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D5.2 Biotic and abiotic conditions favouring HAB development and associated future risks



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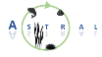
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Abbreviations

ASP	Amnesic shellfish poisoning
AZAs	Azaspiracids
AZP	Azaspiracid poisoning
CCM	Carbon concentrating mechanism
C-HARM	California-Harmful Algae Risk Mapping
CHIME	Copernicus Hyperspectral Imaging Mission for the Environment
DA	Domoic acid
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DSP	Diarrhetic shellfish poisoning
DTXs	Dinophysis toxins
EO	Environmental earth observation
GEE	Google Earth Engine
HAB	Harmful algal bloom
IMTA	Integrated multitrophic aquaculture
IPCC	Intergovernmental Panel on Climate Change
NAO	North Atlantic oscillation
NIR	Red to near infrared
NSP	Neurotoxin shellfish poisoning
OA	Okadoic acid
OCIMS	National Oceans and Coastal Information System
PACE	Plankton Aerosol Cloud and ocean Ecosystem
PDO	Pacific decadal oscillation
PSP	paralytic shellfish poisoning
SBG	Surface Biology and Geology
SST	Sea surface temperature
STX	Saxitoxin
UAS	Unoccupied aerial systems
YTXs	Yessotoxins

1 Summary

This document was created as part of the H2020 All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) project, to support of Work Package 5 Climate – Ocean – Food. The aim of the study was to conduct a literature review towards the identification of the biotic and abiotic conditions favouring the development of harmful algal blooms (HABs) for selected case study sites, commenting both on future HAB-related risks associated with climate change and the recommended HAB-related variables and parameters to be monitored.

Although the ASTRAL project focuses primarily on integrated multi-trophic aquaculture (IMTA) production, this document provides an informative overview with a much broader scope. The document is split into several sections including: An extensive literature study on abiotic and biotic factors effecting HAB were performed; Several factors favouring HABs have been identified with stratification and eutrophication playing an important role; A list was compiled with recommendation for environmental monitoring which was supplemented with case studies on HAB occurrence at the various IMTA labs; Suitability of earth observation data at a regional scale was assessed by a literature study and a case study at IMTA lab South Africa; Vulnerability of IMTA labs to climate change and an in-depth case study of the potential area for the IMTA lab in Argentina was conducted.

The results obtained will be essential to i) boost research and innovation for new and cost-effective monitoring in real-time mode technologies, in line with the uptake, mode of action and consequences, and ii) propose optimal monitoring recommendations to minimise risks related to climate change and environmental stressors of ASTRAL IMTA-systems.

2 Introduction

2.1 Harmful algal blooms

Broadly, harmful algal blooms (HABs) may be defined as algal blooms known to disrupt environmental and ecosystem function, but which may or may not necessarily be toxic (Glibert et al., 2006.). Harmful algal blooms have been documented all over the world, from marine coastal and offshore waters to freshwater lakes and streams. HABs are often associated with toxic events and dense populations of algae; however, the term “harmful algal bloom” is very broad and covers blooms of many types and species, including diatoms, dinoflagellates, raphidophytes, and cyanobacteria, often referred to as CyanoHABs. Though they are more common in fresh water, the occurrence in tropical and subtropical marine waters is increasing (O’Neil et al., 2012). In this report we include cyanobacteria as well when discussing harmful algal blooms. One thing that all HABs have in common is that they cause harm in some way. Blooms can be detrimental environmentally, ecologically, or economically through: 1) production of toxins or harmful metabolites; 2) producing foams, mucilage or scums which can harm fish or bivalves; 3) creating anoxic events via bloom decay; 4) the destruction or alteration of ecosystems, including the shading of submerged vegetation; 5) alteration of trophic structure; 6) or adverse physiological impact on higher trophic organisms (e.g., some HABs have physical structures, such as spines, that can lodge in fish gills and can cause irritation and eventual suffocation (He, 2015) (Anderson et al., 2002; Glibert et al., 2006). HABs may also have socio-economic ecosystem impacts too. For example, some HABs producing mucilage and foams for a nuisance on beaches and are unappealing for tourism.

