseudo-nitzschia* blooms will require sustained monitoring as well as additional efforts in the critical areas of basic research and model development. Where there is sufficient long-term data on *Pseudo-nitzschia* spp. and accompanying environmental information, it has been possible to develop predictive models of toxigenic blooms. These models can incorporate multiple forcing factors including time of year, chlorophyll, silicic acid, water temperature, upwelling index, wind index, cell densities, river discharge and nitrate predict the timing, but not intensity of bloom events. Improvements are still required to predict more accurately the timing of a bloom's appearance and its intensity. Some examples are:

- In Ireland (Europe), *Pseudo-nitzschia* blooms off the coast were linked to upwelling; a statistical model using a wind index, water temperature and recent cell densities helped predict the timing, but not intensity, of bloom events (Cusack *et al.*, 2015). In Bantry Bay in southwest Ireland, the combination of a passive particle tracking model to represent cross-shore advection by upwelling, a circulation model, satellite observations, and *in situ* sensors were used to characterize local water properties and recent toxicity reports (Cusack *et al.*, 2016).
- In northwest Spain (Europe), the presence or absence of *Pseudo-nitzschia* blooms in several coastal embayments was linked to location, day of year, temperature, salinity, upwelling index, and, most importantly, recent bloom occurrence using a support vector machine, which is a common machine-learning algorithm (González Vilas *et al.*, 2014).
- In Portugal (Europe), *Pseudo-nitzschia* blooms were modeled to evaluate the seasonal variation and the relation between all parameters it was applied the Zero-Inflated Generalized Poisson Regression Model, using data of sea surface temperature, upwelling index, and *Pseudo-nitzschia* concentrations to evaluate seasonality and correlation between variables. The results obtained indicate a close relation between upwelling events and *Pseudo-nitzschia* blooms which occurred during spring and summer. The mathematical model shows a lag of 4 d to 6 d between the upwelling events and the presence of *Pseudo-nitzschia* in the monitoring station (Palma *et al.*, 2010).
- In Chesapeake Bay (Virginia, United States of America), a Generalized Linear Model (regression-based approach allowing for both Gaussian and non-Gaussian distributions) was developed with 22 years of cell abundance data and used to make hindcast maps of *Pseudo-nitzschia* bloom probability based on factors including time of year, temperature, salinity, nutrients (phosphate, nitrate, silicic acid), river discharge, dissolved organic carbon, and Secchi depth (Anderson *et al.*, 2010).
- Along the Pacific Northwest coast of the United States of America, the transport of *Pseudo-nitzschia* from formation regions offshore to the coast depending on upwelling or relaxation, was simulated with particle tracking, and the rate of false positives for toxicity events was reduced by incorporating thresholds for overall phytoplankton abundance from an ecosystem model (Giddings *et al.*, 2014).
- In Santa Barbara Channel (California, United States of America) a statistical regression model using satellite ocean colour and sea surface temperature (SST) detected 98 percent of toxic *Pseudo-nitzschia* blooms with less than 30 percent false positive cases (Anderson *et al.*, 2009).
- In Monterey Bay (California, United States of America), a logistic regression model incorporating multiple forcing factors including time of year, chlorophyll, silicic acid, water temperature, upwelling index, river discharge, and nitrate was developed from eight years of observations and used to predict the probability of *Pseudo-nitzschia* blooms (Lane *et al.*, 2009, Bowers *et al.*, 2018); although the

presence of *P. australis* in thin layers (McManus *et al.*, 2008) is one challenge for applying the model).

- On the coast of Washington State (United States of America), a combination of more proactive approaches to monitoring allowed targeted closures. The Olympic Region Harmful Algal Bloom (ORHAB) monitoring partnership uses a simple combination of analytical techniques which includes weekly determination of total *Pseudo-nitzschia* cells using light microscopy and levels of particulate DA in seawater using antibody-based methods to give an effective early warning of shellfish toxification events. To sustain a monitoring program such as ORHAB, progressive integration of newer methods into the state management plans for HABs must occur. By rapidly assisting managers during toxic bloom events, ORHAB partners have effectively demonstrated to state legislators how integral the monitoring program is to effective and timely management of shellfish resources. Resulting legislation has initiated a surcharge on shellfish license fees that will provide enough funding to sustain a state-run program, when the federally funded program ended in 2005. Beach monitoring programs such as ORHAB are now being integrated with fine-scale sampling, using automated devices on moorings, to allow detailed determination of fluctuations in biological, physical, and chemical parameters that influence HAB intensity.
- The EWS for *Pseudo-nitzschia* in the Pacific Northwest is presented and its implementation detailed in the case study in Section 4.9.2.

4.6.2 *Dinophysis* nowcast, forecast and modeling

Data from national monitoring programmes show that contamination above regulatory levels often occur on a timescale less than seven days since, routinely, samples are taken weekly. An ability to predict DSP events becomes highly desirable in the absence of any indication in water samples of when they will occur. A common finding of most, if not all, harmful events caused by *Dinophysis* are strongly linked to meteorological conditions. The consequence of this is that blooms are no more predictable than the weather, limiting forecasts to, at best, five to seven days.

Several modeling approaches have been developed to predict *Dinophysis* blooms:

- In southwestern Ireland, Raine *et al.*, (2010b) used a simple model (fuzzy logic) based on the five-day weather forecast for cross-shore wind and time of year to predict *Dinophysis* import events and DSP toxicity. These model results were used to guide near-term shellfish resource management. Wind-driven circulation during summer months can bring harmful *Dinophysis* spp. from the continental shelf into coastal embayments (Bantry Bay) where they can cause toxic events.
- In the Bay of Biscay (France), Velo-Suárez *et al.* (2010) used a three-dimensional Lagrangian particle-tracking model (LPTM) and identified physical factors, wind-generated turbulence and advection as the primary causes of the decline of a *D. acuminata* bloom. In LPTM models, *Dinophysis* cells are treated as passive particles. To become useful as forecasting tools, more accurate three-dimensional simulations for LPTM models under development require better parameterization of biological processes and behaviour for each *Dinophysis* species under study.
- For the northwestern Iberian coast, several passive particle tracking approaches were used to forecast *Dinophysis* (Ruiz-Villarreal *et al.*, 2016; Pinto el al., 2016; Moita *et al.*, 2016). Simple individual-based “particle tracking” models (IBMs) have been shown to be useful to understand *Dinophysis* bloom development and transport (Reguera *et al.*, 2012).
- In the southern Galicias Rias (Spain), a simple model (fuzzy logic) was used to forecast DSP outbreaks. Once populations of *Dinophysis* are established,

downwelling-promoting southerly winds lead to initiation or intensification of DST contamination, whereas upwelling-promoting northerly winds have the opposite effect (Reguera *et al.*, 1995; Escalera *et al.*, 2006, 2010).

- In Huelva (southwest Spain), a computational framework (artificial neural networks; ANNs) was adopted to predict *D. acuminata* blooms. Instead of using hydrodynamic information, only time series of *D. acuminata* density were used (Velo-Suárez and Gutiérrez-Estrada, 2007).
- In Ría de Aveiro (northern Portugal), using a two-dimensional Lagrangian particle-tracking model (LPTM), Cerejo and Dias (2007) demonstrated the influence of tidal flushing on the horizontal distribution and dispersal of *D. acuta* and *D. acuminata*.
- NPZ (nutrient, phytoplankton, zooplankton) models are not particularly useful to model growth of mixotrophic species where there are no lineal relationships with inorganic nutrients and for which complex predator–prey interactions must be considered.

4.6.3 *Alexandrium* nowcast, forecast and modeling

- In the Gulf of Maine (Maine, United States of America) a model of *A. catenella* that represents cyst germination, growth dependent on temperature, salinity, nutrients, and light, and mortality has been used in diagnostic hindcasts and operational forecasts (Stock *et al.*, 2005; Li *et al.*, 2009). A related model that also imposed diel vertical migration was used to simulate *A. catenella* in an estuary (Ralston *et al.*, 2015) based on a 15-year record of paralytic shellfish poisoning toxins in shellfish tissues. *A. catenella* blooms were associated with warm air and water temperatures, low streamflow, weak winds, and small tidal height variability (Moore *et al.*, 2011). Those models treated the HAB as independent of the broader plankton community by simulating only the species of interest and prescribing the nutrient field (climatology) based on observations rather than having it evolve dynamically. A basic parameterization of germination and growth was formulated for *A. catenella* using *in situ* data coupled with a three -dimensional hydrodynamic simulation. The coupled model allows the simulation of blooms with realistic physical forcing. Shellfish intoxication in the region was due primarily to the introduction of *A. catenella* populations during downwelling favorable wind conditions, followed by south-westerly alongshore transport (Sellner *et al.*, 2003).
- In Puget Sound (Washington State, United States of America) and the Northeast of the United States of America, optimal conditions for *Alexandrium catenella* blooms (warm air and water temperatures in combination with low river discharge and wind speed) have become more common over the past 30 years, as have the frequency and duration of toxic blooms (Moore *et al.*, 2009; Ralston *et al.*, 2014).
- In Section 4.9.3, there is a case study description of the EWS for HABs of the Catalan coast, Spain, with a special focus on *Alexandrium*. Section 4.9 also presents a case study from the Republic of the Philippines of another relevant PST producer, *Pyrodinium bahamense*.

4.7 STAKEHOLDERS AND THEIR NEEDS

The stakeholder groups identified in Chapter 2, Figure 2.3 include several key participants in any EWS for shellfish toxicity including industry, regulators, public health and safety, and society in general.

For an EWS to be effective, actionable observational data for cell and toxin concentrations should be delivered to in real time or near real time (2–3 days' notice at minimum). From the industry and site management point of view, broad spatial and temporal scales are less useful for decision making (Grasso *et al.*, 2019). The system should provide notifications before blooms occur and certainly before harvest begins on location, spatial extent, and trajectory. Growers, harvesters, and managers are making staffing, effort, harvesting, monitoring, business, and safety decisions at shorter periods (daily and weekly timescales) and need information at site-specific levels.

Messages should also incorporate the understanding of the values (easy to interpret results), concerns (interpretation of impacts and risk assessment) and interests of those who will need to act.

The early warning risk methodologies and alerts must provide regulators and industry, the health sector and society, with a product that enables:

- a reduction in the risk of shellfish toxicity to humans;
- a reduction in the economic impact/losses through either shellfish product harvesting delays or product recalls;
- the opportunity to take mitigating action and prevent potential lasting bans;
- help to improve supply coordination;
- help to avoid fishing, swimming, and diving in affected areas.

The regulator's priorities, resources, and policies influence the scope of the early warning system but must reflect the needs of the industry to be implemented and sustained. An effective EWS to assure safe shellfish requires multidisciplinary and multi-agency governmental training, data collection, policy development and management effort in collaboration with members of the shellfish industry.

The engagement of a wider group of stakeholders in management processes and decisions is most beneficial such as public health regulators and practitioners, local communities including Indigenous People and recreational harvesters. EWS may be particularly benefited by Indigenous Peoples and recreational harvesters in remote areas where established regulatory testing may not currently exist. Developing citizen science in these remote areas may increase important observation data and EWS acceptance and utilization.

4.8 BASELINE RISK ASSESSMENT

The harmful effects upon human health and the economic losses to a range of sectors and stakeholders can vary depending on the HAB species (Table 4.1), the toxins involved and the route of exposure (Krahl, 2009; Berdalet *et al.*, 2016; Zohdi and Abbaspour, 2019). This is key information for risk management. Regulatory effects have primarily focused on the ingestion route, particularly that of shellfish, which through feeding efforts can biomagnify toxins in their tissues.

Risk assessment associated with ingestion of toxins include four sequential steps (Hail and Muni-Morgan, 2021):

1) identification of the hazard;
2) a dose–response assessment of the hazard;
3) exposure assessment of the hazard; and
4) a risk characterization of human exposure.

Exposure assessment (step 3) establishes the extent and routes of potential toxin exposures, namely (i) ingestion of contaminated fish, shellfish or drinking water, (ii) exposure to aerosolized HAB toxins, or (iii) dermal contact with a HAB (CDC, 2021).

Effect Risk Characterization merges the exposure assessment (step 3) and risk of human exposure information (step 4) to determine the probability of toxin exposure in sufficiently high concentrations to have a clinical effect (Van Dolah *et al.*, 2001; Krahl, 2009). This is most easily assessed for ingestion of identified toxins. For the majority of toxic pelagic HAB species, the information required to assess risk is poorly understood or unknown. However, for a risk to be assessed it must be acknowledged. Risk perception among stakeholders may vary in the way they obtain and interpret information on HAB causes, risks and impacts on human health. This is critical for communicating, promoting, and regulating public health measures. Workshops, surveys, and protocols of intervention in place are tools and actions to improve the communication between coastal communities and authorities, in particular the health sector, on the link to diarrheic, amnesic, neurologic, and paralytic syndromes produced by HABs. Key information for risk management includes data on:

- Where was the seafood harvested?
- What species were identified?
- What symptoms were reported?
- What is the identification, concentration, and distribution of toxins in seafood?
- How was the seafood processed and cooked (head, gonads, intestines intact)?
- How much was eaten?

Risk assessment can be either qualitative or quantitative. Qualitative risk assessment expresses likelihood estimates in non-numerical terms such as high, medium, low, or negligible and are routinely used for decision making. This approach is suitable for most risk assessments when the lack of toxin identification for some species and of toxin transformations in the environment, lack of biomarkers of exposure, and even the dynamic physical nature of blooms makes both predicting and determining exposure difficult to hamper the health risk assessment of HABS (Hail and Muni-Morgan, 2021).

The first quantitative assessment of the status of HABs around the world and the presence of global trends, the Global HAB Status Report (GHSR), was published in 2021 by Intergovernmental Oceanographic Commission of UNESCO. This unprecedented analysis on Global Harmful Algal Blooms found that:

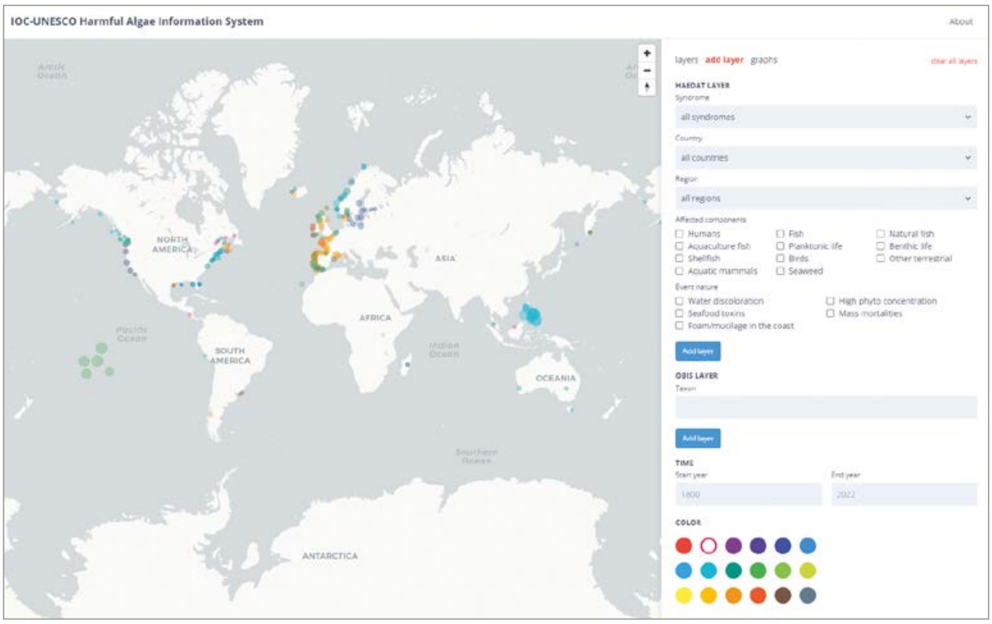
- Regionally -recorded HAB events have increased in Central America/Caribbean, South America, Mediterranean and North Asia; decreased in West Coast of North America and Australia/New Zealand, and had no meaningful change in East Coast of North America, South East Asia, and Europe.
- The impact from the 9 503 events on humans showed 48 percent involved seafood toxins. The event records linked to seafood toxins were as follows: 35 percent paralytic shellfish toxins (PST), 30 percent diarrhetic shellfish toxins (DST), 9 percent ciguatera poisoning (CP) (see Chapter 3 for BHABs), 9 percent marine and brackish water cyanobacterial toxins (see Chapter 6 for cyanobacteria), 7 percent amnesic shellfish toxins (AST), and 10 percent others, including neurotoxic shellfish toxins (NST), azaspiracid shellfish toxins (AZA), and toxic aerosols.
- By region, the largest number of records came from, in order: Europe, North Asia, Mediterranean region, the east and west coasts of North America, the Caribbean region, Pacific/Oceania, Southeast Asia and with more limited data sets for South America, and Australia/New Zealand.
- All geographic regions were impacted by multiple HAB types, but in varying proportions: 50 percent of events in the Caribbean, Benguela, Mediterranean Sea, North and South East Asia are related to high phytoplankton density problems, while seafood toxins and fishkill impacts dominated in all other regions.
- Among toxin-related impacts, PST prevailed in Canadian waters (McKenzie *et al.*, 2021), along the Atlantic coast of the United States of America (Anderson *et al.*, 2021), in the Caribbean and South America and the Phillippines (Sunesen *et al.*, 2021; Yñiguez *et al.*, 2021). DST were the most frequently recorded in Europe (Belin *et al.*, 2021; Bresnan *et al.*, 2021), the Mediterranean (Zingone *et al.*, 2021; Tsikoti and Genitsaris, 2021) and are an emerging threat in the Atlantic coasts of the United States of America (Anderson *et al.*, 2021). ASP -related problems affect of both Atlantic and Pacific coasts of Canada, the United States of America, and the United Kingdom, as Domoic Acid in seafood rarely exceeds regulatory limits elsewhere despite the wide range and intense blooms of *Pseudo-nitzschia* species over many coastal areas. NST were confined to the State

of Florida (United States of America), with a single outbreak also reported from New Zealand (Hallegraeff *et al.*, 2021a).

- For the most part, however, the impacts were confined to shellfish harvesting area closures, rarely to human poisonings. The exception is ciguatera event records that are almost exclusively based on medical reports of human poisonings (see Chapter 3). To date the determination of the human health impacts of HAB toxin exposure has focused on acute impacts of limited exposure. For many HAB routes of exposure, the health effects of chronic (low-level) exposure are unknown and require further research.

The Intergovernmental Oceanographic Commission of UNESCO site of the Harmful Algal Information System (HAIS) provides access to information on harmful algal events (Harmful Algae Event Database (HAEDAT), Harmful Algae monitoring and management systems worldwide, current use of taxonomic names of Harmful Algae, and information on biogeography of harmful algal species (Figure 4.10). Stakeholders can consult the current distribution and risk regarding all syndromes, including cyanobacterial and aerosolized toxin effects, in a global context or for their country or region. Different layers of search are provided as by Affected components (humans, fish, natural fish, aquaculture fish, planktonic life, benthic life, shellfish, birds, other terrestrial, aquatic mammals, seaweed) and Event nature (water discolouration, high phytoplankton concentration, seafood toxins, mass mortalities, foam/mucilage in the coast). HAIS System is being built within the International Oceanographic Data and Information Exchange (IODE) of the Intergovernmental Oceanographic Commission (IOC) of UNESCO, and in cooperation with World Register of Marine Species (WoRMS), International Council for the Exploration of the Sea (ICES), North Pacific Marine Science Organisation (PICES), International Atomic Energy Agency (IAEA)and International Society for the Study of Harmful Algae (ISSHA).

FIGURE 4.10
Intergovernmental Oceanographic Commission of UNESCO
Harmful Algae Information System (HAIS) webpage (https://data.hais.ioc-unesco.org/)



Source: IOC-UNESCO. 2023. Harmful Algal Bloom (HAB) portal (ioc-unesco.org)

4.9 SUMMARY

Food safety is threatened by the presence of toxic pelagic microalgae because toxins accumulate in the food chain, mostly in filter-feeding bivalve molluscs (for example, mussels, oysters, cockles, clams, scallops) and crustaceans (for example, crabs, lobsters, shrimps) that are either farmed or grow in the wild. Humans are affected by consuming contaminated seafood.

There are over 80 toxic pelagic species, grouped in the chapter by their impact on humans, resulting in five human poisoning syndromes, to describe primary symptoms and/or toxins involved. The chapter also includes species that produce toxins although with no effect proven in humans to date, but which may pose a threat.

The most up to date information on the ecology, distribution, monitoring, risk assessment, and stakeholders needs for key toxic species is presented. Blooms are controlled by multiple biological, chemical, and physical processes, which require monitoring of multiple variables. Each HAB species has its own distinct set of variables that regulate its growth and toxin dynamics. Toxic pelagic blooms occur regularly on an annual basis or without a specific pattern, may be of short (2 or 3 weeks) or long (up to 2 months) duration, and species may pose a threat at low concentrations of a few hundred of cells per litre, whereas others must occur in concentrations of millions of cells per litre to cause harm.

The negative impacts of toxins on the seafood industry are well known and widespread globally. The news release An unprecedented analysis on Global Harmful Algal Blooms launched by IOC (unesco.org) (UNESCO, 2021) stated that 48 percent of HAB events with impacts on humans involved seafood toxins and, from those, 35 percent are due to Paralytic Shellfish Toxins (PST) and 30 percent related to Diarrhetic Shellfish Toxins (DST).

The foundations of a toxic pelagic EWS imply the regular monitoring of i) cells in water (and cysts in sediments) to detect the very earliest stages of a bloom, ii) toxins in vector species (that is, molluscan shellfish, crustaceans, finfish), and iii) environmental conditions (for example, wind direction and speed, ocean surface currents, water temperature, chlorophyll, salinity, nutrients) acquired rom a variety of sources (*in situ* time series, sensors, ship based sampling, high frequency radar, oceanographic buoys, satellite and hydrodynamic and biogeochemical model outputs). Data processing and analysis must be immediate (24–48 h, should not exceed 72 h). Preparation of an EWS for pelagic HABs requires region-specific expertise.

The ultimate goal of the EWS is to meet stakeholders' needs for i) prediction of HABs onset and spatial extent, at weekly timescales, ii) which type of species and/or biotoxins are present and the risk associated, iii) knowledge of bloom dynamics over time, iv) identification of hot spots where a HAB is expected to occur, and v) notification of the time of HABs occurrence in neighbouring areas. The target stakeholders are the aquaculture industry, policy makers, resource management, and monitoring agencies; the health sector and managers of water and food security; and, citizens, fishermen and tourists. Warnings must produce a reduction in the risk of shellfish toxicity to humans, a reduction in the economic impact/losses through either shellfish product harvesting delays or product recalls, and the opportunity to take mitigating actions and prevent potential lasting harvesting bans.

Three case studies are presented: Case study 1- Philippine Early Warning System for National Harmful Algal Blooms (*Pyrodinium bahamense*, PSP) (Section 4.10.1); Case study 2- Pacific NW (*Pseudo-nitzschia*, ASP) (Section 4.10.2) and Case study 3- Harmful algal blooms in the NW Mediterranean Sea: the case of the Catalan coast (*Alexandrium* sp., PSP) Section 4.10.3).

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4.10 CASE STUDIES

4.10.1 Philippines Early Warning System for Harmful Algal Blooms

What is the problem caused by the harmful algal bloom?

The Philippines is an archipelagic state where close to 70 percent of the local municipalities are located along a long coastline and 60 percent of the total population reside. Fishing activities in the coastal waters account for one-third of the total fisheries production employing more than 1.5 million Filipinos. Our coastal areas are sources of fish including shellfish and provide a vast area for shellfish cultivation and harvesting. Most of the areas are quite remote and are far from basic government and health services.

The country's coastal waters have been perennially besieged with paralytic shellfish poison (PSP) caused predominantly by the pelagic phytoplankton *Pyrodinium bahamense*. The first recorded toxic red tide incidence or harmful algal bloom (HAB) was in 1983. A monitoring program was initiated in the following year as an essential means to warn of the potential occurrence of HABs with the intention of reducing significant impact on public health and the shellfish industry.

Who are the stakeholders and what were their needs?

The public and private sector stakeholders have interests related to safe shellfish, shellfish production, trade and marketing. They include on- and off-site consumers, shellfish growers and industries. They need timely information on HABs to protect lives and their livelihoods.

On the other hand, key stakeholders that contribute directly to the HAB monitoring and management program are the officials from both local and national levels of government as identified below. The regulators need to strengthen support for infrastructure, that is, laboratories, and build capacities needed to implement a sustainable HAB monitoring and management program. The key regulators are:

- Department of Agriculture (DA). This is the executive department responsible for the promotion of agricultural and fisheries development and growth. Its principal functions are the sustainable development of agricultural and fisheries sectors, formulation of national policies and the promotion of rural development. The Bureau of Fisheries and Aquatic Resources is one of several bureaus under the DA.
- Bureau of Fisheries and Aquatic Resources (BFAR). This bureau is responsible for the development, improvement, management and conservation of the fisheries and aquatic resources. It implements the HAB monitoring and management program.
- Coastal Municipal and City Governments or Local Government Units (LGUs). The LGUs have jurisdiction over the municipal waters, from the shoreline to 15 km offshore, and are responsible for the management, conservation, development, protection, utilization and disposition of all fish, fishery and aquatic resources within their respective municipal waters. Authorization to engage and set-up shellfish growing areas are granted by the LGU that has jurisdiction over the site of the aquaculture operation. Likewise, LGUs are responsible for implementation of controls associated with the construction and operation of fish pens, fish cages, fish traps and other structures for the culture of fish and other fishery products, and closing or opening of fishing ground including shellfish growing areas in times of HABs. A few of the LGUs have set up laboratories to do water analysis to detect the presence of *Pyrodinium bahamense*.

The government agencies also partner with academe in HAB research and development, including efforts to understand bloom species and dynamics, and recently towards an enhanced early-warning system with forecasting capability.

What was the development status of the country or region in terms of monitoring?

At the start, HAB monitoring proceeded with the collection of shellfish meat and water samples from areas where HABs occurred. Aerial surveillances were at times conducted to determine the extent and movement of visible HABs (Bajarias *et al.*, 2006). The residents were then alerted to refrain from collecting and consuming shellfish from the affected area(s). A stringent regulatory limit on PSP in shellfish meat was established and served as the basis for closing or opening coastal areas and/or shellfish growing areas. When toxicity levels in shellfish meat are beyond the regulatory limit of 40 µg STX eq/100 g shellfish meat, the government authorities impose the closure of affected coastal area(s) (NRTTF, 1999). Consequently, the coastal areas are opened when toxicity levels in shellfish meat are below the regulatory limit for three consecutive weeks of sampling. These alerts or shellfish bulletins are published through broadcast media. Information Education Campaigns (IECs) were conducted to educate the fisherfolk and the public about HABs.

An ad hoc task force composed of concerned government agencies with BFAR was subsequently created to evaluate the information gathered concerning HAB incidences to avoid inaccurate reporting. Evaluation results were published through bimonthly updates in the form of a joint statement signed by heads of two departments, the Department of Agriculture and the Department of Health.

However, delays in the issuance of the official notifications were incurred through this approach. Voluminous samples flooded the central office laboratory due to limited manpower and lack of laboratory facilities. In addition, more areas consistently remained closed due to the stringent regulatory standard. The emergence of the present HAB Monitoring and Management Program is the result of BFAR's efforts to improve support infrastructure and build capacities.

The Philippines is the only country in Southeast Asia with a HAB monitoring and management program that is a priority of the government. In fact, the Philippines together with Viet Nam and Japan participated in the IOC HAB Portal development for Southeast Asia which served as reference for subsequent efforts of other international organisations for example, the Southeast Asian Fisheries Development Centre (SEAFDEC) to develop such systems among the member countries of the region.

As HAB-affected sites increase and other types of toxic pelagic HABs and fish kills emerge (Bajarias *et al.*, 2006, Yñiguez *et al.*, 2021), the capacity for monitoring and improved approaches and technologies need to be continually enhanced

What approach/technology was taken to solve the problem?

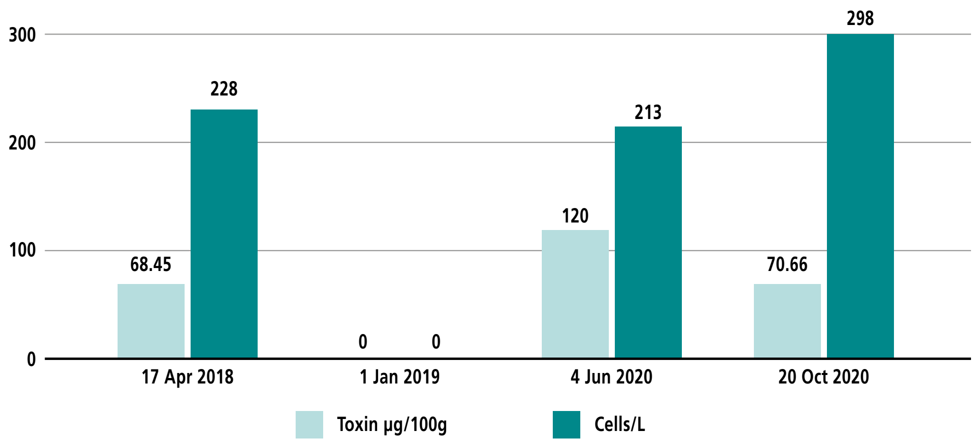
The Bureau of Fisheries and Aquatic Resources is the main management authority over the national HAB monitoring and management program (Arcamo *et al.*, 2017, BFAR, 2010). This is to streamline the decision-making process and hasten the flow of information critical to the implementation of management strategies to address HABs. The shellfish toxin quantification is the standard approach used for regulatory closures of shellfish harvest areas and/or coastal waters while HAB microscopic identification and microscope counts supplement data used in forecasting and monitoring.

The mouse bioassay used to be the standard confirmatory test. However, due to unavailability of saxitoxin (STX) standards to calibrate laboratory mice and the supply of laboratory animals, the BFAR shifted to receptor binding assay. The BFAR regional offices in collabouration with some local governments implement the regular monitoring in the coastal waters, identify phytoplankton in the water samples and submit the shellfish meat for toxin analysis to BFAR central laboratory.

In early 2000, the stringent national regulatory standard was relaxed to a level within the acceptable range from the international standard of 60 µg STX eq/100 g shellfish meat based on long-term data on paralytic shellfish poisoning (PSP) incidence. Subsequently, a standard monitoring schedule was established and predetermined number of sampling sites representative of the number of coastal areas were identified. AOAC accepted screening tests were eventually adopted to reduce the number of samples submitted to the central office laboratory for confirmatory tests. Most importantly, the HAB program is integrated in the BFAR annual work and financial program and incorporated in the office rules of procedure.

What forecast data were used?
Currently the Philippine HAB and Marine Toxin Monitoring program is a nowcast. However, phytoplankton cell counts are an essential part of the monitoring program to support toxicity tests in shellfish meat. They are very important as second line of defence to indicate the potential toxins that may be present in a production area or coastal water and to forewarn shellfish growers of impending HAB. Rapid increases of toxin producing phytoplankton can indicate the need for additional sampling and/or tests to identify potential harmful toxicity in shellfish or the issuance of a localized warning; for example, in the island of Palawan there is direct correlation with toxicity. However, cell count to toxicity link across sites is still being assessed.

FIGURE 4.11
Comparison between the concentration of toxins in shellfish and the concentration of the toxin-producing dinoflagellate, *Pyrodinium*, from 2018 to 2020



Source: Bureau of Fisheries and Aquatic Resources (BFAR, unpublished data). 2022. Philippines.

Increased information on the toxic blooms came from the basic understanding of the biology of *Pyrodinium* (Usup and Azanza, 1998; Lim *et al.*, 2020) to analysis of spatio-temporal trends (for example, Bajarias and Relox 1996; Azanza *et al.*,1998; Yap-Dejeto *et al.*, 2018; Yñiguez *et al.*, 2021). Mechanistic models contributed to comprehending seasonal bloom patterns in particular embayments that can also be applied at least to a certain extent to other *Pyrodinium*-afflicted bays (Villanoy *et al.*, 2006; Yñiguez *et al.*, 2018). Villanoy *et al.* (2006) tracked the development of *Pyrodinium bahamense* blooms in Manila Bay germinating from two cyst beds and influenced by two circulation gyres. Yñiguez *et al.* (2018) linked the life history of *Pyrodinium* with hydrodynamics, nutrients and shellfish toxicity in another bay. Both these models showed the seasonal bloom signal though they were not synchronous due likely to variations in rainfall patterns and circulation features. Remote sensing models have also been developed to try to forecast HABs. These have made use of chlorophyll-a anomalies, normalized Fluorescence Line Height, and particulate backscattering ratio (Almo *et al.*, 2015; Almo, 2016). The chl-a anomaly method was used to develop a Semi-Automated HAB Detection System on the national scale, which has included a feedback system that is important for the calibration of localized thresholds, and to overcome the challenges in using chlorophyll-a remote sensing information. A machine learning approach using random forest algorithm has also been developed for Bolinao-Anda, Pangasinan in the northern Philippines which experiences both fish kills and toxic blooms due to *Alexandrium minutum* (Yñiguez and Ottong 2020). The rare availability of several years of environmental data enabled this effort. These last two models are still being tested and are part of the enhanced early-warning system with forecast capability currently being developed as further discussed below.

What early warning system was put in place?

The BFAR regional offices and some partner local government units are responsible for monitoring occurrences of HABs in the coastal waters. Shellfish and water sample collections are done regularly either on bimonthly or monthly bases while in very few extreme cases it is done twice a week. Toxicity levels in shellfish meat are determined and biotoxin bearing phytoplankton are identified.

Biotoxin monitoring procedure commences as follows (BFAR, 2010):

- collection of shellfish and water samples, and delivery to competent authorities: LGU, BFAR regional and central offices;
- analysis of PSP levels in shellfish meat samples in the BFAR central office laboratory;
- identification of phytoplankton present in the water samples that can be done by trained staff of the LGUs and BFAR regional offices, and BFAR central office at times when identification needs to be validated;
- assigning a status to a production or coastal area whether it be closed or open to harvesting shellfish for food, trade and marketing; and
- communication of results are disseminated through broadcast media.

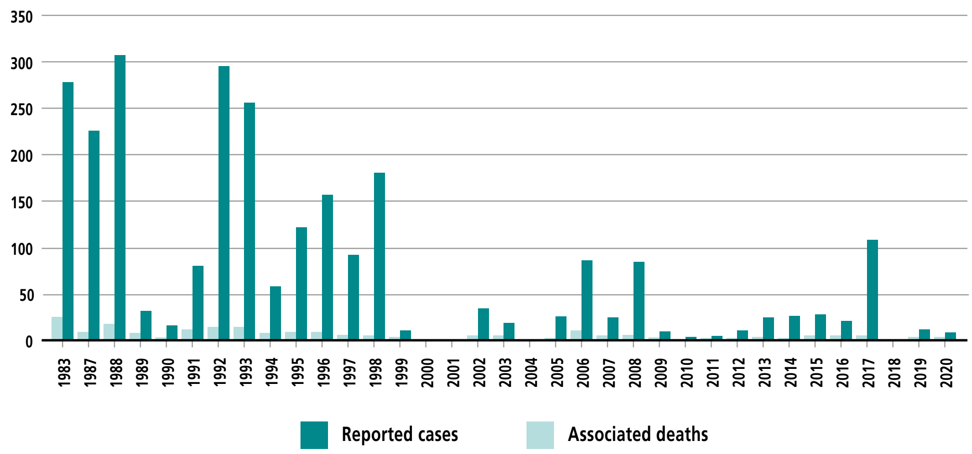
The efforts to develop an enhanced early-warning system with forecasting capacity for HABs has led to an on-going partnership between BFAR, local government units, scientists, as well as the local communities in pilot sites. The framework for this system is based on the Sendai Disaster Risk Reduction framework and is composed of four components:

1) HAB Risk Knowledge;
2) monitoring and Warning Service;
3) dissemination and Communication; and
4) response Capability.

As part of the first component, activities included collation and analysis of historical HAB data and participatory risk assessment with the local communities including marginalized shellfish farmers and their families. For the second component, the development of low-cost real-time environmental sensors are being explored since these can be assimilated in forecast models such as the machine learning model discussed in the previous section. There is still a need though to determine how to obtain and assimilate real-time information on phytoplankton and/or toxicity. The coarser-scale remote sensing model SeAHABS is also a part of this EWS. For the third component, the data and models have been made available at the HABHub web site (https://habhub.whoi.edu/) for dissemination and communication primarily to management and regulators. For the last component, apart from the already existing closures as response to HAB events, the participatory risk assessment activity also identifies some avenues to help mitigate HAB impacts on the stakeholders through changes in policies and governance aspects. This system is still very much in its infancy and many challenges have been encountered and still remain, especially its sustainability since this effort has been supported by a time-bound program funded by the government science agency the Department of Science and Technology.

What were the results for forecast operation?
Substantial decrease in PSP cases and fatalities was noted during the implementation of the early warning system, that is, nowcast. Progress in mitigation efforts has been advanced by the national government through information on HABs in most of the shellfish growing sites and/or coastal waters made available through shellfish bulletins and on-line interactive map posted on BFAR website. Records show that there has been substantial reduction in PSP cases and fatalities over the years.

FIGURE 4.12
Incidence of the Paralytic Shellfish Poisoning Syndrome (PSP) in the Phillipines from 1983 until 2020



Source: Bureau of Fisheries and Aquatic Resources (BFAR, unpublished data). 2022. Philippines.

The enhanced early-warning system with forecast capacity is still in development, and preliminary deployments of the observation tools and forecast models are still under assessment. Dialogue between stakeholders is also on-going to discuss feedback and the path towards integration and sustainability.

What were the consequences of the early warning system for stakeholders?
Due to the existing early-warning system, even without forecasting, the level of awareness on HABs and the appreciation for regular reports on the status of coastal waters in terms of toxic HAB occurrences were raised over time. The informed public rely on the shellfish bulletins and advisories for guidance on access to safe shellfish, trade and marketing. Moreover, the competent authority has maintained its credibility in terms of assigning the status of coastal and/or shellfish production areas. Through developing the enhanced early-warning system with forecasting capacity, greater engagement with the stakeholders is aspired to not only help sustain these efforts, but also build capacities to respond more pro-actively to HAB events in the present and considering the changing climate.

What lessons were learned?

A well-structured early warning system executed with appropriate management measures, streamlined decision making process, and expedited flow of information critical to the implementation of management strategies have effectively reduced/ mitigated negative impacts of HABs on public health and the shellfish industry. In addition, a strengthened support infrastructure and capacitated manpower contribute to the efficiency of the program. Moreover, the adoption of scientifically sound and internationally accepted tests fortified the credibility of the regulator that entice the public to adhere to the shellfish bulletins and advisories. Finally, the fact that the HAB program is included in the yearly work program of the BFAR with appropriate budget ensures sustainable implementation.

As the different agencies, researchers and communities develop newer approaches and technologies to enhance the early-warning system, it is important to facilitate information sharing and building capacity across the stakeholders to operationalize the different components of the EWS.

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4.10.2 Pacific Northwest – Amnesic shellfish poisoning

W hat is the problem caused by the harmful algal bloom?

Beach and harvest closures resulting from toxigenic *Pseudo-nitzschia* (PN) blooms have a severe impact on both coastal economies and on tribal communities. In 1991, the closure of Washington State (United States of America) beaches to recreational and commercial shellfish harvesting resulted in USD 15 million to USD 20 million revenue losses to local fishing communities (Horner and Postel, 1993;, Anderson 1995). The commercial Dungeness crab industry on which Washington's Quileute tribe depends for employment lost 50 percent of their income in 1998 due to harvest closures (Trainer and Wekell, 2000). The entire razor clam harvest of the Quinault tribe, on which they depend for both subsistence and commercial revenue, was also lost in the fall of 1998 (Trainer and Wekell, 2000). In contrast, with timely warnings in October of 2016 that samples were showing slowly increasing levels of *Pseudo-nitzschia* reported by the Pacific Northwest Harmful Algal Bloom (PNW HAB) Bulletin, Washington Department of Fish and Wildlife (WDFW) shellfishery managers had advance warning that the window for razor clam harvest opportunity could be quickly closing. WDFW made the highly unusual decision to increase the daily bag limit from 15 to 25 razor clams per day for the next razor clam harvest opener –the first time such action had ever been considered. As a result of the excitement produced by this significant increase in the bag limit, this short 11-day season at Long Beach resulted in the setting of a record number of one-day digger trips (17 800 diggers on Sunday 30 April 2017) and a total of 55 600 digger trips over the entire opener *and* over USD 5.3 million realized to the local economy. With sufficient warning of an incoming bloom, tribal fishers could seek alternative buyers for eviscerated crab, and shellfish managers might have longer lead times to schedule closures.

The massive toxic algal bloom of 2015, which stretched from central California to the Alaska Peninsula, demonstrated that *Pseudo-nitzschia* blooms, producers of the neurotoxin domoic acid, may initiate outside the Pacific Northwest (PNW) coastal zone and be transported into the Oregon and Washington coastal areas.

The PNW HAB Bulletin early warning system (EWS) is comprised of twice-monthly forecasts that synthesize supporting nearshore monitoring and offshore observations and modeling during spring and fall to help managers do the following: set the timing and location of commercial and recreational clamming and crabbing seasons, set catch/bag limits and determine when closures are necessary to protect public health. For specific upcoming harvest opportunities, the Bulletin provides information on the current areal extent, cell concentration, and geographic location of the HAB as well as a ~72 h forecast of these parameters. It is produced by a collabouration between NOAA, the Olympic Region HAB partnership (ORHAB), the Makah Tribe, the University of Washington (UW), and the Washington and Oregon Departments of Fish and Wildlife and Health.

Who are the stakeholders and what were their needs?

In the near term, shellfish managers require a bimonthly PNW HAB Bulletin that provides early warning of blooms coming to the coast during seasons for harvests (forecast several weeks in advance of scheduled razor clam digs). This allows for early harvest potential or relocation of crabbing and clamming efforts. It may also allow for additional harvest potential prior to an impending bloom. The Washington and Oregon crabbing season openers are in the late fall. Although crab harvests will not be targeted specifically initially, updates (Bulletins) released in October will provide information useful to crab managers and industry. Previous consultation with shellfish managers has determined that they continue to require bi-weekly updates on the pertinent parameters during the razor clam season (April–May and August–October), but the EWS should include flexibility to allow for weekly updates (Bulletins) during intense blooms.

In the longer term, managers may want a seasonal forecast, which will necessitate close collabourations with Olympic Region HAB partnership (ORHAB), Tribes, and State and Federal partners. This would allow for advance planning by coastal shellfish managers (hiring additional staff, planning for additional samples, enhanced collabourations and issuing warnings to crabbers about a high risk of a bloom season). The current Bulletin includes elements of this longer-term outlook and the form and methods for a complete forecast are under development.

In cases of rapid onset or intensification of a bloom, special notification might be needed to alert the public. This could require close coordination of sampling to allow managers to immediately halt fishing as warranted. The states might issue a special closure message and seek the assistance of NOAA to get the word out. In this case, the information dissemination mechanisms of the National Weather Service (NWS) (for example, for NOAA Weather Radio, social media, or other dissemination) and/or the Washington Department of Transportation would provide an important resource. This will require ongoing coordination with NWS/Weather Forecast Office (WFO) Seattle.

The following state and tribal co-managers use information from the PNW HAB Bulletin to guide their management of fisheries:

- Washington Department of Health (WDOH) to guide regulatory testing of shellfish and closures of areas to clamming and crabbing;
- Washington Department of Fish and Wildlife (WDFW) to guide monitoring to support management of clamming and crabbing;
- Washington Coastal Tribes (Quinault Indian Nation, Quileute Tribe, Makah Tribe, Hoh Tribe) to support decisions about tribal subsistence fisheries, guide monitoring to support shellfish management and closures of areas to clamming and crabbing;
- Oregon Department of Agriculture Shellfish (ODA) to guide regulatory testing of shellfish and closures of areas to clamming and crabbing; and
- Oregon Department of Fish and Wildlife (ODFW) to guide monitoring to support management of clamming and crabbing.

The PNW HAB Bulletin will also be shared with other management partners including:

- Department of Fisheries and Oceans Canada
- Canadian Fisheries Inspection Agency
- California Department of Public Health
- Southeast Alaska Tribal Toxin Partnership.

What was the development status of the country or region in terms of monitoring?

The original program that forms the basis of the PNW HAB forecast is the ORHAB partnership which was supported by five years of funding by National Oceanic and Atmospheric Administration (NOAA). The importance of ORHAB in preserving shellfish harvest on the Washington State coast, was presented to the state government which provided funding for monitoring through a tax to state shellfish licenses.

What approach/technology was taken to solve the problem? What forecast data were used?

The demonstration PNW HAB Bulletin is an interpretation and compilation of many distinct observations from a variety of sources, including: Oregon and Washington beach monitoring time-series, *in situ* HAB sensor data, ship-based sampling, HF radar, oceanographic buoy information, and hydrodynamic and biogeochemical model outputs. The level of complexity in preparing the Bulletin is significantly greater than in generating HAB forecasts in other regions of the United States of America and requires region-specific expertise. Figure 4.12 shows an example of the current Bulletin format, issued 27 September 2021.

Bulletin production relies on integrating and interpreting results from:

- *in situ* physical and biological observations (wind direction and speed, water temperature, chlorophyll) and *in situ* HAB-specific biological sampling and observations at the coast (HAB cells and toxins in water and shellfish samples). Because conditions can differ widely between Oregon and Washington, these samples need to be collected weekly from both coasts.
- *in situ* HAB-specific sampling and physical and biological observations via ships and buoys at offshore locations (HAB cells, toxins, water column temperature, salinity and nutrients). With two potential source locations of *Pseudo-nitzschia*, Heceta Bank off the coast of Oregon and the Juan de Fuca eddy off the coast of Washington (see Figure 4.9, offshore samples need to be collected at both sites approximately every two weeks during bloom season (April–November).
- Transport and particle tracks from the UW's LiveOcean (a three-dimensional circulation-biogeochemistry hindcast/forecast model, incorporating carbon chemistry and including particle transport).
- Ocean surface currents provided by high frequency radar (HFR) data from Oregon State University. These observations will be supplemented by the newly funded Integrated Ocean Observing System (IOOS) HFR installations at two locations on the Washington coast.
- Upwelling and other indices are included to provide a view of longer-term conditions and the presence or absence of El Niño conditions.
- Nowcast and forecast weather conditions, especially wind direction and speed from the UW Weather Research and Forecasting (WRF) model.

What early warning system was put in place?

The PNW HAB Bulletin is communicated directly to tribal and state co-managers of the fisheries via e-mail. A public version is made available after a suitable time delay. Current Bulletins over one month old are on the ORHAB website and the Northwest Association of Networked Ocean Observing Systems (NANOOS) website.