Similarly, the harmfulness of HABs is not always directly associated with dense populations of algae. Sometimes even low algal concentrations can cause serious detrimental effects given the degrees of toxicity of the algae involved. Examples include species from the genus *Alexandrium*, which may cause accumulation of paralytic shellfish poison (PSP) toxins in shellfish and can lead to closures of aquaculture sites at concentrations as low as 40 cells/L. Whereas, *Pseudo-nitzschia* spp., which produces amnesiac shellfish poison toxins, cells do not trigger closures till 50,000 cells/L are reached (Coates et al., 2020). Another way HABs are harmful to the environment is when dense blooms run out of nutrients, die, sink, and are decomposed by bacteria which draws oxygen down to hypoxic levels ($< 2 \text{ mg L}^{-1}$) and result in fish and marine invertebrate mortality (Sellner et al., 2003). These hypoxic effects can extend over extensive areas: for instance, the Baltic Sea alone has on average 60,000 km² of hypoxic area each year, which lacks sufficient oxygen to support healthy ecosystem functioning



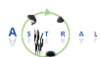
(Conley, 2012); worldwide this has now been reported from >400 systems, affecting a total area of > 245,000 km² (Diaz & Rosenberg, 2008).

2.2 Phytoplankton species associated with harmful algal blooms

Harmful algal blooms can be found worldwide however the phytoplankton species causing these vary by region because of specific environmental conditions related to temperature, salinity, light, and nutrient range. Harmful algae can be found across many taxonomic groups, however dinoflagellates in particular are associated with harmful blooms. Dinophyta (Dinoflagellates) are unicellular eukaryotic organisms, mostly reddish-brown in colour, which swim through of a pair of whip-like flagella. They occur in both saltwater and freshwater. Several subgroups of dinoflagellates frequently develop harmful blooms: Gymnodinoids and Noctilucooids (*e.g.*, *Amphidinium*, *Cochlodinium*, *Gymnodinium* genera), Peridinoids (*Peridinium*), Gonyaulacoids (*e.g.*, *Alexandrium*, *Pyrodinium*, *Gonyaulax*, *Gambierdiscus*, *Ostreopsis*, *Ceratium*), Dinophysoids (*Dinophysis*), Prorocentroids (*Prorocentrum*) (Pettersson & Pozdnyakov, 2013). Species such as *Gonyaulax polygramma*, *Noctiluca scintillans*, *Scropsiella trachoidea*, *Gymnodinium mikimoto*, *Cochlodinium polykrikoides*, are not directly toxic, but can form dense blooms which discolour the water and cause mortality of fish and invertebrates due to oxygen depletion (Pettersson & Pozdnyakov, 2013). Some species such as the dinoflagellate *Alexandrium tamarense* and *A. catenella* have expanded their range over the past decades and can now be found ranging from New Zealand and Australia to Japan and in Europe and North America (Lassus et al., 2016). The geographical expansion of HAB species has been observed in a variety of species among others in the dinoflagellate *Dynophysis* spp. and the diatom *Pseudo-Nitzschia* spp. (Lassus et al., 2016).

Bacillariophyta (diatoms) are ubiquitous throughout the world's oceans and in all aquatic habitats. Several genera are considered harmful due to their mucilage production (*Thalassiosira*, *Coscinodiscus*, *Chaetoceros*), while other can physically damage to fish gills (*Chaetoceros*) or produce toxins (*Pseudo-nitzschia*).

Haptophytes (Prymnesiophytes) are golden-brown flagellates that comprise a heterogeneous group of unicellular algae. Presently only species of the genera *Prymnesium*, *Chrysochromulina*, and *Phaeocystis* are generally recognized as potentially harmful. The species *Prymnesium parvum* and *P. patelliferum* cause damage by clogging fish gills (Pettersson & Pozdnyakov, 2013) whereas *Phaeocystis* is a cosmopolitan genus of foam-producing species.