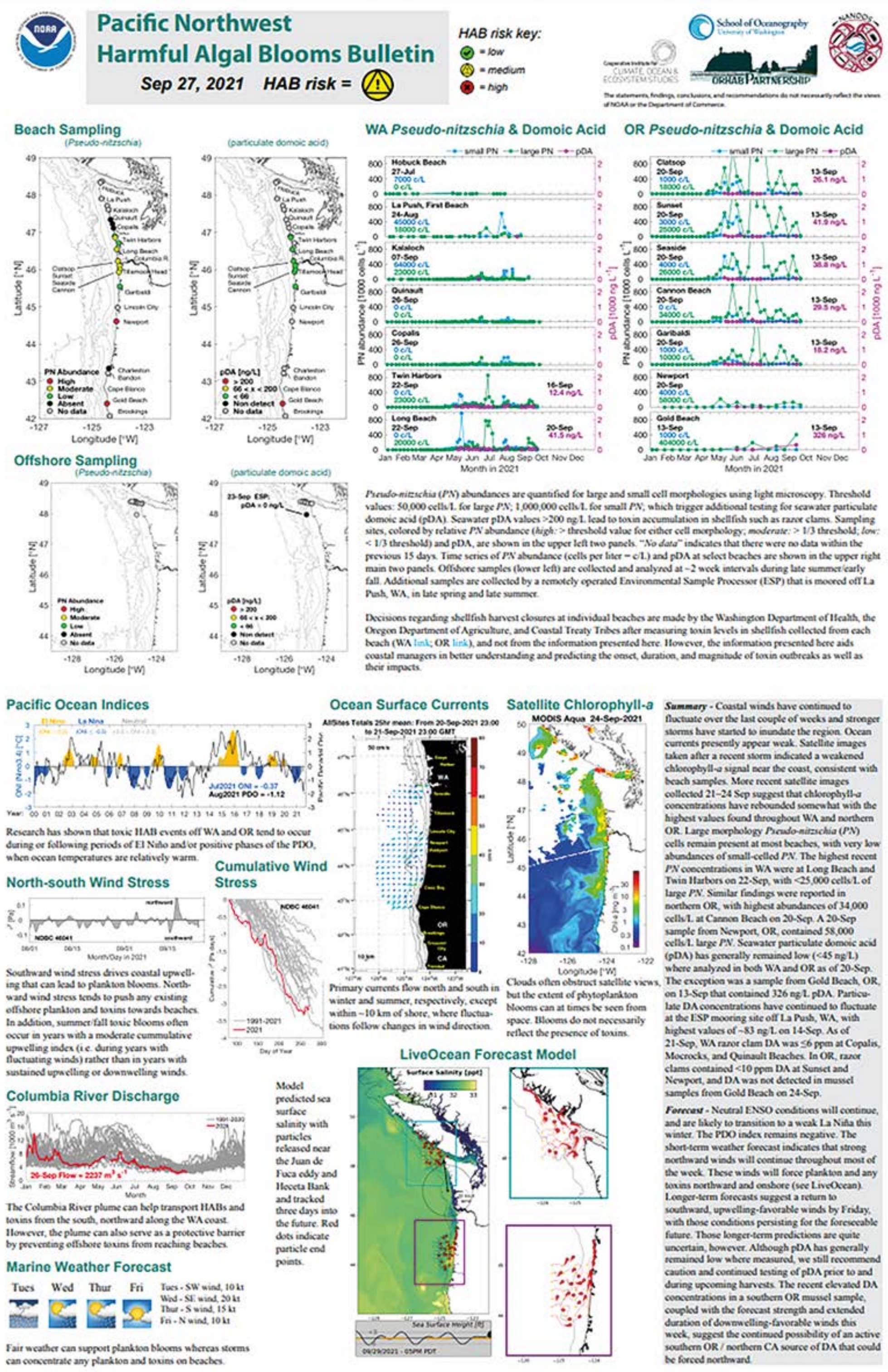
What were the results for forecast operation?

The Bulletin is already fully functional and will transition to NANOOS for sustained service. The transition to operational activities focus on two areas: determining what defines "sustained service" for models and procedures, risk mitigation strategies and implementing these transition tasks; and identifying how and when transitional and sustained funding might be obtained.

What were the consequences of the early warning system for stakeholders?

Frequent local and regional blooms, as well as the massive 2015 west-coast-wide HAB event, have generated high visibility and interest in PNW HAB forecasting products from NOAA and its partners and stakeholders in the region. The HAB issue continues to garner strong congressional interest and support for NOAA efforts to mitigate HAB impacts on coastal communities. For example, State of Oregon Representative Suzanne Bonamici, co-chair of the bipartisan Oceans and Estuary Caucuses, stated,"Coastal communities rely on a healthy ocean and so do shellfish, fish, marine mammals, birds, and ecosystems around the world. Harmful algal bloom and hypoxia events threaten the health of our oceans, lakes and rivers. Our bipartisan legislation will help communities better protect against and respond quickly to harmful algal bloom and hypoxia events."

FIGURE 4.13
PNW (Pacific Northwest) HAB Bulletin from 27 September 2021 showing a moderate HAB risk



Source: Pacific Northwest HAB Bulletin. 2022. Washington

Early warning of future HABs will provide marine wildlife specialists with information about bloom location, allowing them to more rapidly diagnose illnesses in mammals due to HABs. With sufficient warning, fisheries managers including tribal fishers will have the ability to take action, such as finding alternative buyers for eviscerated crab and scheduling selective closures, thereby mitigating effects on local economies.

Having a sustained twice-monthly Bulletin will provide benefits to users by giving them more time to take actions that will mitigate HAB impacts on public health, local businesses, and economies. Advance warning allows state and tribal razor clam managers to fine-tune the number of beaches that must be closed or to allow increased harvesting ahead of a predicted bloom, as was made possible in 2017 by the demonstration Bulletin. The improved ability to forecast and plan for blooms can contribute to decreased economic loss to the local communities and increased public confidence in the safety of harvested clams and crabs. These early warnings can save millions of dollars to coastal economies in a year.

What lessons were learned?

Early and frequent discussions are needed with funding agencies, government officials and stakeholders about the transition of research to operational early warning systems. Partner organisations that will be responsible for operational components of the early warning system may differ from the research entity. Oversight and engagement and collabouration are required to ensure smooth transition of all components of the early warning system to operations and necessary funding to maintain the EWS in operational mode.

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4.10.3 Harmful algal blooms in the northwest Mediterranean Sea: The case of the Catalan coast.

What is the problem caused by the harmful algal bloom?

The Mediterranean Sea (MS) is the largest semi-enclosed sea on Earth and is also among the most oligotrophic areas of the world. This is especially the case for the Eastern Basin, which is considered to be an ultra-oligotrophic system. Nutrient availability in the MS is low and inorganic phosphorus concentrations restrict primary production. However, this limitation may be relaxed and counteracted by inputs from populated coastal areas by river and groundwater discharges, deposition from the atmosphere, and upwelling of deep nutrients showing that biological production is closely coupled to the processes that deliver nutrients to the waters. As a consequence of the oligotrophic nature of the MS, offshore waters are characterized by low amounts of phytoplankton biomass over large areas, with a modest late-winter/early-spring increase observed in some areas, such as in the northwest basin, and relatively high biomass peaks occurring in fronts, upwellings and cyclonic gyres. Phytoplankton blooms in the open sea are induced by natural causes and seasonal cycles and thus are marked by distinct, well-defined patterns.

These characteristics are highly contrasting with those found in coastal Mediterranean areas, where harmful algal blooms (HABs) are abundant, diverse, highly localized, and either recur annually or emerge in a seemingly arbitrary manner, without a defined pattern. Seasonality in coastal blooms is not a constant feature; while most blooms occur in the summer, they may also appear during the winter. The main reason for these coastal blooms is that the abiotic conditions for phytoplankton blooms in the MS coastal area, such as nutrient availability and hydrodynamics, highly differ from those of the open sea, resulting in differences in both the type of phytoplankton species and in the frequency and occurrence of blooms. Nearshore, blooms tend to occur in harbours, marinas, pocket beaches and confined areas such as bays. These blooms may be of short duration (2–3 weeks) or persistent (up to 2 months) depending on several physical, chemical and biological factors which, individually or collectively, influence bloom dynamics (Garcés and Camp, 2012). HABs in those confined areas are denser and occur before blooms affect wider open waters or spread offshore. Confined Mediterranean areas are thus "sentinels" for HABs, because these areas offer the right conditions for dinoflagellate proliferation, such as they present low turbulence, high water residence times (for example, up to 20 days due to the mild effects of tides in the area) and low advection in comparison with open waters. Low turbulence is known to favour dinoflagellate blooms (Margalef, 1978; Margalef *et al.*, 1979). Concurrently, the high nutrient availability in these enclosed areas, which is five to ten times greater than the nutrient concentrations typical of open water areas, maintain phytoplankton biomass and could enhance the growth of toxic species. Moreover, the behaviour and life cycle of dinoflagellates also contribute favourably to the occurrence of blooms under those conditions (Smayda, 1997; Garcés *et al.*, 2000). Resting cyst stages of the meroplanktonic species are concentrated and viable in the sediments of these areas for a long time, with very low physical perturbations. Two nice examples of the "sentinel character" of confined areas can be seen in the detection of *Alexandrium pacificum*, a producer of Paralytic Shellfish Poisoning (PSP), and *Gymnodinium impudicum*. In both cases the bloom started in confined areas before the occurrence of widespread blooms that affected offshore waters. *A. pacificum* provoked an important toxic event in the NW Mediterranean in the spring of 1998 (Vila *et al.*, 2001). The danger to human health posed by the outbreak was worsened by the old practice of both the resident population and tourists of gathering shellfish from natural settlements. In this specific example, the detection of confined blooms of these species was interpreted as the locally confined amplification of wider blooms along the coast, and offshore, and provided an early warning to the local stakeholders. After that event, these species have been frequently reported in harbours, beaches and lagoons in the MS.

The Harmful Phytoplankton Monitoring Programme has been active in the Catalan coast from 1989 to the present, with a weekly sampling frequency at the Ebre Delta bays, the most important shellfish growing areas in Catalonia. Since 1995, a conceptual change has been implemented in the Monitoring Programme, based on the new concept of sampling confined waters as an early warning detection system for the toxic algal species and their proliferation. The objective of this Monitoring Programme is to develop a warning system not only associated to the direct surveillance of shellfish growing areas but also within confined areas with a high risk of occurrence of HABs. Occasionally, sediment samples are taken to map resting cysts in enclosed systems, in order to provide a basal line of the potential toxic species, resting cysts producers, present in the area.

Who are the stakeholders and what were their needs?

In the short term, aquaculture managers, shellfish harvesters and competent authorities require a weekly HAB Bulletin that provides early warning of blooms and risk levels about the presence of marine toxins that can potentially affect shellfish growing areas. In the longer term, stakeholders in the aquaculture and fisheries industries may require a seasonal forecast, which would allow them to plan operations in advance. The current HAB Bulletin includes elements of this longer-term perspective, for example, the display of the available time series of algal abundances and abiotic parameters. The form and methods for HAB forecast proper forecast have been developed for different taxa (Guallar *et al.*, 2016) and are under development for others.

What was the development status of the country or region in terms of monitoring?

The HAB monitoring is fully developed. It has maintained weekly sampling frequency at many sampling stations since 1990 where seawater samples are taken and environmental parameters are measured. The number of sampling stations has increased during the years to cover new areas. Phytoplankton samples are analysed using the method EN 15204:2006. Seawater sampling and phytoplankton analysis are accredited under ISO/IEC 17025:2017 ISO/IEC 17025:2017. Shellfish samples are also taken every week for the analysis of okadaic acid group toxins, pectenotoxins, yessotoxins, azaspiracids by LC–MS/MS (EURLMB, 2015); paralytic shellfish poison toxins by HPLC–FLD (AOAC, 2005) and domoic acid by RP–HPLC using UV detection (EURLMB, 2008). All sampling methods are accredited under ISO/IEC 17025:2017. The sampling frequency for the analysis of domoic acid in some areas has been reduced to fortnightly based on the results of the risk assessment of toxins and phytoplankton.

What approach/technology was taken to solve the problem? Monitoring? What forecast data were used?

The HAB Bulletin is an interpretation and compilation of many distinct observations from a variety of sources including phytoplankton time series in confined waters, in shellfish growing areas, as well as oceanographic information. The preparation of the weekly Bulletin relies on integrating and interpreting results from *in situ* physicochemical measurements (seawater temperature, salinity, oxygen concentration, pH) and biological observations (chlorophyll, pH, phytoplankton abundance and composition) and concentration of toxins in shellfish HAB-specific sampling.

What early warning system was put in place?
The HAB Bulletin is communicated directly by email to diverse stakeholders: competent authorities, fisheries associations, aquaculture industry. Competent authorities (CA) such as the Fisheries Directorate use the information for the management of shellfish growing areas opening or closing the areas, taking into account phytoplankton and toxins analysis results. Another competent authority, the Health Directorate, uses the information to recall and withdraw shellfish products from the market that were harvested between the date of sampling for which the result showed that health standards were not met and the date of closure. The aquaculture industry implements their own checks and decides on harvesting dates taking into account the information in the bulletin. This bulletin is also posted on internet (www.marinemonitoring.org).

What were the results for forecast operation?
The HAB Bulletin is fully functional in what concerns the short-term objective, as it provides the much-needed early warning system for potential toxic blooms. The forecasting ability in the longer term is a work in progress. Several HAB forecast models have been developed using artificial neural networks applied to the time series of phytoplankton monitoring, the models provide a forecast on the abundance of different taxa two weeks in advance with high accuracy, (Guallar *et al.*, 2016). Also, fuzzy logic models have been explored (Estrada *et al.*, 2010). Some other research results are also promising. For example, the sampling of resting stages in confined waters gives encouraging prospects of increased forecasting capacity in the future for the monitoring programme. An example of the importance of a species' life strategies is provided by the dinoflagellate *Alexandrium minutum*, which has been reported in several coastal areas of the MS, specifically in harbours. Resting cysts of this species remain dormant in the sediments of confined sites (Anglès *et al.*, 2010). The persistence of resting cysts in the sediments represent a temporally integrated repository of species diversity; thus, the detection of resting stages of novel and potentially introduced species in the area provides early information about potential future blooms.

What were the consequences of the early warning system for stakeholders?
Current regional legislation includes efforts to mitigate HAB impacts in the Catalan coast and its communities follow the requirements of the European legislation on official control (Regulation 2019/627) aimed at better protecting the communities against, and responding quickly to, harmful algal bloom events. Early warning of HABs is based on the ongoing time series by long-lived observation programmes and provides information about bloom location and timing, allowing management. The weekly HAB Bulletin delivers direct benefits to users by giving them more time by issuing alerts to take actions that will prevent or mitigate eventual HAB impacts on public health, local businesses, and economies. These early warnings can save local businesses and protect human health.

What lessons were learned?
Funding agencies, competent authorities and other stakeholders are using the early warning system provided by the Harmful Phytoplankton Monitoring Programme. The team responsible for the operational components of the early warning system in the Catalan coast is involved in different HABs research projects to improve species identification and deepening the understanding of species ecology.

Monitoring sampling corroborates a scenario in which the risk of HABs in the MS has increased by one order of magnitude in the last 30 years with a tendency to grow. Habitat changes and man-made coastal protection structures, underpinned by regional economic interests and other societal pressures, have also produced an increase in coastline artificialization, with the consequent adaptation of opportunistic harmful algal species to these standardized new habitats such as harbours and recreational marinas. These species stand a greater chance of success, benefitting from this "simplification" of habitat diversity.

The sampling design of the monitoring programme described here, could be applied in other areas with similar properties (that is, highly populated coasts, small tides and many engineered structures that decrease water energy and increase residence times). Similar systems could anticipate widespread harmful events.

Embayments and hydrographically confined areas in the MS act as reservoirs for meroplanktonic dinoflagellates. During stagnant conditions, fluxes of resting stages towards the sediments are favoured. Knowledge of the geographic distribution and density of resting cyst beds of *Harmful Algae* can help to identify risk areas under different ecological and hydrographical scenarios, providing useful information for the management of coastal areas.

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5 High biomass blooms causing fish kills and other environmental impacts

This chapter focuses on the pelagic harmful algal bloom (HAB) species and dynamic processes that cause high biomass blooms, as well as descriptive case studies and technological developments in early warning systems (EWS) to monitoring and mitigate their impacts. In extreme cases, high biomass algal blooms can result in damage to aquaculture operations, causing fish and/or shellfish mortality or recruitment failures. The emphasis herein is on bloom causing fish kills, particularly of species in aquaculture, because of the sudden and dramatic socioeconomic effects on local communities. In other words, the HABs in this chapter are distinct from shellfish toxin producers, which are discussed in Chapter 4. The high biomass blooms discussed in this chapter can have significant negative impacts on wild fish populations and cause marine faunal morbidity and mortality, including of seabirds and marine mammals, through the food webs or via a cascade of environmental stress.

Although such HABs are often collectively referred to as "high biomass", high biomass *per se* or associated indices such as bloom chlorophyll content are generally poor predictors of harmful potential when considered alone. Most HAB monitoring programs are instead based upon cell enumeration of potentially harmful species, and thus typically report massive blooms in terms of cell densities over defined spatio-temporal scales. In fact, such high cell density HABs are usually but not always "high biomass". This distinction is important for monitoring and development of EWS because the typical spring bloom is often high biomass/high chlorophyll but without causing harmful effects. Massive blooms of virtually any algal species can conceivably cause environmental damage due to hypoxia under certain circumstances but such taxa and their untargeted effects should not simply be added to the list of HAB taxa for routine monitoring.

Studies on Harmful Algae have often been focused on species and phycotoxins that primarily impact shellfish aquaculture and human health and safety (see Chapter 4). Perhaps because both shellfish and finfish have a high profile and socioeconomic importance in aquaculture, there has been a tendency to conflate the problem and monitoring strategies for toxins in shellfish and fish and marine faunal mortalities. This has led to a conceptual gap in modelling respective blooms and in designing appropriate monitoring and EWS. Cases of morbidity and mortality of fish and other marine fauna tend to occur only upon exposure to high biomass and/or high cell density blooms and the nature of the toxicity mechanism is often inconclusive – the involvement of known phycotoxins is rarely proven. In spite of occasional overlap between the "shellfish toxin producers" and the "fish killers", this suggests that the bloom phenomena should be considered separately with respect to modelling of blooms and impacts and technological applications for monitoring.

The focus of this chapter is on the harmful high biomass or high cell density blooms for which EWS can be effective in providing advance warning, sufficient for impact avoidance, transference and/or loss mitigation by stakeholders. Stakeholder engagement in the early development of the EWS is critical and continued discussion and input from planning to product is key to achieving the goal of implementing effective operational EWS into monitoring strategies.

5.1 HARMFUL ALGAL BLOOM SPECIES FORMING HIGH BIOMASS BLOOMS AND CAUSING FISH KILLS AND OTHER IMPACTS

Harmful effects of high biomass bloom-forming species are not always well defined but include:

- mechanical damage on the gills, for example, by certain diatoms and dictyochophytes;
- exposure to known ichthyotoxins, mostly from dinoflagellates, but including a single diatom genus; and
- poorly defined toxicity responses elicited primarily by raphidophytes, haptophytes and a few dinoflagellates.

The impacts of Harmful Algae specific on caged and wild finfish can occur via several mechanisms. These mechanisms can include:

- respiratory dysfunction due to mechanical damage to gill epithelium (Albright *et al.*, 1993) caused by abrasive or spiny algal cells, primarily of diatoms and silicoflagellates (Dictyochophytes);
- direct toxic effect of exposure to known phycotoxins, such as neurotoxins or other defined ichthyotoxins, produced primarily by dinoflagellates (Cembella *et al.*, 2002; Martin *et al.*, 2006) (Chapter 4);
- membrane disruption by induction of reactive oxygen species (ROS) and production of unusual polyunsaturated fatty acids (PUFA) causing oxidation of cell membranes (Mardones *et al.*, 2015); and
- disruption of osmoregulatory capacity caused by poorly known ichthyotoxins associated with raphidophytes (for example, *Heterosigma akashiwo*, *Chattonella antigua*, *Pseudochattonella verruculosa*) and some dinoflagellates (for example, *Karenia mikimotoi*) (Díaz *et al.*, 2019).

Causative eukaryotic microalgal species and effects of high biomass and/or high-density blooms are provided in Table 5.1. This table provides examples of the varied microalgal species and their mechanisms of effect but is not intended to be a comprehensive list. Tables of harmful microalgal species known or suspected of causing fish losses in aquaculture are also available (Rensel and Whyte, 2003) but must be revised for nomenclatural changes and for recent HAB events due to global aquaculture expansion.

Although the impacts and mass mortalities of finfish and other marine species caused by Harmful Algae have been recorded for decades (Sellner and Rensel, 2018), there is a growing global concern that these events may increase under global warming and climate change scenarios. Climate change allows for the range expansion of harmful species and creates environmental conditions where these harmful species and their blooms intersect spatially and temporally with coastal aquaculture and marine resources development (Lassus *et al.*, 2016; Trainer *et al.*, 2020; Goes *et al.*, 2020).

The environmental effects of high biomass blooms are integrated beyond the target species and may cause:

- allelochemical effects and responses in aquatic food webs;
- general local disruption of ecosystem function; and
- depletion of oxygen in the water column following the end of a bloom, leading to hypoxic conditions and mortalities (Lassus *et al.*, 2016; Shumway *et al.*, 2018).

Furthermore, increases in both finfish and shellfish aquaculture in coastal regions have led to increased lethal and sub-lethal impacts on fish- and shellfish resources (Hallegraeff *et al.*, 2021; Naylor *et al.*, 2021). Even in cases where a high biomass HAB causes only a non-lethal reduction of dissolved oxygen, this can result in secondary stress on farmed fish, making them more vulnerable to disease (Lassus *et al.*, 2016). In addition to the direct mortalities, sublethal densities of Harmful Algae have led to mortalities due to bacterial or viral infections on weaker or vulnerable fish (Yang and Albright, 1992; Albright *et al.*, 1993).

Algal species causing harm fall into five categories: diatoms, dictyophytes, dinoflagellates, haptophytes and raphidophytes. High biomass blooms of diatoms are generally considered to be non-toxigenic; only one genus *Pseudo-nitzschia*, includes the capacity for producing domoic acid (DA), the amnesiac shellfish toxin (AST) in about half of the species (Chapter 4). Diatoms have silica outer casings (frustules) and some species have spines or barbs (*Chaetoceros concavicornis*, *Chaetoceros convolutes*, *Corethron* spp) that irritate the fish gills causing bleeding of capillaries, dysfunction of gas exchange and suffocation by mucus overproduction (Bell, 1961; Rensel, 1993; Lassus *et al.*, 2016). Other chain-forming diatoms associated with mortality of aquacultured salmon in Chile and easter Canada are *Leptocylindrus danicus*, *Leptocylindrus minimus* and *Eucampia* spp. (Bates *et al.*, 2020).

Similar to spiny diatoms, some Dictyochophytes, known as silicoflagellates (for example, *Dictyocha fibulam*, *Octactis speculum*) also have silica endoskeletons that have also caused fish killing events (Bates *et al.*, 2020; McKenzie *et al.*, 2021). However naked dictyochophytes such as *Pseudochatonella verruculosa* and/or *Pseudochatonella farcimen* have also caused mass fish mortalities in Denmark, Japan, North America (Maryland, the United States of America and British Columbia, Canada), Norway and Sweden apparently due to the production of poorly characterized ichthyotoxic mechanisms. Several groups of marine microalgae contain members capable of producing haemolytic substances (haemolysins) that damage the epithelium of fish gills by destroying blood cells. These groups include the raphidophytes (for example, *Heterosigma akashiwo*, *Chattonella antigua*, *Chattonella marina*), prymnesiophytes (haptophytes) (for example, *Haptolina ericina*, *Haptolina hirta*, *Prymnesium parvum*, *Prymnesium polylepis*), and dinoflagellates (for example, *Karenia mikimotoi*) (Lindahl *et al.*, 1990; Park *et al.*, 2013; Lassus *et al.*, 2016; McKenzie *et al.*,2021). *Heterosigma* blooms occur often in Chile, Japan, New Zealand and the Pacific coast of Canada (Chang *et al.*, 1990; Honjo, 1993), whereas raphidophytes such as *Chattonella* in Japan and haptophytes in northern Europe and Canada are most often responsible for mass fish-killing events (Rosenberg *et al.*, 1988; Bates *et al.*, 2020).

Among dinoflagellates, the unarmoured species *Margalefidinium polykrikoides* (= *Cochlodinium polykridoides*) is often associated with aquaculture finfish mortalities world-wide, particularly in Canada, China, Japan and Korea (Park *et al.*, 2013; Whyte *et al.*, 2001; Lassus *et al.*, 2016; Bates *et al.*, 2020) and has also been associated with shellfish mortality in the Chesapeake Bay, United States of America (Griffith *et al.*, 2019). Certain populations of *Alexandrium monilatum* can produce the polyether macrolide toxin known as goniodomin A and are circumstantially linked to fish kills. *Alexandrium pseudogonyaulax* from northern Europe and A. taylorii from the Mediterranean Sea may also produce goniodomins but are not yet associated directly with fish killing events; these compounds are currently being investigated for their impact on fish and shellfish (Wolny *et al.*, 2020). The phagotrophic dinoflagellate *Noctiluca scintillans* forms high biomass blooms worldwide and, although nontoxic, has also been considered harmful to ecosystem functions and in a few cases mortalities in wild fish populations, either because of oxygen depletion or perhaps high release of ammonium.

Brown tides or blooms caused by the picoplankton *Aureococcus anophagefferens*, a pelagophyte, have caused disruption of ecosystems in shallow estuaries of the United States of America and South Africa for decades. They thrive in low light and low nutrient conditions and are well studied and modeled (Bricelj and Kuenster 1989; Gobler and Sunda, 2012). Toxins produced by this species were found to reduce the feeding rates of juvenile mussels and demonstrated the impacts of this bloom on commercial bivalve populations (Bricelj *et al.*, 2001).

Although not technically algae, blooms of the ciliate *Mesodinium rubrum* have been associated with fish mortalities likely due to oxygen depletion in the water column (Bates *et al.*, 2020; McKenzie *et al.*, 2021). Ciliates are not usually toxigenic but except for those retaining photosynthetic endosymbionts, dense blooms can create high biological oxygen demand. Most harmful events caused by ciliates are associated with hypoxia induced in high density blooms or following the senescence phase.

In Canada, a recent review of three decades of harmful algal events (McKenzie *et al.*, 2021) was conducted as part of a Harmful Algae global status report. Nine phycotoxin-producing algal species were known to have caused, or to have been associated with harmful algal events on the Atlantic and Pacific coasts of Canada. An extensive review of marine harmful algal blooms and phycotoxins of concern for Canada (Bates *et al.*, 2020) provides detailed information on this issue. Among the phycotoxin-producing HAB species known to form occasional high biomass blooms along coastal regions of Canada, most belong to dinoflagellate genera, such as *Alexandrium* with some species producing vegetative cells and cysts containing paralytic shellfish toxin (PST). The toxigenic marine dinoflagellate *Alexandrium catenella* (formerly *A. tamarense*) was the probable cause of mass mortality of caged salmon in Nova Scotia (Cembella *et al.*, 2002), but despite strong circumstantial evidence, it could not be proven that the mortalities were due the high cell paralytic shellfish toxin (PST) content and high cell densities. Although there have been some effects on finfish aquaculture and wild fish and marine mammals caused by high biomass blooms of phycotoxin-producing Harmful Algae (Cembella *et al.*, 2002; Starr *et al.*, 2017), most impacts on caged finfish have been from other species via mechanical damage, as yet unidentified ichthyotoxins, hypoxia due to high biomass or a combination of these factors.

In Oman, HAB events and their impacts on the coastal waters are regularly monitored and documented. Most fish-killing HAB incidents are caused by dinoflagellates such as *Noctiluca scintillans*, *Margalefidinium polykrikoides*, *Tripos* (previously *Ceratium tripos*) acount for about 80 percent of these occurrences (Al-Hashmi *et al.*, 2014) dominated by *Noctiluca scintillans* (Al-Azri *et al.*, 2012; Al-Gheilani *et al.*, 2011; Harrison *et al.*, 2017; Thangaraja *et al.*, 2007). The blooms caused by *Noctiluca scintillans* have been observed in coastal waters of Oman almost annually. Although their species abundance varies (A. Al-Azri *et al.*, 2007), they are generally responsible for more than 50 percent of HABs in coastal waters of Oman (Al-Gheilani *et al.*, 2011; Harrison *et al.*, 2017).

The HABs reported along the coast of Oman often exhibit intense cell accumulation in surface waters causing water discolouration, unpleasant foam and odor, and resulting in fish mortality due to oxygen depletion during the bloom and following bloom decay. These bloom events disrupt coastal recreation and operation of desalination plants, as well as causing deterioration of coastal water quality and economic losses to fisheries (Al-Gheilani *et al.*, 2011; Sarma *et al.*, 2013). Other microalgal species have been reported to occur in high cell density without discolouring the surface waters such as species of the diatoms *Psudo-nitzschia* and *Chaetoceros*. Some potentially toxigenic species of harmful phytoplankton have been recorded on the Omani coasts, but except for the PSTs (saxitoxins: STXs), toxigenicity has not been verified in laboratory studies.

In 2001, blooms of several HABs species (including *Pseudo-nitzschia pungens*, *Prorocentrum arabianum* and others) caused widespread fish and green sea turtle (*Chelonia mydas*) mortalities in the southern region of Oman. About 284 tons of fish during March and April and 40 tons of fish along with 250 turtles, some dolphins and birds were killed during November and December of the same year. In addition, in 2005, there was a massive fish kill caused by HABs at Masirah Island, primarily comprising *Noctiluca scintillans*, *Prorocentrum micans* and the cyanobacterium *Trichodesmium erythraeum*. The bloom of *Margalefidinium polykrikoides* in 2008–2009 was one of the worst HABs incidents that occurred in the region. The bloom lasted for eight months and caused severe ecological and economic impacts on recreational activities and environmental resources. The HAB affected 1 200 km coastline of several countries, including Iran, Kuwait, Oman and the United Arab Emerites all of which are located around the Arabian Gulf and the Sea of Oman (Boerlage and Nada, 2015; Richlen *et al.*, 2010).

TABLE 5.1
Causative eukaryotic microalgal species and effects of high biomass and/or high-density blooms

Causative species[a]	Mechanism of harm	Effects on marine species and/or environment	Associated toxigenic factors	Impacts
Diatoms				
Cerotoneis closterium	Hypoxia	Foam, mucilage, water discolouration, odor Asphyxiation due to hypoxia		Coastal tourism: Maryland (USA)
Chaetoceros concavicornis, Chaetoceros convolutus Chaetoceros debilis, Chaetoceros peruvians Chaetoceros wighamii Corethron spp. *Eucampia* spp. *Leptocylindrus danicus, Leptocylindrus minimus*	Gill damage, (mechanical) 5 cells/ml mucus production	Farmed salmon bleeding of capillaries, dysfunction of gas exchange, suffocation		Farmed fish mortality: British Columbia (Canada), Chile Scotland (United Kingdom) France Scotland (United Kingdom) New Brunswick (Canada), Chile
Pseudo-nitzschia spp. *P. australis P. pungens*	Gill damage, Exposure to algal neurotoxins,	Lethal or sub-lethal impact on fish, marine mammals, seabirds, invertebrates Lethal impact on fish, dolphin and green turtle	AST (Domoic Acid; DA)	Widespread environmental and economic impact: North America (Pacific coast), Oman and surrounding areas
Dictyophytes				
Dictyocha fibula Octactis speculum	Gill damage Mechanical (silicate exoskeleton)	Gill damage, clogging gills, mucus production response and Suffocation		Farmed fish, benthic invertebrate mortality: British Columbia (Canada), Denmark, France,
Pseudochato-nella farcimen Pseudochato-nella verruculosa	Toxin production (no exoskeleton)	Wild and Farmed Fish mortalities	Ichthyotoxins	Fish mortality: British Columbia (Canada), Japan Maryland (USA), North Sea
Dinoflagellates				
Akashiwo sanguinea	Mechanical (Gill clogging, removal of feather protection in seabirds)	Fish kills, oyster mortalities, foam production, seabird mortalities	surfactant-like proteins	Fish mortality: Asia, North America, South America; oyster mortalities: British Columbia, (Canada), Puget Sound (USA); foam production, seabird mortalities: west coast of the USA
Alexandrium catenella,	Gill lesions, hypoxia, mucus production, membrane lysis Exposure to algal neurotoxins	Fish Kills (NA, SA) Morbidities and sub-lethal cumulative effects	Ichthyotoxicity (haemolytic activity) (SA) PSTs (Saxitoxins; STXs), recurring blooms, cyst reseeding blooms	Mass mortality of fish/ other marine organisms, feeding interruption: Chile, Quebec (Canada)
Alexandrium monilatum	Mechanical and toxins	Fish Kills, oyster mortality	Haemolytic products Goniodomines	Fish: Costa Rica, Florida (USA); Shellfish mortality: Massachusetts (USA) Skin irritation[b]

TABLE 5.1
Causative eukaryotic microalgal species and effects of high biomass and/or high-density blooms (cont.)

Causative species[a]	Mechanism of harm	Effects on marine Species and/or environment	Associated toxigenic factors	Impacts
Karenia brevis *Karenia mikimotoi*	Toxins Toxicity causing Gill and digestive membrane tissue damage, Mucus production, Oxygen depletion in fish, marine organisms	Fish kills, invertebrate, marine mammal mortality due to food chain accumulation	Brevitoxins, (aerosol) Ichthyotoxins Extracellular haemolytic substances Asphyxiation due to hypoxia Possible brevetoxins	Wild fish kills: Florida, North Carolina (USA); marine mammals, dolphins and manatees: Florida (USA); Breathing and health issue Florida (USA), New Zealand Farmed and wild fish mortality: China Hong Kong SAR, France, Japan, Korea, Norway; and wild fish, scallops, oysters, extensive mortalities of marine species: Egypt, France, Ireland, Japan, New Zealand Scandinavia Scotland Breathing and Health issue, skin irritation[b]: New Zealand
Karlodinium veneficum	Toxins Fish gill tissue	Lytic effect on membranes of marine fish and invertebrates	Ichthyotoxins, karlotoxins-broad lytic effect on membranes	Farmed fish mortality: Norway; wild fish mortality: Australia, Maryland (USA), North Carolina (USA), Texas (USA)
Margalefidinium polykrikoides	Toxins effecting gills and other tissue	Recurring blooms, cyst reseeding blooms	Ichthyotoxins	Finfish and shellfish, coral mortality; severe ecological and economic impacts Worldwide events e.g. California (USA), Japan, Korea, Maryland (USA), Massachusetts (USA), Oman, the Philippines, Virginia (USA) Impact on water quality, desalination plants and tourism[b].
Noctiluca scintillans	Oxygen depletion	Suffocation and decay, cascading ecosystem impacts	Asphyxiation due to hypoxia	Fish kills, discolouration, disruption of desalination plants[b], recreational and commercial fishing
Haptophytes				
Chrysochromulina leadbeateri *Haptolina ericina* *Haptolina hirta* *Prymnesium parvum* (brackish water) *Prymensium polylepis*	Gill damage Toxins and gill damage Toxins	Damage the epithelium of gill tissue/destruction of blood cells Mortality of fish, tadpoles and gill-breathing invertebrates. Suffocation and decay, cascading ecosystem impacts	Haemolysins Ichthyotoxins, haemolytic and cytotoxic compounds Haemolytic compounds	Farmed fish mortality: Norway Farmed fish mortality: British Columbia (Canada) Farmed and wild fish, benthic flora and fauna, ecosystem disruption: Denmark, Norway Farmed and wild fish, invertebrate mortality: Denmark, Norway, Sweden

TABLE 5.1

Causative eukaryotic microalgal species and effects of high biomass and/or high-density blooms (cont.)

Causative species[a]	Mechanism of harm	Effects on marine Species and/or environment	Associated toxigenic factors	Impacts
Raphidophytes				
Chattonella antigua, *Chattonella marina*	Ichthyotoxins	Damage the epithelium of gill tissue/destruction of blood cells Also water discolouration, foams	Ichthyotoxins including ROS (reactive oxygen species) Haemolysins and neurotoxins	Farmed fish mortalities: Japan Wild fish mortalities: Egypt Farmed fish mortalities: Norway Wild fish mortalities: Algeria, France, India Wild fish, invertebrates mortalities, octopus: Mexico
Heterosigma akashiwa	Gill clogging by mucus and gill damage by haemolytic substances	Damage the epithelium of gill tissue/destruction of blood cells	Ichthyotoxins Haemolysins	Farmed fish mortalities: British Colombia (Canada), Chile, France, Japan, New Zealand, Washington State (USA) Impacts on feeding management

[a] Representative common species not an exhaustive list (see Lassus et al., 2016 for additional species)
[b] Human health impact.
Source: Elaborated by the authors.

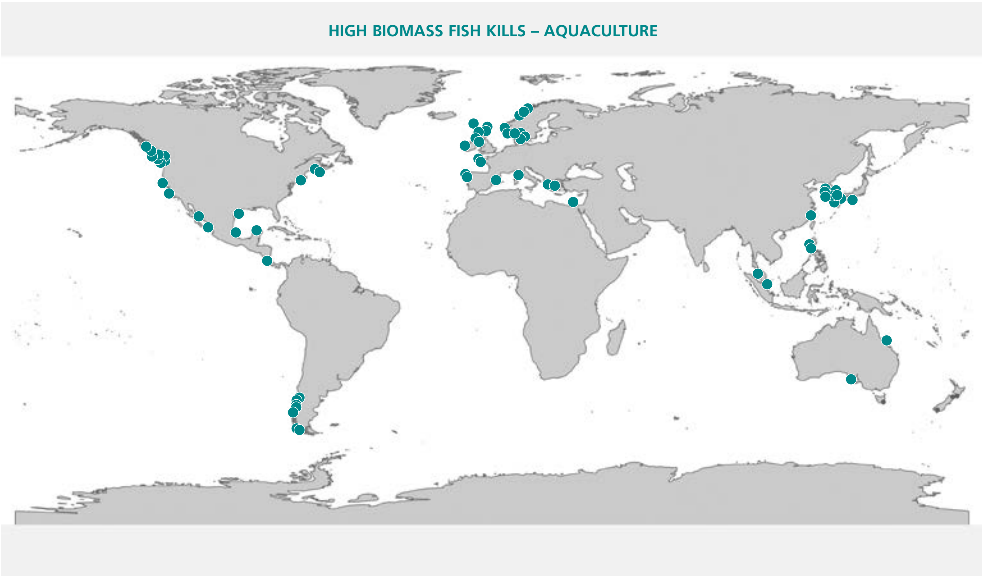
5.2 HARMFUL ALGAL BLOOM DYNAMICS AND FORCING FACTORS

Several emerging anthropogenic pressures are impacting the natural biological, chemical and physical drivers of HABs including climate change, ocean acidification, nutrient input, coastal development and the vectors for species transport (McKenzie *et al.*, 2021). Case studies of three extreme weather events resulting in three extreme HABs with catastrophic impact on the United States and Canadian Pacific Northwest, Australia and Chile (Trainer *et al.*, 2020) led the authors to caution that such events could be an indicator of future effects of climate change in these areas. Coastal ecosystems are particularly vulnerable to change in HAB distribution in response to climate change (Glibert *et al.*, 2014). Although there are no comprehensive reviews specific to high biomass bloom responses to climate change, the dynamics and forcing factors of pelagic diatoms and dinoflagellates are discussed in detail in Chapter 4, Section 4.3.

5.3 OBSERVATIONS OF HARMFUL ALGAL BLOOM FISH KILLING SPECIES AND HIGH BIOMASS EVENTS

Two global maps indicating reported HAB mass mortality fish killing events were created using the IOC -UNESCO Harmful Algae Information System (HAIS)) (https://data.hais.ioc-unesco.org). This system is based on ICES IOC HAEDAT information provided voluntarily by countries around the globe on HAB events and may not be complete. The first map indicates the global events of mass mortalities of fish associated with aquaculture (Figure 5.1). These events could be caused by any of the mechanisms discussed above due to exposure from organism indicated in Table 5.1. The diatoms *Chaetoceros convolutus* and *C. concavicornis* have been reported to cause fish kills (caged lingcod and salmon) on the Pacific coast of the United States and Canada since 1961 (Bell, 1961; Albright *et al.*, 1993). The mortality of aquaculture fish due to blooms of these diatom species in British Columbia (Canada) has been reported frequently but not annually from 1986 to 2017 (McKenzie *et al.*, 2021). In Scotland (United Kingdom), a *Chaetoceros* species and local flagellate blooms caused salmon mortalities and feeding issues at several fish farm locations (Treasurer *et al.*, 2003). In Chile, similar mortalities of farmed salmon and sea trout were recorded for *C. convolutus* (Clement and Lembeve 1993; Lassus *et al.*, 2016). In 1998, and 2003, mass mortalities of aquaculture fish in Passamaquoddy Bay (Bay of Fundy, New Brunswick, Canada) were linked to a bloom of the ciliate *Mesodinium rubrum*, which also caused water discolouration, hypoxia and anoxia (Martin *et al.*, 2007).

FIGURE 5.1
World distribution of reported mass mortality fish kill events associated with aquaculture sites; last updated in April 2022



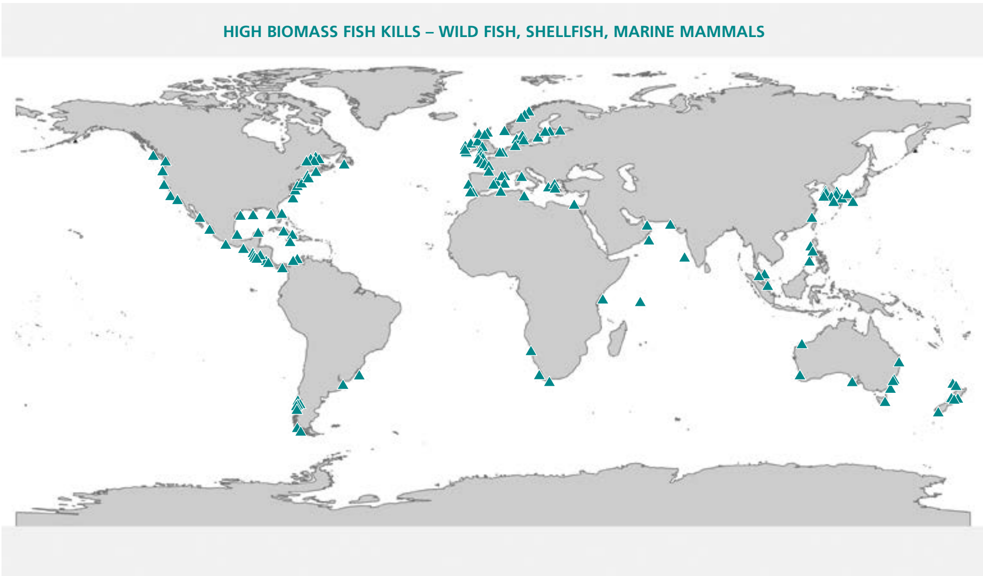
Source: Created by Fisheries and Oceans Canada (DFO) with data from IOC-UNESCO. 2023. Harmful Algae Information System. Harmful Algal Bloom (HAB) portal (ioc-unesco.org)

In Chile, the PST-producing dinoflagellate, *A. catenella*, caused a high impact bloom in the summer of 2002 with major losses for the salmon industry (Fuentes *et al.*, 2008). However, in the spring of 2009 an even larger bloom (6 000 cells x mL^{-1}) covered a geographical area from the Aysén region (46 °S) to the south Chiloé Archipelago (42 °S) (Mardones *et al.*, 2010) causing millions of dollars in losses to the aquaculture industry (Díaz *et al.*, 2019). Blooms of *A. catenella* caused mass mortalities of Atlantic salmon near Shelburne, Nova Scotia at finfish aquaculture sites in 2000 (cell densities >700 000 cells x L^{-1}) (Cembella *et al.*, 2002) and in the Bay of Fundy, New Brunswick (Canada) in 2003 and 2004 (Grand Manan Island 888 000 cells x L^{-1}; southwest coast, 3 million cells x L^{-1}) (Martin *et al.*, 2008). The Nova Scotia (Canada) bloom was detected after growers observed fish swimming near the surface, in the opposite direction to the school and acting lethargically. Deceased fish exhibited irritation around the gills and low levels of PSTs were detected in gill tissue (Cembella *et al.*, 2002). Salmon exposed to various concentrations of *A. catenella* cells in laboratory experiments determined that exposure to 100 000 cells x L^{-1} or fewer had no lethal effect on salmon (Burridge *et al.*, 2010).

The raphidophyte *Heterosigma akashiwo* has been responsible for the mortality of aquaculture fish in the Pacific region of Canada since the 1970s (Gaines and Taylor, 1986; Black *et al.*, 1991; Haigh and Esenkulova, 2014). A bloom of *H. akashiwo* on the upper west coast of Vancouver Island, in Kyuquot Sound and Nootka Sound in late August 1992 affected several aquaculture operations in the area, resulting in the mortality of ~250 000 kg of salmon and over USD 1.5 million in economic losses. In June 2018, two salmon farms lost ~250 000 fish, nearly half of their product, because of a bloom of *H. akashiwo* (Bates *et al.*, 2020). In Washington State (United States of America), *H. akashiwo* blooms have been reported in 1989, 1990 and 1994 with associated farmed salmon mortalities (Horner *et al.*, 1997). In Japan, frequent *H. akashiwo* blooms in the Seto Inland Sea were clearly linked to eutrophication (Honjo, 1993). In 2002, another ichthyotoxic raphidophyte, a species of the genus *Chattonella*, was responsible for the mortality of almost 1 000 metric tons of farmed Atlantic salmon in Esperanza Inlet, on the upper west coast of Vancouver Island (Bates *et al.*, 2020). In March 2001, *Chattonella marina* was responsible for a bloom in Norway that led to 1 100 metric tons of destroyed farmed salmon (Lassus *et al.*, 2016). Ichthyotoxic dictyochophytes (*Octactis speculum*, *Dictyocha fibula*, and *Pseudochattonella verruculosa*) have also been linked to mortalities in British Columbia (Canada) salmon aquaculture facilities (Haigh *et al.*, 2014; Haigh *et al.*, 2018). A *P.* cf. *verruculosa* bloom in Chile in 2016 caused the death of 39 942 metric tons of fish or about 27 million fish (Clément *et al.*, 2016). Extreme climate conditions were considered to be contributing factors for this bloom (Garreaud 2018; León-Muñoz *et al.*, 2018).

The second global map created from the IOC HAIS indicates the reported mass mortalities or fish kills in wild fish, shellfish or marine mammals (Figure 5.2).These events are often broader in scope and impact the ecosystem and human activities. These events include those fish kills discussed earlier (Section 5.1) in Oman, Canada, the United States, South Africa, Chile, Japan and several other global locations due to the causative species listed in Table 5.1.

FIGURE 5.2
World distribution of reported mass mortality fish kill events impacting wild fish, shellfish and marine mammals; last updated in April 2022



Source: Created by Fisheries and Oceans Canada (DFO) with data from IOC-UNESCO. 2023. Harmful Algae Information System. Harmful Algal Bloom (HAB) portal (ioc-unesco.org)

5.4 DATA CONSIDERATIONS

Developing an EWS for fish killing HABs events requires access to data on the timing, duration, movement, spatial extent of the bloom and on associated risks to fish and shellfish.

Understanding the characteristics and behaviour of targeted HAB species and limitations of sampling programs and platforms are important considerations when designing an early warning system (see Chapter 2). In general, however, data needs to include:

- Taxonomic identification of harmful algal species;
- Quantitative measurements of HAB cell abundance;
- Quantitative measurements of ichthyotoxins and dissolved oxygen associated with blooms of these algal species or an understanding of how mechanical damage (from spines, mucous) causes mortality;
- Guidance on alert levels for cell abundance, toxins, dissolved oxygen, or potential for mechanical damage;
- Information on the timing, duration, and geographic scope of a HAB event; and
- Ancillary data on oceanographic and atmospheric conditions.