Raphidophytes are small golden-brown flagellates that can pose a serious threat to finfish aquaculture due to both physical clogging of gills by mucus excretion as well as gill damage by hemolytic substances. The following genera/species are most frequently associated in this connection: *Chattonella*, *Heterosigma*, *Olisthodiscus lutes*, *Fibrocapsa* (Pettersson & Pozdnyakov, 2013).

Cyanobacteria have long been known to cause toxic blooms in lakes, but there is increasing evidence that marine cyanobacteria blooms are on the rise. Marine cyanobacteria are mostly found in warm tropical and subtropical waters, and to some extent in brackish waters and estuaries. There are over 30 species of cyanobacteria that are associated with toxic blooms (Paerl & Fulton, 2006). Species such as *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, *Nostoc*, and *Oscillatoria* handle most of the toxic episodes (Pettersson & Pozdnyakov, 2013).

One type of HAB which gets a lot of attention are the toxic HABs, especially when they are related to human health issues or aquaculture closures. A variety of microalgae across several taxa are associated with HABs *Table 2*. Such as, *Pseudo-Nitzschia* spp. (diatom), *Dinophysis* spp. (dinoflagellate), *Synechococcus* (cyanobacteria) *Heterosigma akashiwo* (Raphidophyceae). This large diversity is also seen in the wide variety of toxins that are produced by HAB species. Toxin groups such as Okadaic acid (OA), dinophysis toxins (DTXs), azaspiracids (AZAs), saxitoxin (STX), domoic acid (DA) and the brevetoxin groups are known to cause illness in humans, while yessotoxins (YTXs) cause illness in fish and abalone. These toxins can cause harm to a variety of organisms in different ways (*Table 2*). Those toxins are responsible for illness such as diarrhetic shellfish poisoning (DSP), azaspiracid poisoning (AZP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP) and neurotoxin shellfish poisoning (NSP).

Table 1 The Main harmful microalgae and the toxins they produce

Microalgae	Toxins	Disease	Abbreviation	Toxins found in
<i>Gymonodinium</i> spp. <i>Alexandrium</i> spp. <i>Pyrodinium</i> spp.	Saxitoxin	Paralytic shellfish poisoning	PSP	Shellfish, scallops, mussels, clams, oysters, cockles; moon snails some fish and crabs
<i>Dinophysis</i> spp. <i>Prorocentrum lima</i>	Okadaic acid	Diarrhetic shellfish poisoning	DSP	Shellfish, scallops, mussels, clams, and oysters
<i>Pseudo-nitzschia</i> spp.	Domoic acid	Amnesic shellfish poisoning	ASP	Shellfish, scallops, mussels, clams, and oysters; possibly some fish species
<i>Karenia</i> spp. <i>Chattonella marina</i> <i>Fibrocapsa japonica</i> <i>Heterosigma akashiwo</i>	Brevetoxins	Neurotoxic Shellfish Poisoning	NSP	Fish, marine mammals



<i>Proroperidinium</i> <i>Azadinium</i> spp.				
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Information obtained from: (CDC, 2020; Leftley & Hannah, 2009)

2.3 HABs and ecosystems

Quantifying the total of harm from algal blooms to the ecosystem can be extremely difficult. Particularly when a bloom goes unnoticed because it is not in close proximity to aquaculture sites. HAB monitoring sites located in less densely populated coastal areas or offshore. In addition, the harmful effect might not always be clearly visible for instance when it relates to microbial community changes or when effects do not become visible until bioaccumulation occurs in higher trophic levels. Toxins, other nuisance compounds or structures produced by harmful microalgae affect many trophic levels. It can cause harm or death either directly or indirectly to the organisms which are feeding on HABs, or it can go up the food chain, accumulate, and cause death in shellfish, fish, marine mammals, birds, or humans.

In other cases, HABs may harm, but not kill, other members of the food web, leaving them stressed and therefore more susceptible to other sources of mortality, such as infection by pathogens (Glibert et al., 2006). Zooplankton impaired by ingesting harmful algae may be more susceptible to predation and thus may become an important vector for transferring toxins in the pelagic food web (Glibert et al., 2006). Alternatively, zooplankton faecal pellets may be important sources of toxin to benthic communities. In both cases the toxins can be transferred to other trophic levels where their accumulation might cause additional harm.

2.4 Economic costs of HABs

Estimating the economic costs of HABs is difficult due to the variability in size, toxicity, location, and duration of HABs as well as how well it is documented. It is challenging to assess societal, ecological, and other auxiliary impact of HABs (Trainer, 2020). Economic losses from harmful algal blooms can make up a significant portion of the annual aquaculture industry turn over. For instance, economic loss in the culture of bivalve molluscs due to *Dinophysis* toxins in Scotland costs about 1.37M GBP/year over a national annual industry turnover of ~ £ 12.4M (Martino et al., 2020). Though the industry value is comparatively small, it is critical for many of the rural communities. In addition, these losses in production effects the whole of the shellfish aquaculture supply chain and many other industries including commercial and recreational fisheries, seafood markets, tourism, and tourism-related businesses.



Within the aquaculture and fishing industries, HABs have an array of economic impacts, including the costs of conducting monitoring programs for shellfish and other affected resources, short- and long-term closure of harvestable shellfish and fish stocks. In addition to reputational damage which may cause reductions in seafood sales including the avoidance of “safe” seafood as an over-reaction to health advisories and, mortalities of shellfish and wild and farmed fish or costs incurred from medical treatment of exposed populations.

Therefore, estimating the total economic costs of HABs is complex and requires consideration of many different issues. Not only the accurate cost of loss of income from harmful algal blooms need to be considered, but also precautionary closures of fisheries and aquaculture farms to prevent human poisoning and the ongoing monitoring systems for detecting HABs. As such, a very broad range of values have been suggested when estimating the cost of harmful algal blooms.

Total economic cost of harmful algal blooms amount to approximately 8 \$billion/year globally, due to mass mortalities in finfish, harvesting bans preventing the sale of shellfish that have accumulated unsafe levels of HAB phycotoxins and human health costs (Brown et al., 2020). A large part of those costs is due to precautionary closures of fisheries and aquaculture farms to preserve human health. Annual costs of precautionary closures are estimated at \$3–4 billion (Brown et al., 2020). If we look at a specific area and break down these numbers, we can get a picture of where the biggest economic impacts are. An excellent economic assessment was done by Jin et al. (report Pices edited by Trainer, 2020) who estimated the economic cost of HABs for a variety of sectors in several regions. For instance, economic impact from HABs in Korea showed costs vary widely. Estimated economic effects to the aquaculture sectors caused by HABs in Korea from 2010-2018 was on average 4.5M USD/year but ranged from 0.1 to 20.8 million dollar. While loss of income from tourisms was estimated to be 8.1M USD/year, for marine fisheries it was 20,000 USD/year and recreation fisheries is 16.1M USD/year. This demonstrates the wide impact HABS can have on a variety of industry sectors. In addition, annual red tide monitoring and management costs in Korea was estimated to be around 3.2M USD/year. In addition, Kouakou & Poder (2019) calculated the cost of digestive and respiratory human illness from HABs, which ranged from \$86 to \$14,600 per case depending on the severity of the illness.

2.5 HAB causes and trends

HABs have been steadily increasing over the past 2-3 decades. Increases in nutrient loading, changes in agriculture and aquaculture practices, overfishing, ballast water discharge, and global climate change may all be important in the global increase in HABs (GEOHAB, 2006). Nutrient enrichment of

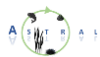


coastal water due to the influx of industrial, urban, agricultural runoff and intensive animal farming, and increased use of fertilizers has been indicated as leading causes in the increase in HAB incidence. However, nutrient loading alone is not sufficient to explain the increase in the number of HAB occurrences and a specific set of circumstances need to come together to provide the optimal conditions for bloom formation such as stable water column, optimum temperature, salinity, and light conditions. In addition, monitoring of coastal areas and aquaculture sites have significantly increased over the past few decades and thus also the likelihood of detecting HABs.