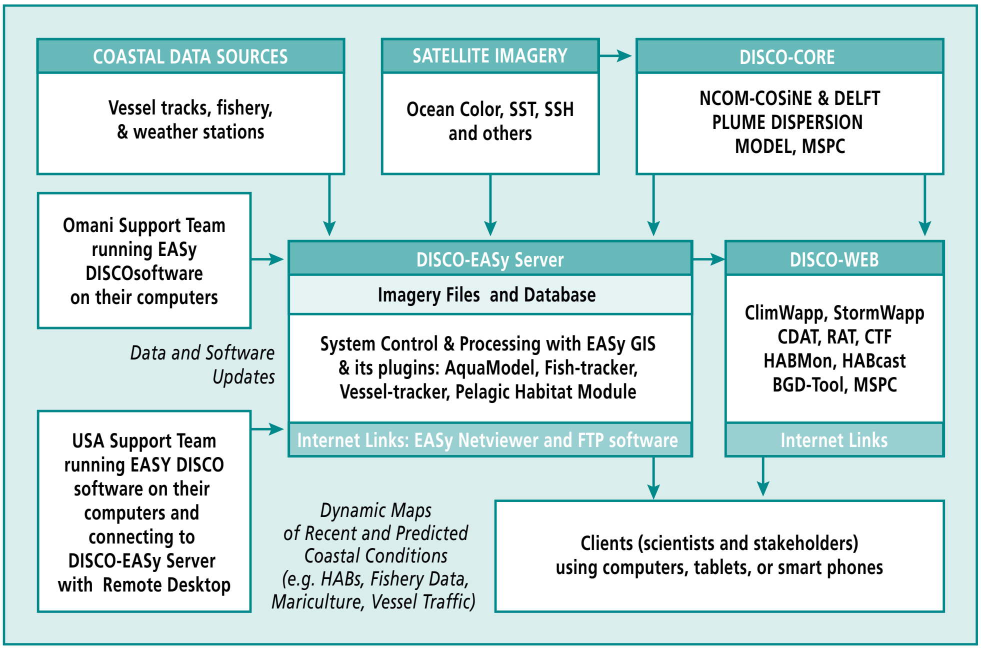
The Imaging Flow Cytobot (IFCB) is a submersible optical cytometer with acceptable image capture resolution for identification of many HAB species (>5 µm). Such systems are currently deployed on shellfish farms in Massachusetts (United States of America) to provide early warning of *Margalefidinium* blooms. The IFCB generates images of individual phytoplankton cells at a rate of up to 12 per second. Image recognition algorithms identify and count the species. The IFCB sends alerts when critical thresholds are reached for particular harmful species. This allows farms to turn off incoming water flows to nursery tanks, switch to recirculating mode, and oxygenate the water to sustain the shellfish until the bloom threat has passed. A region-scale network of IFCBs and other sensors called the HAB Observing Network–New England (HABON-NE) is being piloted. Data are fed to a central web portal, the WHOI HAB Hub (https://habhub.whoi.edu/), which presents HAB data within interactive maps and plots for farmers, resource managers and other stakeholders. This imaging technology allows early warning for possible mitigation activities on oyster farms, not for public health (Chapter 4), but rather for the health and protection of the oysters themselves from HAB impacts. Examples of monitoring for harmful algal species, abundance and phycotoxins are considered in Chapter 4. Several fish farms have developed and organized their own harmful algal and environmental data collection and monitoring programs for early detection and response (for example, Canada, Chile, Norway).

An example of the use of these data considerations is provided by the EWS case study of the Sultanate of Oman in Section 5.7.1 in which the EWS called Decision and Information System for the Coastal waters of Oman (DISCO) is deployed. The details of the system are outlined in this data section as an indication of how available data can be integrated to form an effective EWS.

DISCO was designed primarily as a tool for forecasting recurrent outbreaks of *Noctiluca* blooms. However, it can be adapted for any other bloom-forming species if sufficient historical and eco-physiological information is available. DISCO can be adapted for any other region of the world where HAB outbreaks are a problem, the environmental drivers of the HABs are well known and field monitoring is in place for validation.

The architecture of DISCO operational system, Figure 5.3 shows the transformation and spatial-temporal integration of four categories of coastal environmental information and human and natural infrastructure that can be integrated with dynamic maps of past and current coastal conditions as well as seven-day forecasts that include threats of HABs. These maps and supplemental information are then provided to stakeholders via a file transfer program (FTP) that can be programmed to fetch the data at any time of the day preferably during low internet traffic hours.

FIGURE 5.3
Schematic of the DISCO Operational Monitoring and Forecasting System showing components and data flow



Source: Elaborated by Dale Kelfer, Department of Biological Sciences, University of Southern California.

Environmental data relevant for forecasting HABs and their impact comes from:

- coastal data sources such as historical scientific surveys of water quality, currents, fisheries, and planktonic community;
- a sophisticated three-dimensional, biogeochemical model of circulation and plankton dynamics for coastal and offshore waters, which provides time-series data of ocean conditions as well as seven-day forecasts of algal blooms;
- satellite imagery of coastal conditions that includes sea surface measurements of water temperature, chlorophyll a concentration and associated bio-optical variables, topography, and wind speed and stress.

Field survey and satellite imagery provide information to test the validity of the biogeochemical model. Taken together, this information helps to determine the vulnerability of these coastal resources to HABs. Such model-derived time series simulations are particularly important to improve knowledge of the causes of HABs and the threats of these blooms to coastal infrastructure and ecosystem services including operations of fish farms, fishing grounds, water treatment plants, harbours and shellfish beds.

In Chile, the laboratory Plancton Andino is unique in that it identifies the HAB_f INDEX and not the algae total abundance. HAB_f INDEX is a metric for average water column ratios accounting for all Harmful Algae species and their weighted factor of risk to fish. The HAB_f INDEX has proven to be of great importance to salmon farmers, who can easily evaluate the risk of their fish-farm using an online applicaction (app plankton andino). Additionally, the HAB_f INDEX allows for comparison between monitoring programs (Clément *et al.*, 2020). Classical machine learning (ML) development has focused on new and more advanced algorithms that can analyze datasets in Excel. Each iteration contributes to the improvement of the algorithm architecture and hyperparameter data, commonly known as the "model centric approach". Furthermore, innovative ML methods include the new "data centric approach" which focuses on improving the data rather than solely focusing on the importance of the model. At Plancton Andino, their enhanced knowledge of the problem provides the ability to focus on data improvement. Currently a model is being developed that uses water data from fish farms and Programa Oceanografico y Ambiental en Salmonidos (Oceanographic and Environmental Program for Salmonids, POAS) monitoring programs. This model takes into account variables such as solar radiation in the visible spectrum of the phytoplankton maximum absorption, sea surface temperature from *in situ* sensors, weather data from meteorological models, and finally, phytoplankton ecological information including species richness and abundancy, and functional groups.

5.5 NOWCAST, FORECAST AND MODELING

Once the data considerations are reviewed and access to the required data is in place, modeling for nowcasting and forecasting can proceed. Harmful algal blooms their causes, and the species that comprise them have been studied and modelled for nowcasting and forecasting (Gilibrand *et al.*, 2016; Flynn and McGillicuddy, 2018; Glibert and Burkholder, 2018). Continuing with the DISCO example from Oman, the modeling portion of the EWS is considered here. The key processing components of DISCO are the biogeochemical model that is a three-dimensional, data assimilation and simulation model of circulation and nutrient/plankton dynamics (Figure 5.3). The model runs on a remote, super-computer that streams output to the operational system. If the biogeochemical model were to provide accurate maps of current and forecast HAB distribution and intensity, there would have been little need to include supporting information from satellites and field surveys in the system. Unfortunately, in most regions forecasting models of such accuracy are not available, or even when available, they need a robust dataset of *in situ* data to continuously test the validity of the outputs and improve model performance.

The DISCO biogeochemical model is an atmosphere–ocean, biogeochemical simulation model. It is essentially a regional and local down-scaling of the operational global Navy Coastal Ocean Model (NCOM, ocean circulation), coupled to the CarbOn Silicate Nitrate Ecosystem (COSiNE) plankton model (deRada, 2009; and references therein). The model is forced by atmospheric fields (wind, heat and water fluxes), and boundary conditions prescribed by NOAA global atmospheric models that are tuned using all available data from Global Meteorological Data ground observation.

This regional biogeochemical model is configured as a threee-nest grid system: 1/12° Global > 1/25° Regional (Arabian Sea) >1/50° Coastal (Oman). The 1/12° "Oman" grid equates to an effective resolution of about ~11 km covering the entire coastal waters of Oman and is eddy-resolving. Enhanced capabilities within the model include the ability to configure and run high-fidelity metre-scale resolution grids using DISCO Environmental Analysis System–GIS (EASy–GIS). This typical nested modeling approach allows smooth downscaling of lower-resolution models to produce accurate 5-day to 7-day simulated forecasts at scales effective for coastal decision making. The model generates the primary physical constituents for the air/sea boundary layer and sea-state, as well as three-dimension ocean physical and biogeochemical properties at hourly frequencies. These variables are stored in a data-archive at the Naval Research Laboratory and then transferred each day to DISCO–EASy–GIS. The coupled physical-biogeochemical model simulates sea level, temperature, salinity, and currents, and the flow of carbon, nitrogen, phosphate, silicate, and oxygen through the planktonic community, which consists of two size categories of phytoplankton and zooplankton. A subroutine to describe and incorporate the ecological and physiological dynamics of *Noctiluca* is currently being built (Huang *et al.*, 2015; Gomes *et al.*, 2014). The final version of this subroutine will be complex since *Noctiluca* is a mixotroph that feeds phagotrophically at the sea surface during the winter and photosynthetically using its endosymbionts within the seasonal thermocline during the summer. Furthermore, the cells can float and swim and thus are capable of vertical migration. The vertical and horizontal distribution is linked to the depth of the hypoxic layer as well as cross-shelf transport by eddies. The outputs of the model are checked for quality, and then cataloged and stored. Once the required data, which includes observations for data-assimilation and forcing fluxes from global models, are ready, the model runs, take about one hour to complete, and the results are archived and then sent to DISCO–EASy–GIS via automated web FTP services and data-transfer agents.

DISCO's current satellite data of SST and Chlorophyll a are obtained from the Moderate Resolution Imaging Spectrometer-Aqua (MODIS–Aqua), the NOAA Suomi National Polar-orbiting Partnership (NPP) Visible Infrared Imaging Radiometer Suite (VIIRS) and the new VIIRS-20 sensor. All are processed at their highest resolution at Naval Research Laboratory into end-user-ready geo-corrected and geo-referenced Level-3 and Level-4 maps specifically for Oman. These data are available to end-users in Oman directly via automated transfer or via DISCO–EASy–GIS. Work is currently being done to develop ocean colour algorithms specifically to detect *Noctiluca* blooms. The algorithms are awaiting further testing before they are cleared for operational use. In addition to these datasets, the GIS is set up to automatically access, grab and transfer via FTP, other satellite datasets from National Aeronautics and Space Administration (NASA), National Oceanic and Atmospheric Administration (NOAA) and European Space Agency (ESA) data portals – specifically altimeter measurements of sea surface height. At present the DISCO archive of satellite imagery exceeds one terabyte and contains times series that date to 2000.

The control and analysis centre of the operational system is the Environmental Analysis System–GIS (EASy–GIS) platform (Tsontos and Kiefer, 2002, 2003) (Figure 5.3). As shown in Figure 5.3, the four categories of streaming and static data are imported or streamed into the marine geographic information system (GIS) that stores this information, displays it in a four-dimensional (latitude, longitude, depth and time) geodetic framework, provides many tools for processing and analysis, and supports dissemination over the internet. EASy–GIS was developed by System Science Applications, Inc. with research support from NASA, NOAA, FAO, National Science Foundation (NSF), United States of America Department of Agriculture, and the European Union. One of the early applications of this software was in 1997 when a small team of marine scientists assembled by FAO and the European Union built the Integrated Coastal Analysis and Monitoring System (ICAMS) that integrated satellite imagery, field surveys, and vector files of bathymetry and coastal infrastructure to address concerns about water quality at coastal sites in Ireland, Italy, Greece, and Egypt.

EASy–GIS runs on Windows personal computers (PCs) which are relatively easy to run and maintain since it draws on standard features of Microsoft Office applications. The ability to use this EWS on PCs is particularly important for countries and states where advanced computational resources are not available. EASy–GIS requires no custom preprocessing of source data so that imagery and vector files can be stored in their native format. When running on a PC, the GIS can be run in three modes: beginner, intermediate and advanced. The beginner mode supports viewing all imagery and analysis data in the created project, but without the ability to delete or destroy previously created project information. The intermediate mode adds the ability to add to or modify project data and settings. Finally, the advanced mode provides the access to sophisticated analysis and support capabilities that can enhance the ability to do research, as well as the full capability to build new projects or to modify existing projects. The GIS also includes a capability for developing custom analysis algorithms and models that can be incorporated into projects using the software's applications programming interface (API). When projects are completed, they can be displayed and used for created for generating imagery that can be disseminated over the internet and for statistical data analysis on local computers The EASy–GIS coupled with the Netviewer plug-in provides interactive access for multiple internet clients. Clients use standard browsers such as Chrome, Firefox, Internet Explorer, or asdf on iPads or iPhones. It also provides access to project defined imagery, source data, and analysis results. Figure 5.4 shows a schematic of the components of the project's GIS, each of which is described below.

In Figure 5.4, five categories of input data files are stored, visualized, and processed by EASy–GIS:

- **Imagery** files from satellites and output from the biogeochemical model. These are the main sources of streaming information needed to monitor and predict the distribution of HABs. The GIS provides an interface to schedule and automatically download new satellite and model imagery from Internet portals.
- **Vector** and **ARC Shape** files of bathymetry, coastline, and human and natural infrastructure. Bathymetric topography and shoreline shape contribute to coastal flow and associated transport of HABs. The location of coastal resources is of course a key determinant of vulnerability to HABs.
- **Sample Data** files are gridded numerical information from field surveys on water quality and related biogeochemical variables. Water sampling and subsequent microscopic counts by eye or camera, gene isolation and sequencing, HPLC pigment measurement, or toxin detection are in most cases in many cases the only means to identify HAB species. This information is stored in the GIS's relational database. Gridded data from spreadsheets, CSV files, and relational databases are imported manually into the GIS using EASy's database wizard. Examples of this type of data include not only measurements from water samples but information from sensors in the field such as CTDs, current meters, optical instruments or electronic tags.
- **Analysis** files, which are shown at the bottom of the figure, store the results of processing and analyses. Examples of these results include images of seven-day forecasts calculated with the biogeochemical model, matching in space and time gridded data from field surveys with imagery to build habitat models of fish and to characterize the environmental conditions leading to HABs. The particle tracking interface of the GIS is used to trace the movement of water carrying algal blooms, the movement of water at intake and discharge lines of water treatment plants, and the movement of water at eddies and fronts. All input and results files reside on either the PC's internal or external hard drives or its local area network.

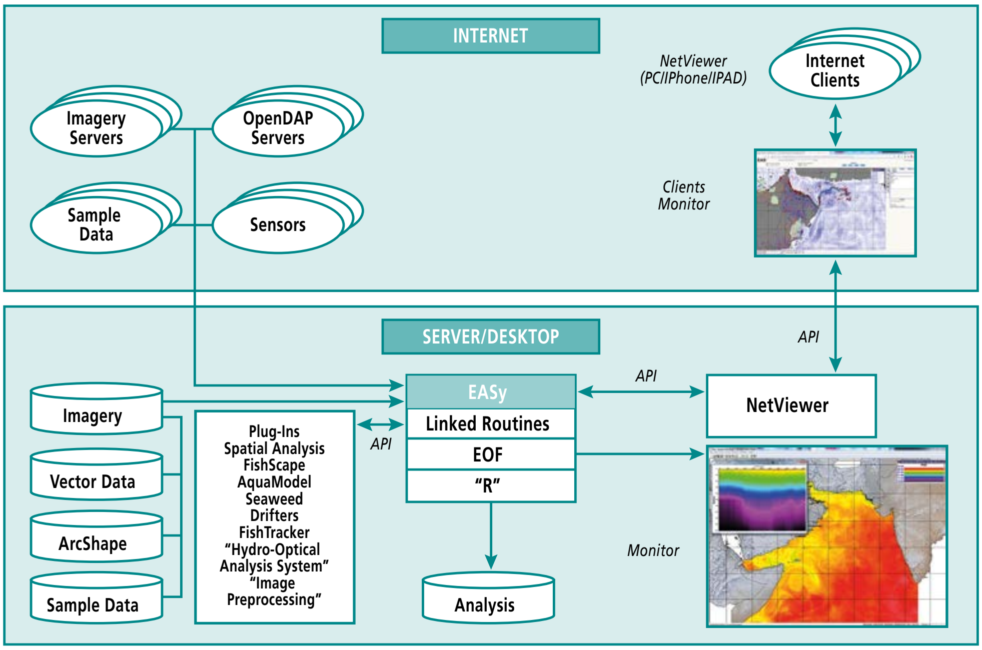
As shown in the top left corner of the figure, data flow into the GIS project is of course available over the internet. Four categories are listed as examples:

- **Imagery Servers** provide satellite data and output from models including of course the DISCO biogeochemical model. As stated previously, the GIS provides an interface to schedule and automatically download. Imagery is stored in their original format and when called for display are translated into the temporal and spatial co-ordinate system of the GIS. EASy handles roughly 100 different imagery formats.
- **Sample Data** are generally available as CSV or NetCDF files and transferred to the project's ACCESS relational database. This transfer is manual, but during the initial transfer with the Wizard, one can record and save the process, allowing automated transfer subsequently.
- **OpenDAP**, which has become a popular tool of portal-to-portal transfers. The data is stored locally rather than over the Internet because the project runs faster, and external hard drives are inexpensive.
- **Sensors** can be connected to EASy–GIS through a simple interface so that the data stream can be stored, processed, and integrated into the project.

The GIS's tools for processing the imagery and data discussed in the previous paragraph, consist of the customary capabilities of EASy–GIS such as contouring gridded data, sampling pixel values of image variables, measuring distances between locations, drilling three-dimensional fields to obtain vertical profiles of a, drawing transect line through a three-dimensional field to provide a colour coded curtain plot of a variable's value with depth along the transect, and measuring the area and mean pixel value of a selected region. It also includes custom modules of greater complexity.

In addition, the processing and analysis tools in the EASy–GIS include "linked routines" which allows for automated file exchange between EASy–GIS and external R- statistical software packages. One that is used routinely is the R-EOF (empirical orthogonal function) routine that is used to extract information on climate events such as the ENSO and Pacific Decal Oscillation from satellite imagery.

FIGURE 5.4
A schematic of the components and processing of the DISCO GIS



Source: Elaborated by Dale Kelfer, Department of Biological Sciences, University of Southern California.

The processing and analysis tools also include custom modules that run within the GIS. These modules are listed in the box labeled "Plug-Ins":

- **Spatial Analysis** is a search and processing routine that matches in space and time gridded measurements from field surveys with pixel values found in satellite imagery. This matching yields a matrix that is used as a look-up table or data for models of fishery habitat, but it will also be used for example to determine environmental conditions prior and during *Noctiluca* blooms.
- **Fishscape** is a simulation of the population dynamics and purse seine fishery of tuna of the eastern tropical Pacific Ocean – developed to assess the impact of climate change on the fishery.
- **AquaModel** and **SeaweedModel** are simulation models of the operations and environmental impact assessment of fish farms (AquaModel) and seaweed farms, developed to improve management of these growing industries of food production that are at times threatened by eutrophication and by HABs.
- **Drifters** provide an interface to track horizontal water movement – virtual drifters are launched at specified locations and time steps – develop to track transport of HABs and pollutants as well as complex water motions such as vortices and divergence and convergent fronts.
- **FishTracker** processes archival and satellite pop-up tags by integrating

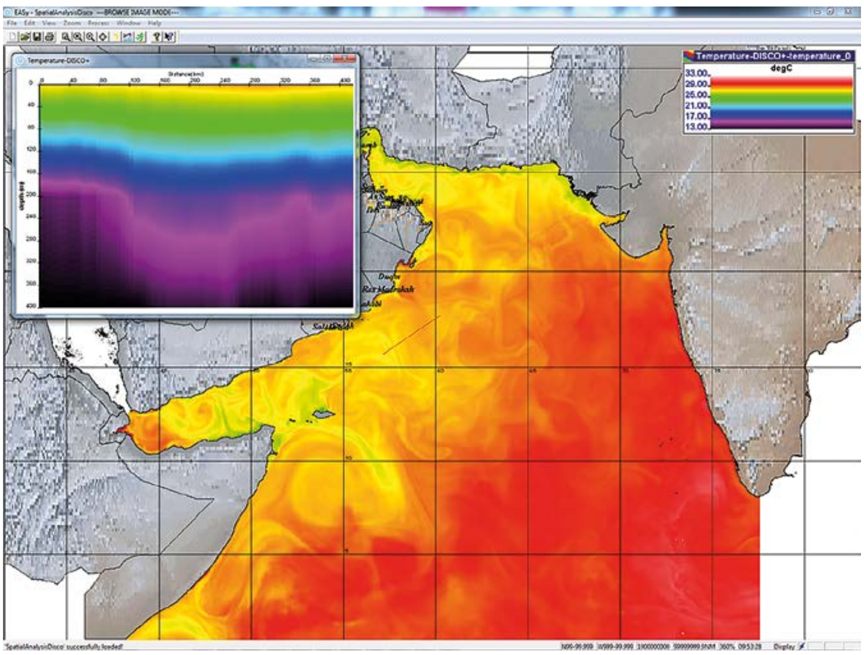
environmental data from the tags on pelagic species with satellite imagery to improve geolocation of tags that lack GPS.

- The **Hydro-Optical Analysis System** calculates concentration of water quality constituents by iteratively searching for the best fit between calculations of ocean colour with radiative transfer code with a bio-optical model of constituents and measurements from satellite ocean colour imagery. This system will be employed to help build a bio-optical algorithm for satellite mapping *Noctiluca* blooms in the Sea of Oman.
- **Image Pre-processing** provides an interface to transform images from satellites and biogeochemical models into images providing new information. These processed images are then imported into EASy–GIS for display and analysis. Examples of such transformations include mapping vorticity and divergence from images of current velocity, and mapping water density from images of temperature, salinity, and depth. In the case of DISCO this processing will support simulations of the depth distribution of *Noctiluca*.

The screen shot in the “SERVER/DESKTOP Frame” of Figure 5.4 is also found in Figure 5.5. The base map in Figure 5.5 is the sea surface temperature calculated from the DISCO biogeochemical model at a selected time step. The panel in the upper left of this panel is a curtain plot of the vertical distribution of temperature along the transect line found in the base map.

FIGURE 5.5

A screenshot of sea surface temperature simulated with the DISCO biogeochemical model and displayed in the projects GIS running on a laptop. The upper left panel is a curtain plot of the vertical distribution of temperature along the transect indicated by the red line in the base map



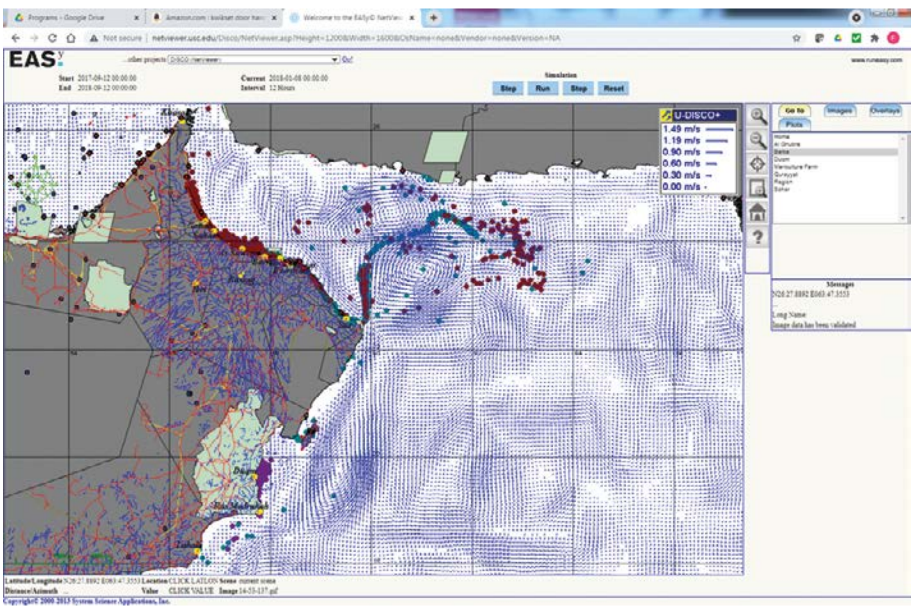
Source: Elaborated by Dale Keifer, Dept of Biological Sciences, University of Southern California.

As shown in the upper right corner of Figure 5.4, when EASy–GIS along with the Netviewer module are run on a Windows® server, stakeholders can view data in the project with an Internet browser from a PC, iPad, or iPhone. Viewing and analyzing the project's maps and plots over the web provides capabilities similar to those when the project is run on a PC. The stakeholder is free to view and analyze data and imagery that are mixed and matched in space and time and use the information for decision support. In addition, maps and plots can be called and viewed either sequentially in play/video mode or in browse mode in which data and images are selected for a given date. If desired, projects running with Netviewer can also be run with Google Earth or Google Maps.

The Netviewer screen shot in the "Internet Frame" of Figure 5.4 is also found below in Figure 5.6. The figure shows the flow field of surface water off Oman on 8 January 2018, as simulated by the DISCO biogeochemical model. The image also shows the position of surface drifters that were previously "launched" form several sites along the coast of Oman to track the movement of water and associated dissolved and suspended material from selected terrestrial sources. This tracking algorithm can be initiated within EASy–GIS for mapping bloom evolution and trajectories, both fundamental to an EWS EASy–GIS user interface allows that the client among other capabilities has freedom to select data, view it as maps or plots at selected levels of spatial resolution, and sample the three-dimensional field with vertical and horizontal profiles and curtain plots.

FIGURE 5.6

A screen shot for 8 January 2018 of surface currents and drifters launched from coastal sites as simulated by the DISCO biogeochemical model and as displayed in Chrome internet browser linking to the Netviewer client interface. Such simulations have supported monitoring and forecasting of HAB transport along the coast as well as the threat of oil spills and cyclones to coastal infrastructure



Source: Elaborated by Dale Keifer, Dept of Biological Sciences, University of Southern California.

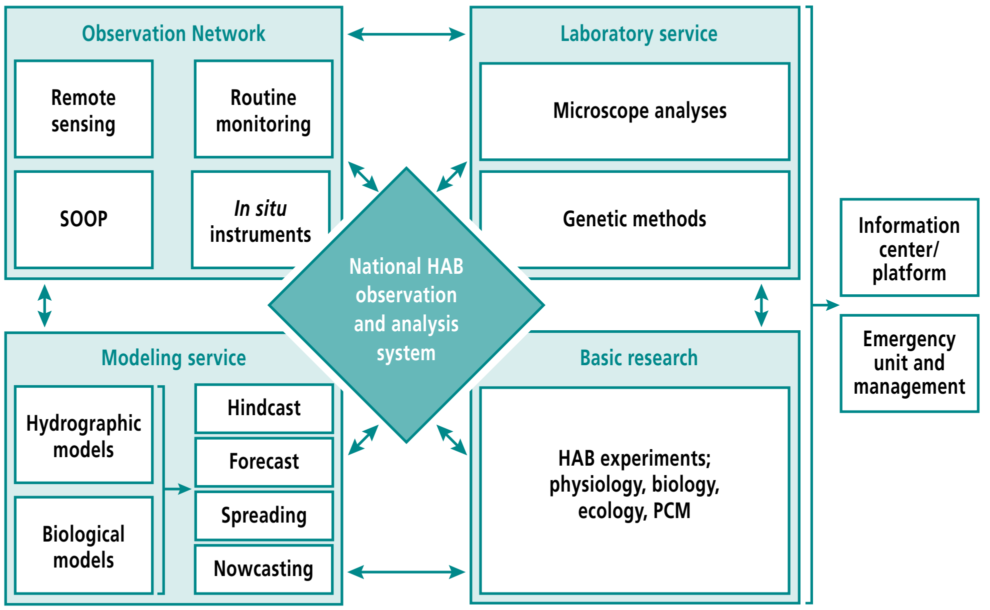
5.6 STAKEHOLDERS AND THEIR NEEDS

The stakeholder groups identified in Chapter 2, Figure 2.3 include several key participants in any EWS for high biomass fish killing blooms and their impacts. These stakeholders include the aquaculture industry, water quality industries including desalination plants, public health/safety and environmental regulators and society in general. Identification and engagement with stakeholder groups in advance of development of monitoring strategies is critical to successful implementation. Since most such programmes are decided on a national level the appropriate design is largely dependent on the financial model for the monitoring and the structure of the resource industry. For example, in Norway, the *HAB-mussel* monitoring programme focusing on toxigenic HAB species and their associated toxins in bivalve shellfish is government-administered and developed with input from research and monitoring authorities, with selective contributions from the shellfish industry. In contrast, in the emerging *HAB-Fish* program, the fish aquaculture industry, and associated insurance companies, are to a large extent the primary stakeholder. The high value and capital investment of the private industry has driven the monitoring agenda and finance priorities in consultation with government agencies and other stakeholders. In such circumstances, the role of state agencies is limited to developing general guidelines and negotiating and assuring compliance with national and international agreements and initiatives (for example, the European Water Framework Directive (WFD)) for environmental issues.

Although Norway has experienced sporadic fish killing HABs over several decades, the widespread high cell density *Chrysochromulina* bloom in 2019 in northern Norway caused huge, massive direct losses (USD >105 million) of aquaculture salmonids. This suddenly increased stakeholder and public awareness led to establishment of *HAB-Fish*, a program focusing on blooms causing fish mortality. Much of the advisory contribution to monitoring and EWS development is derived from historical data on species, presence, and events. In this geographical approach, the aim of retrospective data analysis has been to identify periods and locations of increased risk for the various HAB species/events. Recent efforts concentrate more on "coordinating" datasets on monitoring activities, to ensure as much synergy as professionally possible. The existing biological experience is then compared with the dominant water transport within or between fish aquaculture areas (currently 13 production areas for salmonids) for comprehensive assessment of the spread of HABs. Here, part of the challenge for coherent progamme design as shown in Figure 5.7 has been that the various bloom monitoring activities have been built up incrementally over decades, but with a shifting focus depending on different needs of industry, regulatory and health agencies and public stakeholders. At the moment, remote sensing data is not included in the reporting database. Data from remote sensing will be eventually available on the information portal, but as a separate data source (see preliminary webpage; https://algestatus.hi.no/).

In order for an EWS to be effective, actionable observational data for high cell concentrations should be delivered to in real-time or near real-time (2-day to 3-day notice at minimum). From the industry and site management point of view, broad spatial and temporal scales are less useful for decision making (Grasso *et al.*, 2019). The system should provide notifications, before blooms occur, likely time of arrival at locations where management and mitigation decisions such as feeding suspension and/or product harvest are required Growers, harvesters, and managers are making staffing, effort, harvesting, monitoring, business, and safety decisions at shorter periods, daily and weekly timescales and need information at site-specific levels.

FIGURE 5.7
Schematic overview of the monitoring and early warning system (EWS) under development for Norwegian waters, with focus on fish-killing harmful algal blooms (HAB-Fish). The new program is designed to include existing monitoring of other HAB events, hellfish toxicity (HAB-Mussel) and general high-biomass blooms and effects (HAB-high biomass) to form one large HAB EWS.



Source: Elaborated by Lars Naustvoll, Institute of Marine Research.

The early warning risk methodologies and alerts must provide regulators and industry, the health sector and society, with a product that enables:

- a reduction in the economic impact/losses through either information on feeding risk, finfish product harvesting delays and other mitigation efforts (see case study Section 5.7.2);
- a reduction in the environmental stress of lethal and sublethal high biomass blooms on fish, marine mammal and ecosystem food webs;
- the opportunity plan for impacts on desalination system (see case study Section 5.7.1);
- for public safety to avoid fishing, swimming and diving in affected area (see case study Section 5.7.1)

The regulator's priorities, resources and policies influence the scope of the EWS but must reflect the needs of the industry to be implemented and sustained. An effective EWS for high biomass fish kill bloom requires multidisciplinary and multi-agency governmental training, data collection, policy development and management effort in collabouration with members of the affected industries, regulators and the public. In Case Study 2 from Chile discussed below in Section 5.7.2, a schematic was developed to indicate the flow of the interaction with stakeholders and users when developing and utilizing the EWS.

Their simple strategy uses network monitoring stations to determine HABf INDEX. Methods include high frequency sampling in time and space, analysis of fresh samples (that is, no chemical fixatives) and a rapid response time with online results and computer tools to share information with stakeholders. Additionally, they can share online information with Business Intelligence via system connected servers, smartphone apps, and data visualization dashboards that support EWSs.

5.7 CASE STUDIES

Two case studies are provided to show how current EWS can be used in real world situations. Case study 1 is from Oman where annually recurring blooms of *M. polykrikoides* and *Noctiluca scintillans* led to the development of the DISCO system for that coastal environment, which has been described in previous sections. The second case study examines the HABf INDEX and ML used in Chile for early warning of HAB risk to finfish and shellfish aquaculture sites.

5.7.1 Early warning system – Oman

What is the problem caused by the harmful algal bloom?

Different coastal regions of the Oman vary in their susceptibility to HABs (Al-Gheilani *et al.*, 2011). Most events recorded are associated with high biomass impacts. The worst HAB incident that occurred in the region was the bloom of *Margalefidinium polykrikoideswhich* lasted for eight months (August 2008–April 2009) and caused severe ecological and economic impact on several recreations and marine environment resources. The appearance of this species in 2008–2009 was the first on record for the Omani coastal waters. The bloom of this species was rare in the region, however this HAB event was the major and longest-lasting ever recorded in the coastal waters of Oman (Al-Azri *et al.*, 2014). After 2009, there was no further record for *M. polykrikoides* bloom in the region. In 2018, high cell abundance of *M. polykrikoides* was reported in some areas along the Sea of Oman such as Muscat, Al Gubra, Barka and Sohar. Although no fish mortality was observed, these events disrupted the operations of some desalination plants located along the coast such as Al Ghubra desalination plant and Majis desalination plant. This HAB initiation may be due to the presence of cysts of this species, as a study conducted by Al-Kharusi (2020) showed the persistence of *M. polykrikoides* cysts at Sohar Industrial Port.

Besides *M. polykriokoides*, one of the most notorious HAB forming organism along Oman's coast is the species of the green mixotrophic dinoflagellate *Noctiluca scintillans* which occurs every year with almost predictable regularity during the winter monsoon (Goes *et al.*, 2018, 2020). Over the past 12 years, *Noctiluca* blooms have become more intense and widespread and are usually followed by swarms of salps and jellyfish, their only known predators. The increasing intensity of *Noctiluca* blooms, accompanied by the rise in instances of salp and jellyfish swarms and water quality deterioration issues along Oman's coast are now recognized as tangible threats to the health and vitality of Oman's coastal ecosystem. Among one of *Noctiluca* unique features is its capacity to accumulate large amounts of ammonium and lipids within its central cytoplasm, making individual cells highly buoyant and easily dispersible by currents and fine scale oceanographic features. Thus, satellite ocean colour data as well as other satellite oceanographic data such as sea surface temperature, sea surface winds, sea surface height are useful for studying *Noctiluca* blooms and their evolution in time and over space.

Who are the stakeholders and what were their needs?

These bloom incidents typically result in massive fish mortality of marine pelagic and benthic organisms, caused damage to coral reefs, loss of traditional fishery jobs, impact coastal tourism, force the closure of many desalination plants and refineries in the region. The bloom of *M. polykriokoides* in 2008–2009, forced the closure of several schools located near the coast because of the intense odors of the methyl sulfide compounds that was produced by the *M. polykrikoides* bloom (Al-Azri *et al.*, 2014; Al-Gheilani *et al.*, 2011; Berktay, 2011; Boerlage and Nada, 2015; Harrison *et al.*, 2017; Richlen *et al.*, 2010; Piontkovski *et al.*, 2011). This outbreak, in particular, raised the need to achieve an effective management of the HABs using new technologies of observation and monitoring in order to understand their causes, predict their occurrences, and mitigate their effects on public health, food safety and the environment, involving all the related stakeholders. Due to these blooms, there is new recognition by local resource managers and scientists that if these changes are not managed, their long-term consequences could be detrimental to local artisanal fisheries, fresh water supply, public health, tourism and the livelihoods of coastal communities who depend on the sea (Al-Azri *et al.*, 2014; Al-Hashmi *et al.*, 2014, Belwal *et al.*, 2015).

What was the development status of the country or region in terms of monitoring?

The Sultanate of Oman has attempted to address the problem of HABs at different levels through implementing regular monitoring programs at different coastal regions. Typical measurements include temperature, salinity, dissolved oxygen, inorganic nutrients, and numeration and identification of phytoplankton using compound and inverted microscopes. In addition, satellite images are utilized during HABs outbreaks. Following this incident, the Ministry of Agriculture, Fisheries Wealth and Water Resources have worked to build the capacity for an effective management of HABs by cooperating with different national and international agencies and institutions such as IAEA, IOC, FAO, Sultan Qaboos University (SQU), Ministry of Higher Education, Research and Innovation, and Environmental Authority. In addition, a reference laboratory for harmful algal blooms was established with cooperation with IAEA using nuclear technology (RBA) for detection of marine biotoxin.

What approach/technology was taken to solve the problem? Monitoring? What forecast data were used?

During *M. polykrikoides* blooms, all species of fish and shellfish killed were identified and their number recorded along with all available metadata including date of occurrence and location. Physical parameters were recorded using Hydrolab and an instrument that measures conductivity, temperature and density (CTD) at different depths and stations. Phytoplankton samples were collected and identified using light and microscopy scanning electron microscopy. In addition, to understand the mechanism of fish kill by *M. polykrikoides*, the histopathological alterations in normal and affected fish gills were studied using light and electron microscopy. For species toxicity evaluation, water samples were sent to the Universita degli Studi de Napoli Frederico II laboratory in Italy to test several toxic compounds. Fish were collected and analyzed for microbial flora, heavy metals and parasites. During *M. polykrikoides* blooms, drinking water samples were collected from different sites in the Oman and Arabian Seas and analyzed for organic and inorganic compounds.

Remote sensing technology and satellite images were used to observe and track the movement and spreading of the HABs event.

What early warning system was put in place?

The *M. polykrikoides* incident helped identify several stakeholders and initiate efforts to bridge communication gaps between these stakeholder and decision support managers via a national effective communication network. The Ministry of Agriculture, Fisheries Wealth and Water Resources coordinated with several governmental and academic institutions to prepare national emergency and rapid response plans. The purpose was to aid in resource loss mitigation, water quality management, as well as protecting aquaculture farms and desalination plants and other industries related to the coastal zone. Following a wide-spread, high-impact *Noctiluca* bloom, plans for an EWS for HABs for Oman were conceived. With funding support from NASA, plans for developing a Decision and Information System for the Coastal Waters of Oman (DISCO) came to fruition (see Figure 5.3 for a schematic of the system). DISCO was designed primarily as a tool for forecasting recurrent outbreaks of *Noctiluca* blooms.

However, it can be adapted for any other bloom forming organism if sufficient historical and eco-physiological information is available about the HAB forming organism. DISCO's operational framework takes into consideration several factors that are unique to Oman's coastal ecosystem:

- the Sea of Oman is a marginal sea, and the coastal waters of Oman are heavily influenced by monsoonal-driven circulation and biogeochemical processes in the adjoining Arabian Sea;
- dynamic circulation processes, including summer coastal upwelling, winter convective mixing and the seasonal formation of mesoscale eddies contribute to the shoaling of deep nutrient-rich, oxygen poor waters along Oman's coast making it a conducive environment for HAB outbreaks;
- unlike many other regions of the world, nutrient inputs from river discharge or run-off from land do not play a significant role in HAB formation;
- HABs first form offshore and outbreaks in coastal bays are largely the result of cross shelf transport processes; and
- the dispersal of HABs along the coast of Oman and eventually throughout the northern Arabian Sea is largely due to mesoscale eddies that form along the coast of Oman.

A detailed explanation of the operation of DISCO is provided in Section 5.4 Data considerations and Section 5.5 Nowcasting, forecasting and modeling.

What were the consequences of the early warning system for stakeholders?

The EWS that was implemented played an important role in enhancing HABs awareness among different sectors and developing action protocol to have a quick response during HABs incidents in order to mitigate socio-economic losses. For instance, desalination plants were considered one of the most important beneficiaries from the established EWS, as they were able to use new techniques to reduce the impact of the phenomenon on filtration systems. In addition, some desalination plants used satellite images to monitor the phenomenon. Also, they built effective collabouration with research and academic institutions to work together in finding technical solutions.

What lessons were learned?

The increasing intensity of HAB outbreaks that mostly affected coastal fisheries, aquaculture, desalination plants and other coastal industries which have a role in the country economy has raised the need to develop an effective EWS using advanced technology (for example, data assimilation and numerical modeling tools, mobile smart applications, and so on) for forecasting bloom.

The need to improve HAB monitoring and detection systems including systematic surveys all along the coast became evident, including continuous monitoring using fixed moorings and autonomous vehicles.

Building and strengthening partnerships ensured stakeholders' alignment and engagement, created stakeholders and public awareness and cooperation to achieve an effective national network and strategy for the management of HABs.

In DISCO, the effectiveness of satellite data was greatly enhanced with the help of outputs from a circulation-biogeochemical model. It provided forecasts of sea-state variables that can be used for mapping the trajectories of the bloom that is first visible in the satellite ocean colour data. However, routine handling, analyses and interpretation of model outputs or satellite data by coastal resource managers for effective decision making requires training of personnel to interpret the data. In general, even when adequate computational resources are available, handling large datasets provided by satellites and models and their interpretation can be challenging. To circumvent these and other problems, DISCO was deliberately built as a user-friendly, menu driven, windows-based GIS platform that can be used with a modest amount of funds and training.

5.7.2 Aquaculture, fish kills and harmful algal blooms in southern Chile

Description of the problem (what type of HABs)

Within the framework of the study and monitoring of HABs, laboratories in southern Chile have been dedicated to the identification and quantification of the different species of Harmful Algae within the ecosystem of fjords and channels in the inland sea, particularly Plancton Andino of Puerto Varas, Chile.

Aquaculture in the southern region of Chile is frequently affected by HABs that threaten fish behaviour and their associated tissues (Clément and Lembeye 1993; Mardones *et al.*, 2019; Diaz *et al.*, 2019; Clément *et al.*, 2021). The heterogeneity and complexity of algae blooms poses the need for the salmon industry to have a unified index that measures risk and can be used as a tool for EWS.

For several years, the aquaculture of southern Chile has developed monitoring programs that focus on the study of water column properties and algae identification and abundance (Figure 5.8). This approach is vital for the sustainable development of fish farming in this region; however, given its reactive nature, lacks the ability to anticipate future HABs events (proactive approach).

Who are the stakeholders and what were their needs?

- Aquaculture and fisheries industries: The most impacted groups are salmon farmers, feed and insurance companies, and certain suppliers especially during the flagellate blooms in autumn of 2016 and 2021.
- The Santiago Stock Exchange market: The Chilean salmon industry share value fell by nearly 10 percent per event of HAB by the end of the trading day on Tuesday, 1 March 2016 as recorded by salmon farmers. The most affected salmonid producer was AquaChile, with a decrease in 9.2 percent (USD 217.9); followed by Australis with -8.9 percent (USD 13.4); Camanchaca with -5.9 percent (USD 16.9); Invermar with -2 percent (USD 49); Blumar with -1.7 percent (USD 101.0); and finally, Multiexport with -1.5 percent (USD 90.6).
- Local government: Aquaculture and fisheries officials and public health agencies had to manage a strong social and political crisis.

What was the development status of the country or region in terms of monitoring?

Marine activities in Southern Chile have an adequate development status in terms of phytoplankton monitoring programs, with several organisations including public and private interacting at different levels. IFOP (Instituto de Fomento Pesquero) and CREAN (Centro de Estudios de Algas Nocivas) have an intensive phytoplankton and seafood toxin monitoring program in the south and along the austral coastline of Chile. Additionally, the National Fisheries Service manages the PSMB (Programa de Sanidad de Moluscos Bivalvos or Bivalve Molluscs Sanitary Program). In the private sector, INTESAL has conducted data reports for their members over a long period of time. Lastly, the POAS program and some fish farm companies have their own phytoplankton monitoring systems.

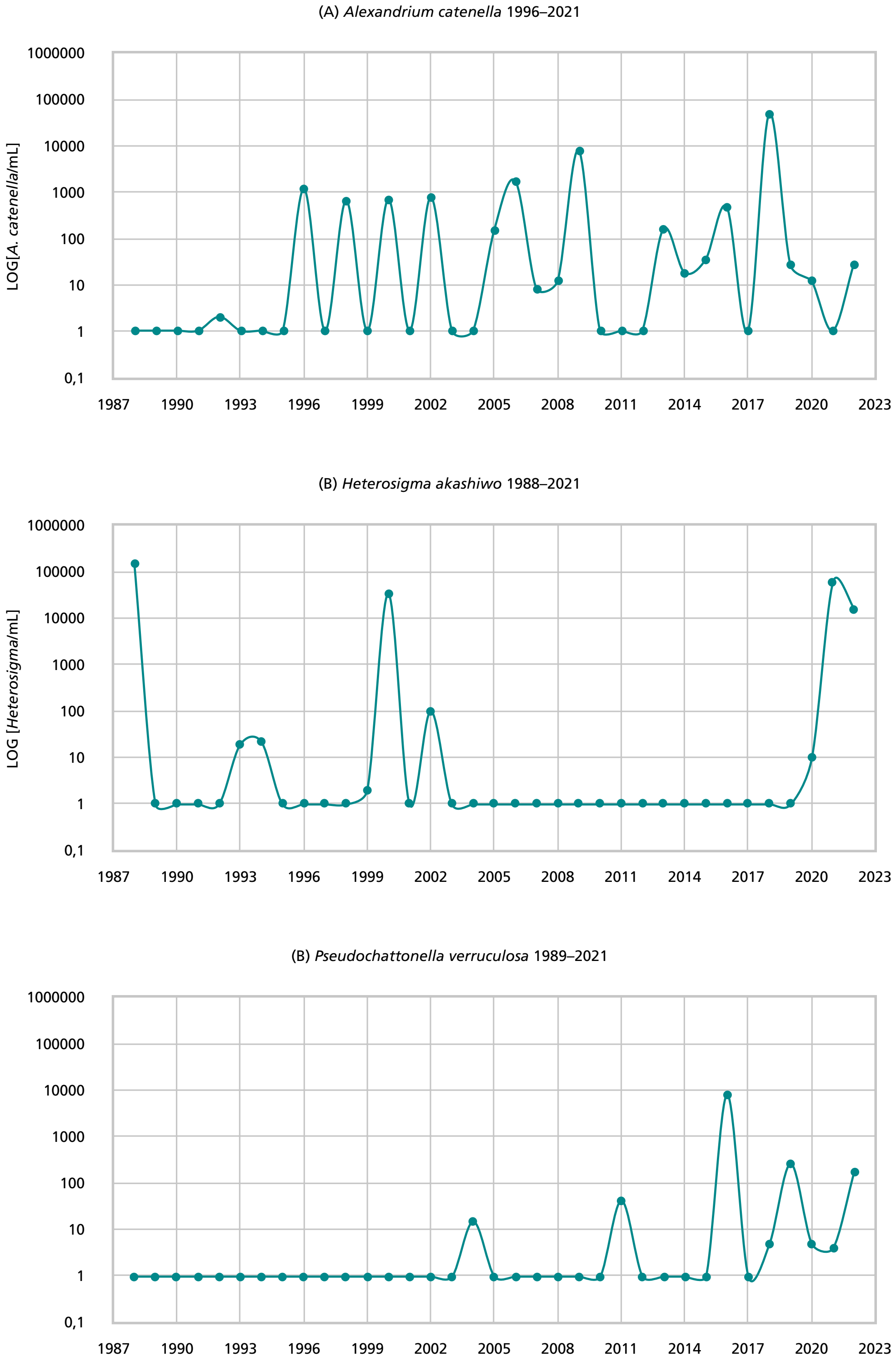
What approach/technology was taken to solve the problem? Monitoring? What forecast data were used?

One of, if not the most important, foci of the Chilean aquaculture industry is to forecast future HAB events before they occur. With the rise of new technology (for example, machine learning, ML), faster and less expensive access to technology, and the increase in data-driven decision-making companies – the possibility of forecasting HABs is closer than ever before.