The specific causes of HABs are complex, vary between species, time and season, and locations, meaning that HAB formation mechanisms are not all well understood. In general, algal species proliferate when environmental conditions (e.g., nutrient and light availability, temperature, and salinity) are optimal for cell growth. Other biological (e.g., vertical migration, grazing, viral infection, and parasitism) and physical (e.g., transport, currents, stratification) processes determine if enhanced cell growth will cause biomass accumulation. Further, cell physiology, genetic variation of toxin production, life cycles, and strain specificity adds to the challenge of understanding HABs dynamics. The challenge for understanding the causes of HABs stems from the complexity of these biological, chemical, and physical interactions and their variable influence on growth and bloom development among different species. Overall, HAB bloom formation is very complex meaning that their prediction and management is a significant challenge (GEOHAB, 2006).

2.6 HAB initiation, formation, and progression

A harmful algal bloom has 3 stages: initiation, maintenance, and demise. No single factor is responsible for bloom formation, but rather a complex interaction of biological, physical, and chemical factors is synergistically required for bloom formation. Bloom initiation requires an inoculum of cells to initiate the bloom. This can be obtained from several different sources and may involve many different life stages and species (Steidinger & Garccés, 2006). Holoplanktonic species, such as *Karenia mikimoto* (Raine et al., 2018), are present year-round and over winter in low numbers (<100 cell/l) and only become a nuisance when conditions are favourable, generally in spring, with increasing temperature and light conditions. While other species are using various life stages as a survival strategy and bloom seeding. For example, *Alexandrium* spp. produces a resting stage during suboptimal growth conditions which sink to the bottom and after an obligate dormancy period which can then excyst to release vegetative cells that swim to the surface and re-seed the water column (Sellner et al., 2003). This resting stage therefore provides *Alexandrium* spp. with an essential prolonged survival and blooming strategy over populations that cannot persist under unfavourable biotic or abiotic conditions.



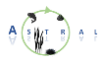
Furthermore, McGillicuddy et al., 2011 showed a correlation between the abundance of cysts of *Alexandrium fundyense* and the magnitude of the bloom. Whilst upwelling or periods of inclement weather can prevent the formation of a bloom (e.g., by means of advection of cells before sufficient aggregation to form a bloom), they may reseed the resting stages cyst back into the water column where they can excyst and induce bloom formation. Meanwhile, currents can transport phytoplankton over long distances while rivers provide nutrient input for blooms. These factors are often seasonal such as upwelling, increased river discharge or current direction and intensity. Identifying where cysts beds are in combination with monitoring oceanographic properties can aid in predicting where and when bloom initiation might occur.

Once the bloom starts, its succession depends on having a gross growth rate in excess of losses due to physical (e.g. advection, mixing) and biological factors (predation, allelopathy and infection). In addition, for a single species to dominate the bloom, successful competition over other phytoplankton needs to be achieved by: (i) higher maximum growth rate, (ii) higher substrate affinities and/or mixed feeding strategies, (iii) higher photosynthetic efficiency, (iv) increased or improved motility (enabling movement to advantageous locations in the water column), (v) allelopathic capability (Flynn, 2008). Even small differences in any of these can have a profound effect explaining competitive advantage in growth capacity. HAB species do not often exhibit all these capabilities. The factors that seem to play a critical role in the success of bloom is motility, which will be of value only under certain stratifying conditions (see section 3.1.2.1 Stratification), allelopathy once a significant algal density is reached (see section 3.2.2) and the use of a wide range of feeding strategies. It is not uncommon for HAB species to be mixotrophic or phagotrophic (see section 3.1.1 and 3.2.1).

The cause of bloom termination cannot always be deterrent. Blooms can be advected or dispersed by currents and storms. Other possible contributors are nutrient depletion, zooplankton predation, and life-stage transitions, such as vegetative planktonic form to benthic resting form. The cause and effect and interactions of these various factors will be discussed in more detail in chapter 3.