With Plancton Andino's recognition of proactive monitoring as a necessity and the Data Specialist Department's recent development in ML models, future HAB events can now be forecast.

FIGURE 5.8

Temporal distribution of the Log year maximum abundance (cell/mL) of each HAB species in the inland sea and fjords of Southern Chile. (a) *Alexandrium catenella*, (b) *Heterosigma akashiwo* and (c) *Pseudochattonella verruculosa*



Source: Modified from Programa Oceanográfico y Ambiental en Salmónidos and Plancton Andino. 2023. https://plancton.cl

The approach to solving this problem includes a recent ML wave that differs from classical ML currents. Classical ML development has focused on new and more advanced algorithms that can analyze datasets in Excel®. Each iteration contributes to the improvement of the algorithm architecture and hyperparameter data, commonly known as the "model centric approach".

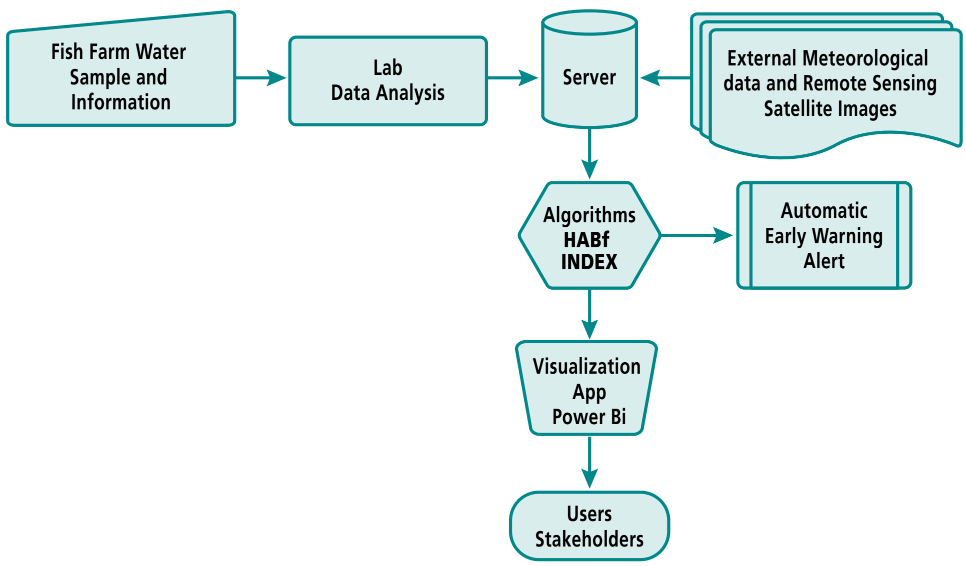
Furthermore, innovative ML methods include the new "data centric approach" which focuses on improving the data rather than solely focusing on the importance of the model. Currently, a model is being developed that uses water data from fish farms and POAS monitoring programs. This model takes into account different variables such as solar radiation in the visible spectrum of the phytoplankton maximum absorption, sea surface temperature from *in situ* sensors, weather data from meteorological models, and lastly, phytoplankton ecological information including species richness and abundancy, and functional groups.

Some salmon farm companies use mitigation techniques such as the upwelling system or use of microbubble barriers to dilute harmful cells concentrated in the surface layer (Anonymous, 2021). Some of these techniques have been tested in hydraulic channels but several assessments are required in the sea and sea-farms.

What early warning system was put in place?

In a joint effort with the main stakeholders in the salmon and Mytilidae industry, the POAS was created. This allows the generation of information for an EWS through the analysis of meteorological, oceanographic conditions and phytoplankton structure, among other variables, to support the decision-making process in the face of the risk of HABs (Figure 5.9).

FIGURE 5.9
Flow diagram of the interaction with stakeholders and users



Source: Modified from Plancton Andino. 2023. https://plancton.cl

Other projects consist of the use of new technologies and bio-optical sensors to be able to detect the variations of the fluorescence with different channels and the state of the phytoplankton biomass in the water. For this, in a project co-financed by CORFO (see the video in Youtube: www.youtube.com/watch?v=2l4-sincd6k), an IOT solution for the measurement of PAH; chlorophyll a, phycocyanin and turbidity with pollution-free sensors is proposed. The use of this type of solution allows a continuous time series, which generate a large amount of information, and allows the use of statistical models and time series analysis for a possible prediction model.

What were the results for forecast operation?

With the automated algorithm, the refined database and a correct visualization of the information regarding the HABf INDEX, the efforts are focused on generating predictive models in order to be able to warn in advance a possible Harmful Algae bloom.

Therefore, modern predictive ML algorithms are being tested. In a study still in development, statistical models of convolutional neural networks and assembly models such as Random Forest or Gradient Boosting Machine are being tested to predict possible increases in the HABf INDEX and thus be able to alert salmonid farming sites to take action before a HAB phenomenon occurs.

What were the consequences of the early warning system for stakeholders?

Our simple strategy uses network monitoring stations to determine HABf INDEX. Methods include high frequency sampling in time and space, analysis of fresh samples (i.e. no chemical fixatives), and a rapid response time with online results and computer tools to share information with stakeholders. Additionally, online information can be shared with Business Intelligence via system connected servers, smartphone apps, and data visualization dashboards that support EWS, for example, Table 5.2.

TABLE 5.2
Values of HABf INDEX for an EWS

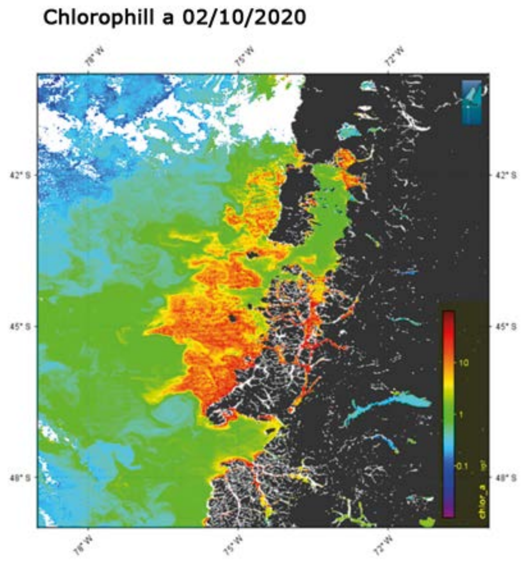
HABf index and early warning systems			
COD	**Range**	**Colour**	**Risk**
1	[0,00-1.00]	GREEN	NONE
2	[1,01-3.00]	YELLOW	LOW
3	[3,01-25.00]	ORANGE	MEDIUM
4	> 25,01	RED	HIGH

What lessons were learned?

Remote sensing imagery of ocean colour and sea surface temperature are important tools and techniques for studying and monitoring HABs, particularly from a large-scale point of view (Figure 5.10) (Amin *et al.*, 2009). However, spatial resolution and cloud coverage at mid latitude (42–52 °S) has restricted the quality of the images.

In addition, special algorithms considering bio-optical properties of local waters, and functional groups are required (Bracher *et al.*, 2017).

FIGURE 5.10
Example of a satellite image obtained from sensors like MODIS or VIIRS (Moderate Resolution Imaging Spectroradiometer and Visible Infrared Imaging Radiometer Suite, respectively) onboard satellites Aqua, Terra, Suomi-NPP and NOAA-20 from NASA OceanColor project



Source: NASA Official. 2023. Ocean Color Web. https://oceancolor.gsfc.nasa.gov

The application of online real time calibrated *in situ* bio-optical sensors, like chlorophyll a, phycobilin, turbidity, among others, are relevant data to understand short term phytoplankton variability and distribution. However, there is also a need to focus on *in situ* nutrient technologies, particularly for silicate, nitrate and phosphate detection. There is an interesting recent development of *in situ* marine technology of reagent less electrochemical detection systems (Legrand *et al.*, 2021) and Lab on Chip Chemical Sensor Technology (Mowlem *et al.*, 2021) for silicate measurements. The use of online real time silicate technology to determine driving variables for diatoms and flagellate distribution is proposed.

Furthermore, the Bio-Aqua Sensors (BAS) system can be used to measure phytoplankton bio-optical properties in fish farms and onboard well boats.

Mitigation Techniques: Mardones *et al.*, (2019) state ichthyotoxic flagellates are more toxic to fish gill cells upon rupture in laboratory conditions. Therefore, more scientific information is required to observe cell lyses in the sea, and during the application of mitigation techniques such as microbubbles, upwelling systems, among others.

Modelling: Our forecast model is in an internal testing stage and is aimed to be launched soon to stakeholders. This first development has taught us some valuable lessons. Because the model is based on the POAS monitoring program data, constant sample delivery is needed. Due to scarce samples taken in the winter and increased samples in the summer, samples are not taken consistently. A need has been identified for the development of a forecast program with involved stakeholders to assure constant delivery. More information will be learned after the model enters a deployed status.

5.8 SUMMARY

High biomass blooms that cause fish kills and other environmental impacts are the focus of this chapter. Examples of HAB species that cause high biomass blooms, as well as descriptive case studies and technological developments in early warning systems (EWS) to monitoring and mitigate their impacts are provided. In extreme cases, high biomass algal blooms can result in damage to aquaculture operations, causing fish and/or shellfish mortality or recruitment failures. The emphasis herein is on bloom causing fish kills, particularly of species in aquaculture, because of the sudden and dramatic socioeconomic effects on local communities. The high biomass blooms discussed in this chapter can have significant negative impacts on wild fish populations and cause marine faunal morbidity and mortality, including of seabirds and marine mammals, through the food webs or via a cascade of environmental stress and associated human impacts.

Although such HABs are often collectively referred to as "high biomass", high biomass *per se* or associated indices such as bloom chlorophyll content are generally poor predictors of harmful potential when considered alone. Most HAB monitoring programs are instead based upon cell enumeration of potentially harmful species, and thus typically report massive blooms in terms of cell densities over defined spatio-temporal scales. The typical spring bloom is often high biomass/high chlorophyll but without causing harmful effects. Massive blooms of virtually any algal species can conceivably cause environmental damage due to hypoxia under certain circumstances but such taxa and their untargeted effects should not simply be added to the list of HAB taxa for routine monitoring. This suggests that the bloom phenomena should be considered separately with respect to modelling of blooms and impacts and technological applications for monitoring.

The impacts of Harmful Algae specific on caged and wild finfish can occur via several mechanisms depending on the type of Harmful Algae causing the bloom. These mechanisms can include:

- respiratory dysfunction due to mechanical damage to gill epithelium caused by abrasive or spiny algal cells;
- direct toxic effect of exposure to known phycotoxins, such as neurotoxins or other defined ichthyotoxins;
- membrane disruption by induction of reactive oxygen species (ROS) and production of unusual polyunsaturated fatty acids (PUFA) causing oxidation of cell membranes; and
- disruption of osmoregulatory capacity caused by poorly known ichthyotoxins.

The environmental effects of high biomass blooms are integrated beyond the target species and may cause:

- allelochemical effects and responses in aquatic food webs;
- general local disruption of ecosystem function; and
- depletion of oxygen in the water column following the end of a bloom, leading to hypoxic conditions and mortalities.

In cases where a high biomass HAB causes only a non-lethal reduction of dissolved oxygen, this can result in secondary stress on farmed fish, impacting feeding activites and making them more vulnerable to disease. In addition to the direct mortalities, sublethal densities of Harmful Algae have led to mortalities due to bacterial or viral infections on weaker or vulnerable fish.

Developing an EWS for fish killing HABs events requires access to data on the timing, duration, movement, spatial extent of the bloom and on associated risks to fish and shellfish. Understanding the characteristics and behaviour of targeted HAB species and limitations of sampling programs and platforms are important considerations when designing an early warning system. In general, however, data needs to include:

- taxonomic identification of harmful algal species;
- quantitative measurements of HAB cell abundance;

- quantitative measurements of ichthyotoxins and dissolved oxygen associated with blooms of these algal species or an understanding of how mechanical damage (from spines, mucous) causes mortality;
- guidance on alert levels for cell abundance, toxins, dissolved oxygen, or potential for mechanical damage;
- information on the timing, duration, and geographic scope of a HAB event; and
- ancillary data on oceanographic and atmospheric conditions.

Harmful algal blooms their causes, and the species that comprise them have been studied and modelled for nowcasting and forecasting. An example is provided using the Decision and Information System for the Coastal Waters of Oman (DISCO) example from Oman. The key processing components of DISCO are the biogeochemical model that is a three-dimensional, data assimilation and simulation model of circulation and nutrient/plankton dynamics

Stakeholders for high biomass fish killing EWS include the aquaculture industry, water quality industries including desalination plants, public health/safety and environmental regulators and society in general. Identification and engagement with stakeholder groups in advance of development of monitoring strategies and EWS is critical to successful implementation. In Norway a suddenly increased stakeholder and public awareness of HAB related fish kills, led to establishment of *HAB-Fish*, a program focusing on blooms causing fish mortality. Much of the advisory contribution to monitoring and EWS development is derived from historical data on species, presence, and events. In this geographical approach, the aim of retrospective data analysis has been to identify periods and locations of increased risk for the various HAB species/events. In order for an EWS to be effective, actionable observational data for high cell concentrations should be delivered to in real-time or near real-time (2-day to 3-day notice at minimum). From the industry and site management point of view, broad spatial and temporal scales are less useful for decision making. The system should provide notifications, before blooms occur, likely time of arrival at locations where management and mitigation decisions such as feeding suspension and/or product harvest are required. Growers, harvesters, and managers are making staffing, effort, harvesting, monitoring, business, and safety decisions at shorter periods, daily and weekly timescales and need information at site-specific levels.

Two case studies are provided to show how current EWS can be used in real world situations. Case study 1 is from Oman where annually recurring blooms of *M. polykrikoides* and *Noctiluca scintillans* led to the development of the DISCO system for that coastal environment. A second case study examines the HABf INDEX used in Chile for early warning of HAB risk to finfish and shellfish aquaculture sites.

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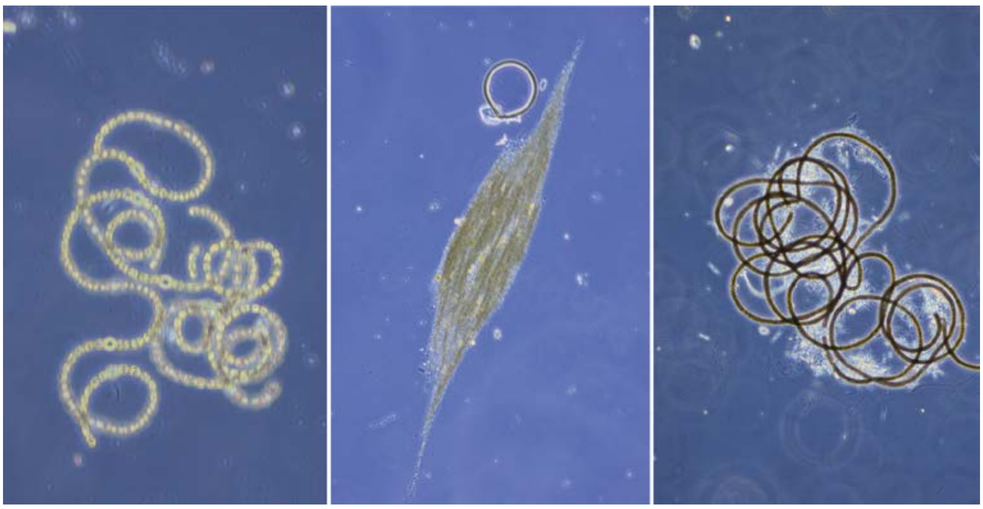
6 Early detection and early warning of harmful cyanobacteria blooms

This chapter will provide an overview of harmful cyanobacteria blooms, forcing factors, ecology and methods for early detection and early warning of these blooms. The scope of the chapter is limited to marine and brackish water. Cyanobacteria blooms in freshwater are sometimes referred to. A comprehensive book on toxic cyanobacteria in water (Chorus and Welker, 2021) is available open access.

Cyanobacteria are important primary producers in the sea. The term blue-green algae was formerly used but has been abandoned by most since cyanobacteria are prokaryotic organism while algae such as seaweeds (macroalgae) and microalgae, for example, diatoms, are eukaryotic. The eukaryotic organisms have their DNA organised in a nucleus. This is not the case for prokaryotes. It is worth noting that the term Harmful Algal Blooms (HABs) does include the cyanobacteria blooms. Planktonic marine and brackish water cyanobacteria span a size range from c. 0.7 µm to a few mm. The smallest are picoplankton such as *Prochlorococcus* and *Synechococcus* while the largest are colonies of filamentous genera such as *Trichodesmium* and *Aphanizomenon* that are visible to the naked eye. At present 5145 species of cyanobacteria are recognised in the database AlgaeBase (Guiry and Guiry, 2021).

FIGURE 6.1
Examples of cyanobacteria: Left. *Nodularia spumigena*, middle: *Aphanizomenon flosaquae* and right: *Dolichosperum* sp.



6.1 AN OVERVIEW OF HARMFUL CYANOBACTERIA AND THEIR EFFECTS

6.1.1 Examples of toxin producing cyanobacteria

In the UNESCO Taxonomic Reference List of Harmful Micro Algae (Churro and Moestrup, 2021) 45 toxic cyanobacteria are listed. Many of these occur in freshwater only. Most of the harmful cyanobacteria belong to the order Nostocales. Common toxin producing cyanobacteria are listed in Table 6.1. A few toxin producing cyanobacteria belong to the Oscillatoriales, for example, *Planktothrix rubescens* or the Synechococcales, for example, *Snowella lacustris*.

TABLE 6.1
Causative common toxic cyanobacteria and effects

Toxin(s)	Causative organism	Syndrome	Note
Anatoxin-a and PSP	*Aphanizomenon flosaquae* Ralfs ex Bornet and Flahault, 1886	Neurological	Strains occurring in the Baltic Sea show no toxin production
Lyngbyatoxin	*Lyngbya majuscula* Harvey ex Gomont, 1892	Dermatotoxical	"Swimmer's itch" when in direct contact with human skin
Microcystins	*Microcystis aeruginosa* (Kützing) Kützing, 1846	Possibly carcinogenic to humans	
Nodularin	*Nodularia spumigena* Mertens ex Bornet and Flahault, 1888	Tumor promoter (hepatotoxin)	Accumulates in food chain, including blue mussels, zooplankton, fish. Human swimming in a bloom may cause a rash, probably caused by lipopolysaccharide endoxins.
Cylindrospermopsin	*Raphidiopsis raciborskii* (Wołoszyńska) Aguilera, Berrendero Gómez, Kaštovský, Echenique and Salerno, 2018	Hepatotoxin	Freshwater

Source: Churro, C., and Moestrup, Ø. 2021. Cyanobacteria, in IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Available online at www.marinespecies.org/hab. Accessed on 2021-12-12. Retrieved 2021-09-24 from www.marinespecies.org/hab

6.1.2 Ecology, physiology and behaviour of selected harmful cyanobacteria

Cyanobacteria vary in size and shape and also in their physiology and ecology. Here some examples of traits relevant to cyanobacteria early warning are provided.

Several cyanobacteria within the Nostocales, for example, *Aphanizomenon*, *Dolichospermum* and *Nodularia* can utilise dissolved nitrogen gas as a nitrogen source, they are nitrogen fixers (diazotrophic). This gives them a competitive advantage compared to phytoplankton in general during conditions when nitrate or ammonium are limiting factors. This means that in phosphate replete waters, the nitrogen fixers can keep on growing when nitrate and ammonium are depleted. Another effect is that nitrogen fixing cyanobacteria contribute significantly to the nitrogen budget in some areas (Adam *et al.*, 2016; Karlson *et al.*, 2015; Niemisto *et al.*, 1989; Olofsson *et al.*, 2021). It also implies that monitoring the concentrations of inorganic nutrients in the water column is relevant for early warning of certain cyanobacteria blooms. High temperature favours the growth of cyanobacteria in general (Paerl and Huisman, 2008). This applies to many phytoplankton but to cyanobacteria in particular. For many cyanobacteria species there is a positive correlation between abundance and water temperature.

Some cyanobacteria have the capability of controlling their position in the water column utilising gas vesicles (Hajdu *et al.*, 2007; Walsby *et al.*, 1995; Walsby *et al.*, 1997). Near surface observations of these cyanobacteria during different times of the day may give different results. This may need to be considered when designing observing systems. Positively buoyant cyanobacteria accumulate at the sea surface during calm weather (Figure 1). This is usually the time when the public notices the blooms. Weak onshore winds may cause accumulation of the surface scums of cyanobacteria in archipelagos and on beaches.

FIGURE 6.2
Surface accumulations of cyanobacteria in the southern Baltic Sea proper in July 2013. A polarizing filter was used on the camera.

Akinetes, a type of resting stage, are produced by some cyanobacteria (Wood *et al.*, 2021). The akinetes may act as seed population for cyanobacteria blooms. This may need to be considered when modelling the development of cyanobacteria blooms. The chance, or risk, for the akinetes to reach surface waters where conditions for growth is favourable, will vary depending on water depth, upwelling and other physical oceanographic conditions.

6.1.3 Effects of harmful cyanobacteria

The cyanobacteria blooms of the Baltic Sea are used as the main examples. *Nodularia spumigena* (producer of nodularin), *Aphanizomenon flosaquae* (probably non-toxic in the Baltic Sea) and *Dolichospermum* spp. dominate summer cyanobacteria blooms in the Baltic Sea (Figure 6.2). They often occur together.

Nuisance blooms – beach fouling

During calm weather, positively buoyant cyanobacteria accumulate at the sea surface. Weak winds may transport the surface scums to beaches. In the Baltic Sea, this often occurs during summer vacations. Since it is not possible to evaluate if the cyanobacteria are toxic without sophisticated methods, authorities often issue warnings to the public applying the precautionary principle. The warnings have effects on leisure activities such as swimming and also on tourism in general. Mobile tourists may choose to go to an area free from cyanobacteria blooms.

Toxin accumulation in bivalve molluscs, fish, and so on

Nodularin have been shown to accumulate in blue mussels (*Mytilus edulis* L.), in zooplankton, and in fish, such as cod and flounder (Kankaanpaa *et al.*, 2005; Karjalainen *et al.*, 2008; Karlsson *et al.*, 2003; Mazur-Marzec *et al.*, 2007; Sipia *et al.*, 2001; Van Buynder *et al.*, 2001). Eider ducks, feeding on blue mussels, have also been shown to accumulate nodularin (Sipiä *et al.*, 2004; Sipiä *et al.*, 2006).

Human health issues

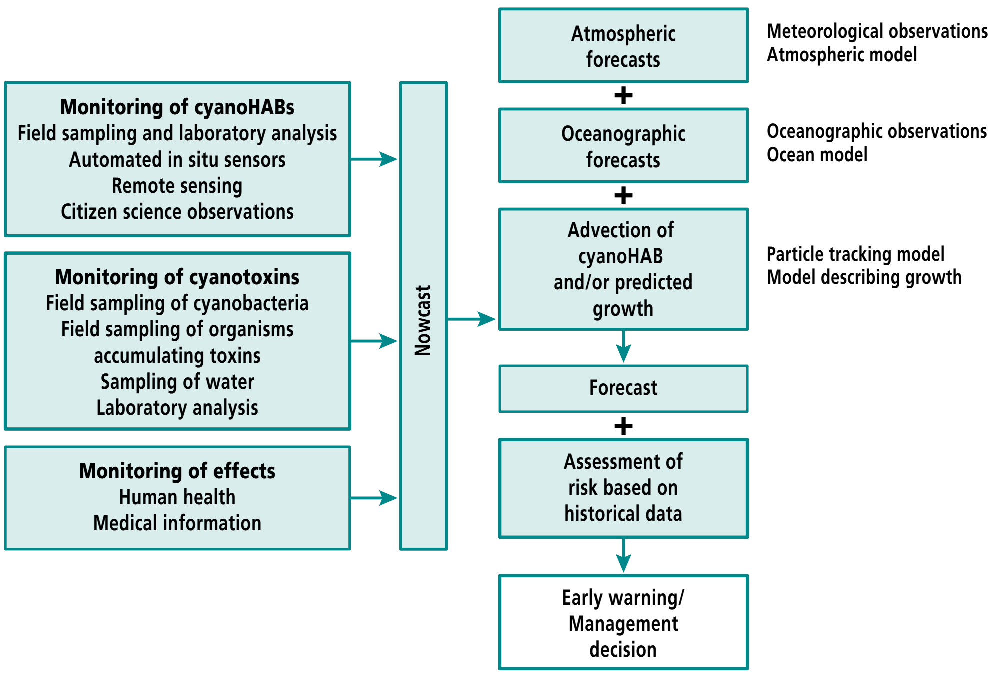
Cyanotoxins in seafood is a potential risk for human health. The toxins may affect for example, the central nervous system, liver, kidney and skin (Table 6.1). In addition, cyanotoxins may occur in aerosols from lakes and from the sea affecting lungs of person inhaling the aerosols (Plaas and Paerl, 2020; Schaefer *et al.*, 2020; Wood and Dietrich, 2011). Cyanotoxins in drinking water are largely outside the scope of the chapter but some information is provided (Codd *et al.*, 2016; Codd *et al.*, 2020). Cyanobacteria toxins in drinking water is mainly a problem in freshwater since this is used directly in plants producing drinking water. An example comes from the Laurentian Great Lakes in Canada (Miller *et al.*, 2017). In addition, there is information about toxins produced by phytoplankton in water produced by desalination plants. (Hess *et al.*, 2017; Villacorte *et al.*, 2015).

Mortalities of dogs

Mortalities of dogs due to nodularin have been reported for example, from Finland (Simola *et al.*, 2012), the Netherlands (Algermissen *et al.*, 2011) and South Africa (Harding *et al.*, 1995).

FIGURE 6.3

The main components of a cyanobacteria early detection and warning system



Source: Elaborated by the authors.

6.2 AN OVERVIEW OF A CYANOBACTERIA EARLY WARNING SYSTEM

To establish an early warning system for harmful cyanobacteria some, but not all, of the components illustrated in Figure 6.3 are needed. Available resources and infrastructure will limit the content of the warning system. Nowcast is the current situation based on observations. A forecast is based on the nowcast and predictions from meteorological and oceanographic modelling. A prediction of the advection of a cyanobacteria bloom is an example. In addition, a biological model describing growth of the cyanobacteria may be included. Also, loss from grazing and so on may be added. An early warning and a management decision should be based on the nowcast, the forecast and on an assessment of risk based on historical data.

6.3 STAKEHOLDERS AND STAKEHOLDER NEEDS

Stakeholders include the public, coastal management, the tourism industry, the aquaculture industry, fisheries, food health managers, desalination plant operations and ocean health. The direct target of products from a cyanobacteria early warning system may be ocean information offices that cover a specific region or coastline. The information office would have the responsibility to distribute information to media and to end users in a form fit for purpose. Web sites with information and weekly bulletins is one way to distribute information. For example, the Lake Erie Harmful Algal Bloom Forecast is accessed by a link: https://coastalscience.noaa.gov/research/stressor-impacts-mitigation/hab-forecasts/lake-erie/

6.4 BASELINE RISK ASSESSMENT

Long-term monitoring of the presence of harmful cyanobacteria is essential to understand where and during which time period the risk for harmful blooms occurs. If long-term data are unavailable, a pilot study covering at least one year or the bloom period is recommended. Species identification and measurements of toxins are essential. Ideally cultures of several local strains of toxin-producing cyanobacteria should be established and investigated in the laboratory. In addition to the detailed information on species and toxins, the spatial and temporal distribution of high biomass blooms can be investigated using tools such as satellite remote sensing of ocean colour and *in situ* fluorescence of pigments indicative of the cyanobacteria in focus. In addition to the investigation of the cyanobacteria, the risk for effects should be investigated. Medical aspects are important. If information from health care on intoxications and so on are available, this information should be utilized to investigate risk.

6.5 NOWCASTS – SAMPLING STRATEGY AND PLATFORMS

6.5.1 General aspects

There are a number of different ways to observe cyanobacteria as described below. It is recommended that a combination of different methods and observing platforms are used. Using only tools for observing cyanobacteria biomass, the detailed information about toxin producing species and toxin content would be lost. Using only the traditional tools aimed at species identification, the general overview of bloom distribution in time and space would be lost. The novel automated *in situ* imaging methods for automated near real time analysis of phytoplankton, including cyanobacteria, can give a very high spatial and temporal information on the distribution of cyanobacteria at the species or genus level.

Research vessels

Many research vessels are well equipped for carrying out observations of cyanobacteria blooms. The negative aspect is that these ships often are dedicated to short term studies making regular, high frequency observations difficult. Cost for ship time is often a limiting factor. In these cases, the samples collected from research vessels will complement other observations. The *in situ* observations made using research vessels are very important as sea truth data for satellite remote sensing of ocean colour.

Ships of opportunity (FerryBox systems)

Ships of opportunity (SOOP) are often merchant vessels or passenger ferries that have been outfitted with sensors and automated water sampling devices(Petersen *et al.*, 2017; Seppälä *et al.*, 2021). The term ferrybox is often used for a set of instruments and water sampling devices mounted on a ship (Ainsworth, 2008). In the Baltic Sea, the use of Ferrybox systems for observations is widespread (Karlson *et al.*, 2016) and started in the 1990s (Leppänen and Rantajärvi, 1995). An advantage with ferrybox systems is that they are usually very low cost compared to research vessels. In addition, the routes of ferries often cover large geographical areas frequently. Disadvantages includes that only near surface water is sampled and that frequent access to the ship is needed for collecting water samples, cleaning of sensors and general maintenance.

Satellite remote sensing

Many cyanobacteria have bio-optical features that makes it possible to discriminate them from phytoplankton in general. Satellite remote sensing of ocean colour is frequently used to observe near surface blooms of cyanobacteria (Bernard *et al.*, 2021; Groom *et al.*, 2019; Mishra *et al.*, 2019, Page *et al.*, 2018), see also Figure 6.4. In Table 6.2, satellites and sensors widely used for observing cyanobacteria are listed. The frequency of satellites passing a certain sea basin varies depending on latitude and width of swath. In general, the polar orbiting satellites pass about once a day. Geostationary satellites may provide hourly data (Choi *et al.*, 2012; Yang *et al.*, 2014).

The high reflectance of near surface blooms was utilised to determine the distribution cyanobacteria in the Baltic Sea (Kahru, 1997). The method was implemented in the Baltic Algae Watch System (BAWS) in Sweden in 2002 (Öberg and Karlson, 2014), and it is still used operationally with daily updates in summer (www.smhi.se/data/oceanografi/algsituationen). BAWS has been adapted to new satellites and sensors.

Another approach is to use hyperspectral data that in some cases makes it possible to discriminate between different cyanobacteria genera (Kudela *et al.*, 2015). In the remote sensing community, there are high hopes of applying hyperspectral sensors for observing algal blooms (Dierssen *et al.*, 2021).

The main advantage with satellite remote sensing is that the geographical coverage is very high during cloud free conditions. Geostationary satellites provide data with a very high frequency, minimizing the problem with cloud cover. Disadvantages include that no, or very limited, information on species composition is included, cloud cover often makes observations impossible, and only near surface blooms are observed. Sea truth data on species composition and so on are essential in a satellite-based early warning system.

Beach sampling

Accumulations of cyanobacteria on beaches can be a nuisance and also a risk for human health. Thus, sampling of cyanobacteria and analyses to determine if toxic species are present are important. In addition, analysis of toxins in the cyanobacteria and in water is recommended. In Europe, a directive concerning the management of bathing water quality regulates observations (Directive, 2006). The directive is implemented differently in different countries. Often only superficial optical observations using the human eye are made. In some countries regular sampling of cyanobacteria and of cyanotoxins at beaches are made (Karlson *et al.*, 2021). Citizen science observations of cyanobacteria may complement the sampling by authorities (Burford *et al.*, 2020).

TABLE 6.2
Examples of satellites widely used for observing near surface cyanobacteria blooms. Satellites with narrow swaths are not included. Acronyms are explained in table at the start of this publication

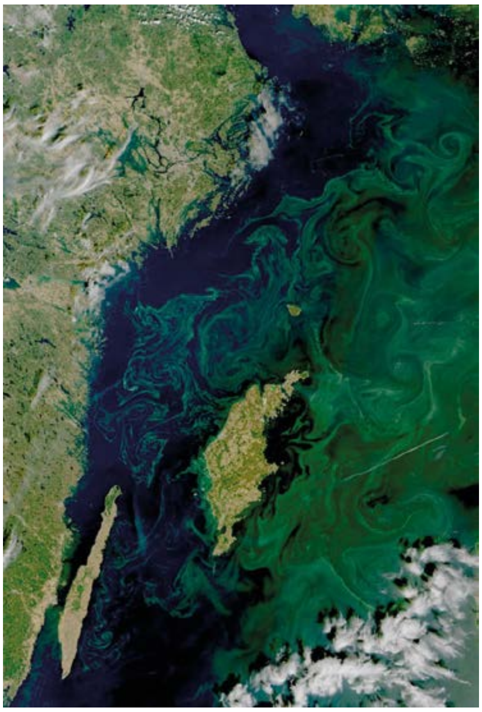
Satellite(s)	Sensor	Data repository	Start year	End year
Aqua and Terra (NASA)	*MODIS*	*https://oceancolour.gsfc.nasa.gov*	2002	-
EnviSAT (ESA)	*MERIS*		2002	2012
NPP SUOMI (NASA)	*VIIRS*	*https://oceancolour.gsfc.nasa.gov*	2011	-
Sentinel 3A and 3B (ESA)	*OLCI*	*https://coda.eumetsat.int*	2016 and 2020	-
Communication, Ocean and Meteorological Satellite (KARI/KORDI)	*GOCI*	*https://ioccg.org/sensor/goci/*	2010	-

Source: **Churro, C., and Moestrup, Ø**. 2021. Cyanobacteria, in IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Available online at www.marinespecies.org/hab. Accessed on 2021-12-12. Retrieved 2021-09-24 from www.marinespecies.org/hab

Airplanes and drones

Airplanes have been used for observing near surface cyanobacteria blooms for many years. A photo of a bloom in the Baltic Sea is presented in Figure 6.5. Advanced hyperspectral sensors can also be used (Hunter *et al.*, 2010; Vander Woude *et al.*, 2019). A novel development, mainly in lakes and reservoirs, is to use drones fitted with hyperspectral sensors, to observe bio-optical properties of cyanobacteria. (Wu *et al.*, 2019).

FIGURE 6.4
Satellite image of surface accumulations of cyanobacteria in the Baltic Sea, 14 August 2018, Sentinel 3A, OLCI, processed by SMHI. (See also Table 6.2)



Source: Image created by Satellite Sentinel 3A, with sensor Ocean and Land Colour Instrument, European Space Agency and EUMETSAT. Ocean colour data was processed by the Swedish Meteorological and Hydrological Institute.

FIGURE 6.5
Surface accumulations of cyanobacteria in the Baltic Sea, south of the island of Gotland

6.6 METHODS FOR OBSERVATIONS OF HARMFUL CYANOBACTERIA

6.6.1 Abundance and identification of species

Water sampling and microscopy

Water sampling using Niskin-type bottles, tubes (Lindahl, 1986) or automated sampling in ferrybox systems is the traditional way to obtain samples for analysing the composition and abundance of planktonic cyanobacteria. The sedimentation chamber/inverted microscopy method (Edler and Elbrächter, 2010; Utermöhl, 1958) is frequently used for investigating species composition, cell numbers and biomass of phytoplankton including cyanobacteria. In early warning systems it is essential to analyse samples shortly after sampling.

Automated imaging flow cytometry

Automated imaging in flow methods for analyses of phytoplankton provide near real-time data on diversity, abundance and biomass. The Imaging FlowCytoBot (McLane Research laboratories Inc.) (Olson and Sosik, 2007; Sosik and Olson, 2007) was used to investigate the cyanobacteria community in the Baltic Sea as part of an operational oceanographic observing system (Kraft *et al.*, 2021). Other *in situ* imaging instruments include the FlowCam (Yokogawa Fluid Imaging Technologies, Inc.) and the Cytosense (CytoBuoy b.v.).

Molecular methods (qPCR)

Molecular methods are developing quickly. One example is metabarcoding which is becoming popular tool to investigate the diversity of plankton. The 16S part of rDNA can be used as a taxonomic marker for identifying bacteria, including cyanobacteria (Hu *et al.*, 2016). However, the process of filtering samples, extracting DNA, preparing libraries, sequencing and the bioinformatic process is quite time consuming. This means that metabarcoding is, at present, not suitable to be part of an early warning system. Another molecular method, qPCR, is faster and also gives quantitative data, but only for a few taxa in a sample. qPCR has been applied to cyanobacteria (Al-Tebrineh *et al.*, 2011; Chiu *et al.*, 2017; Koskenniemi *et al.*, 2007; Lu *et al.*, 2020). It is potentially a useful tool in early warning systems.

6.6.2 Biomass

General aspects
Biomass estimates based on satellite remote sensing of ocean colour was described earlier in this chapter. Many cyanobacteria have photosynthetic pigments such as phycocyanin and phycoerythrin, that are rare in other groups of phytoplankton. One has to keep in mind that representatives of the algal class Cryptophyceae do have similar pigments. Also, some Dinophyceae (dinoflagellates) belonging to the genus *Dinophysis* and some ciliates, for example, *Mesodinium rubrum*, have similar pigments since they have acquired chloroplasts from the cryptophycea as kleptoplastids. Concentrations of phycocyanin and phycoerythrin can be used as proxies for the biomass of certain cyanobacteria

Phycocyanin fluorescence – a proxy for cyanobacteria biomass
The bloom-forming filamentous cyanobacteria in the Baltic Sea contain phycocyanin. The fluorescence of phycocyanin can be used as a proxy for the biomass of these species as a group (Seppala *et al.*, 2007). Sensors can be mounted for example, on oceanographic buoys, on CTD instruments, in ferrybox systems and in water inlets of desalination plants. When using phycocyanin fluorescence as a proxy for the biomass of cyanobacteria, it is essential to investigate the fluorescence properties of the local cyanobacteria and to choose excitation and emission wavelengths of sensors according to the results. Sensors from different manufacturers vary in this respect and sometimes wavelengths are not specified by manufacturers.

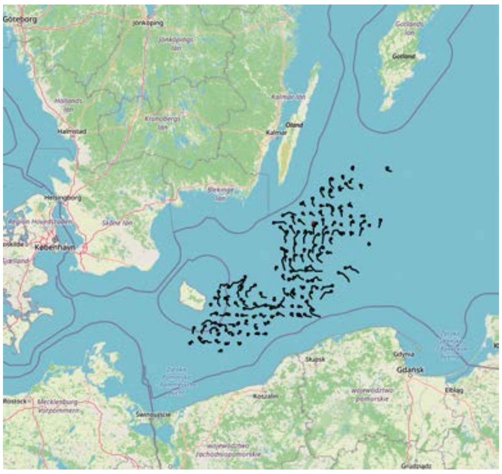
Other bio-optical measurements
Multi spectral fluorometers and hyperspectral absorption detectors (Wollschlager *et al.*, 2013) are available commercially as oceanographic instruments. Manufactures sometimes claim that they can estimate the biomass of different phytoplankton groups based on piment signatures. The results should be used with caution. On the other hand, the results can be quite useful when combined with other methods.

6.7 SHORT TERM PREDICTION OF ADVECTION OF CYANOBACTERIA

In Figure 6.3, it is indicated that short-term predictions rely on observations of the cyanobacteria and on forcing environmental factors. To make predictions about the advection of cyanobacteria blooms an atmospheric three-dimensional model forcing a three-dimensional physical oceanographic model can be used together with models for particle tracking. Popular physical oceanographic models include NEMO (Hordoir *et al.*, 2019; Madec *et al.*, 2017) and ROMS (Shchepetkin and McWilliams, 2005). Both can be used to simulate particle drift. It is also possible to give properties or traits to the particles to simulate cyanobacteria behaviour, for example, buoyancy. The drift model used together with NEMO-Nordic, a setup of NEMO for the Baltic Sea and the North Sea area, is built around a Lagrangian particle model known as PADM (Particle Advection and Dispersion Model) (Liungman and Mattsson, 2011). The PADM is a Lagrangian particle spreading model, which means that the substance or object being simulated is represented as a cloud of particles. The trajectory of each particle is calculated based on the spatial-temporal evolution of flow fields. An example of results is presented in Figure 6.6.

FIGURE 6.6

An example of result from prediction of cyanobacteria drift in the Baltic Sea. Satellite observations of near surface bloom of cyanobacteria were combined with NEMO Nordic model and a particle drift model PADM (see main text and references for details)



Source: Elaborated by Bengt Karlson.

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APPENDIX 1. Existing resources for biotoxin monitoring andmanagement

Biotoxin monitoring and management is based on monitoring of phytoplankton and on shellfish toxicity testing.

FAO, IOC AND PARTNERS

- Global Harmful Algal Bloom Status Report
- Guidelines for the study of climate change effects on HABs IOCX manual and guides no. 88)
- IOC-UNESCO Harmful Algal Information System (HAIS), 2021. https://prod.hab.ioc-unesco.org/hais-2
- IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Lundholm *et al.*, 2021)
- GlobalHAB: Evaluating, Reducing and Mitigating the Cost of Harmful Algal Blooms: A Compendium of Case Studies. PICES Scientific Report No. 59, 2020.
- Code of practice for fish and fishery products (FAO and WHO 2020a)
- Report of the Expert Meeting on Ciguatera Poisoning (FAO and WHO 2020b)
- E-learning training course: Monitoring and preventing ciguatera poisoning (Dechraoui Bottein *et al.*, 2020)
- Solutions for managing cyanobacterial blooms: A scientific summary for policy makers, 2019.
- Technical Guidance for the Development of the Growing Area Aspects of Bivalve Mollusc Sanitation Programmes (FAO and WHO 2018)
- Harmful Algal Blooms (HABs) and Desalination: A Guide to Impacts, Monitoring and Management, Donald M. Anderson, Siobhan Boerlage and Mike Dixon, Eds. UNESCO 2017
- Food safety risk management: Evidence-informed policies and decisions, considering multiple factors (FAO 2017)
- Toxicity Equivalency Factors for Marine Biotoxins Associated with Bivalve Molluscs (Botana *et al.*, 2017; FAO and WHO 2016)
- *IOC Manuals and Guides*, no. 59. Reguera B, Alonso R, Moreira A, Méndez S,Dechraoui-Bottein M-Y (Eds). IOC-IAEA Guide for Designing and Implementing a Plan to Monitor Toxin-Producing Microalgae. 2016. 2nd Ed. Intergovernmental Oceanographic Commission (IOC) of UNESCO and International Atomic Energy Agency (IAEA), Paris and Vienna. *IOC Manuals and Guides*, no. 59. 66 pages. (Spanish and English)
- Toxic and Harmful Microalgae of the World Ocean, Nicolas Chomérat, Philipp Hess, Elisabeth Nézan and Patrick Lassus, *2016 by ISSHA and IOC of UNESCO.*
- Assessment and management of biotoxin risks in bivalve molluscs (J. Lawrence *et al.*, 2011)
- *IOC Manuals and Guides*, no. 55. Karlson B, Cusack C and Bresnan E (Eds). Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis. Paris, UNESCO. 2010.
- Standard for Live and Raw Bivalve Molluscs (Codex Committee on Fish and Fishery Products 2008)

- Food safety risk analysis: A guide for national food safety authorities. (FAO and WHO 2006)
- Marine Biotoxins, Food and Agriculture Organisation of the United Nations, Rome, 2004.
- Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters, Anderson D.M. *et al.*, (Eds.) APEC Report # 201-MR-01.1, Asia Pacific Economic Programme and Intergovernmental Oceanographic Commission of UNESCO, Technical Series No. 59, Paris, France, 2001.

AUSTRALIA

- Seafood Handling Guidelines (Sydney fish Market 2013)
- Risk assessment of the seafood safety scheme (New South Wales Government 2017)
- Monitoring and Sampling Manual Environmental Protection (Water) Policy 2009 (DES 2018)
- MARINE BIOTOXIN MANAGEMENT PLAN NSW shellfish program www.epa.gov/cyanohabs/state-habs-monitoring-programs-and-resources
- Marine Biotoxin Management Plan VICTORIAN MARINE BIOTOXIN MANAGEMENT PLAN 5th edition https://vfa.vic.gov.au/aquaculture/publications/shellfish-quality-asurance/marine-biotoxin-management-plan

EUROPEAN UNION

- The European Union Reference Laboratory for Marine Biotoxins have two good guidance documents (one published and one in prep) in the last couple of months which might be good to include here on biotoxins (waiting on endorsement and publication) and phytoplankton (published) - Monitoring of Toxin-producing Phytoplankton in Bivalve Mollusc Harvesting Areas. Guide to Good Practice: Technical Application of the European Union www.aesan.gob.es/en/CRLMB/docs/docs/procedimientos/Phyto_Monitoring_Guide_DEC_2021.pdf

IRELAND

- Code of Practice for the Irish Shellfish Monitoring Programme (Biotoxins) www.fsai.ie/uploadedFiles/About_Us/Industry_Fora/MSSC/CoP_Biotoxin_Monitoring.pdf

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

- www.foodstandards.gov.scot/downloads/Guidance_Document_%282%29.pdf
- www.food.gov.uk/business-guidance/biotoxin-and-phytoplankton-monitoring#phytoplankton-monitoring

UNITED STATES OF AMERICA

- Cyanobacterial Harmful Algal Blooms (CyanoHABs) in Water Bodies, US Environmental Protection Agency, 2021.
- Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 - Fish Sampling and Analysis, Third Edition, US Environmental Protection Agency, 2000.
- National Shellfish Sanitation Program (NSSP) Model Ordinance (MO) Standardization Field Guide, US Food and Drug Administration, 2020.
- Fish and Fishery Products Hazards and Controls Guidance, Chapter 6: Natural Toxins, US Food and Drug Administration, 2019.
- Guide for the Control of Molluscan Shellfish, National Shellfish Sanitation Program (NSSP), 2019.
- US State HABs Monitoring Programs and Resources

Globally, there are 3 400 to 4 000 described species of marine microalgae but only 1 to 2 percent are considered to be harmful. Harmful algal blooms (HABs) have significant impacts on food safety and security through contamination or mass mortalities of aquatic organisms.

The impacts and mass mortalities of marine species caused by harmful algae are not new and have been recorded for decades. However, there is growing concern that these events will increase due to accelerating global warming, climate change and anthropogenic activities.

Indeed, if not properly controlled, aquatic products contaminated with HAB biotoxins are responsible for potentially deadly foodborne diseases and when rapidly growing, HAB consequences include reduced dissolved oxygen in the ocean, dead zones, and mass mortalities of aquatic organisms. Improving HAB forecasting is an opportunity to develop early warning systems for HAB events such as food contamination, mass mortalities, or foodborne diseases. Surveillance systems have been developed to monitor HABs in many countries; however, the lead-time or the type of data (i.e. identification at the Species-level, determination of toxicity) may not be sufficient to take effective action for food safety management measures or other reasons, such as transfer of aquaculture products to other areas. Having early warning systems could help mitigate the impact of HABs and reduce the occurrence of HAB events.

The Joint FAO-IOC-IAEA Technical Guidance for the Implementation of Early Warning Systems (EWS) for HABs will guide competent authorities and relevant institutions involved in consumer protection or environmental monitoring to implement early warning systems for HABs present in their areas (marine and brackish waters), specifically those affecting food safety or food security (benthic HABs, fish-killing HABs, pelagic toxic HABs, and cyanobacteria HABs). The guidance provides a roadmap for stakeholders on how to improve or implement an EWS for HABs and biotoxins, where appropriate.

It is important to note that not all countries and institutions can implement the same level of EWS for HABs, and this guidance is intended mainly for those who seek to broaden existing early warning systems, or who are just beginning to consider putting a system in place.

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