2.7 Environmental factors and HAB likelihood

HAB species require certain environmental conditions to form harmful blooms. Several factors effecting the likelihood of a bloom occurring have been identified such as run-off, stratification, closed hydrodynamic features and weather and climatic patterns. One of the most often proposed causes for the increase in frequency of algal blooms is nutrification (Anderson et al., 2002; Glibert et al., 2010; Zhou et al., 2017). However, environmental changes are multi-faceted and as such, the effect on HAB



success is difficult to isolate to a single factor due to the overlapping and coinciding direct and indirect factors. For instance, an increase in nutrient concentration and ratio is a consequence of anthropogenic input (e.g. increase in terrestrial run-off), altered precipitation, modified biogeochemical remineralization, and weather changes. HAB responses are further influenced by hydrodynamic features of the local geography such as river, estuarine, bay, and coastal geomorphology. Dinoflagellates a group of phytoplankton often associated with harmful blooms prefer stratified waters (Smayda, 1997) which are warm, possible low salinity, with limiting water exchange (Pitcher et al., 2010). Stratified waters with a closed or semi-enclosed hydrodynamic features such as eddies, estuaries and bays can form ideal environments for toxic blooms to develop (Pettersson & Pozdnyakov, 2013). To complicate the issue, developed blooms may be moved to a different location by wind, current, and/or upwelling events, so where the blooms are found, may not be the location of a bloom origin. In addition, future projections of more intense precipitation events will influence the timing and magnitude of terrestrial run-off, increased sea temperature may enhance stratification, etc, adding further complexity to the projections.

The biotic variables are even more perplexing. Zooplankton grazing can regulate phytoplankton biomass, but only during part of the bloom life cycle (Turner, 2006). In addition, many HAB-species are mixotrophic and therefore grazers themselves and thus while grazing can reduce the biomass of some phytoplankton species it can cause the increase in other phytoplankton species (Stoecker et al., 2006b). The interaction between bacteria, viruses and HAB species is highly species and even strain specific (Amin et al., 2015, Brussaard & Martinez, 2008; Imai et al., 2006) and it impacts many areas from HAB growth, toxin production to cyst formation. A more in-depth study of all the various biotic and abiotic factors will be presented in Chapter 3.

2.8 Methods for monitoring and predicting HABs

One way to mitigate the potential impact of these blooms is to improve their early detection. Historically, detection of these organisms has relied on microscopic methods, but microscopic determination of the phytoplankton species composition and cell density is time-consuming, low-throughput, and requires a high degree of taxonomic expertise. More modern advances have been done in the development of molecular probes, thereby enabling detection of lower concentrations of cells. These have the potential to allow for discrimination of unique strains and even for assessment of the metabolic activity of target species. However, they are also expensive and time-consuming. Finally, there is increasing research into the development of a suite of sensors or *in situ* technology to detect and identify the various phytoplankton species responsible for HAB formation. This can make



for a rapid, real-time, *in situ* and operationally inexpensive HAB monitoring. A biosensor directly measuring HABs is currently under development in ASTRAL WP3. A low-cost vision-based sensor with built-in Deep Learning models for phytoplankton (>10 μ m) classification and identification is under development. It may provide significant advances to the accessibility of phytoplankton identification technology.

Although regular monitoring for HAB and water quality at aquaculture sites or from ships can provide very detailed information on phytoplankton bloom composition, concentrations and environmental conditions, it can only provide one point in space and time while being very expensive at the same time. ASTRAL is developing a low-cost IoT Kit with several probes for key water quality parameters, long range communication protocols, data storage functionality, and optimized self-powering capabilities for remote deployment. It is integrated to an innovative AI data analytics platform (AIDAP) for predictive modelling of physico-chemical parameters and biological water quality indicators. An overview of physico-chemical water quality parameters and devices used to measure water quality can be found in D3.6 Technology User Guide for Training Courses and Apprenticeships. For a comprehensive overview of the monitoring recommendations on the most appropriate sensor observing platforms, sampling strategies, and approaches at different IMTA sites, see ASTRAL deliverable 5.5.

As discussed in section 2.6, blooms can be transported to other locations by wind, wave, and currents making them highly variable in space and time. Satellite-based remote sensing technology can be an ideal instrument for HAB detection, especially in episodic blooms, because they provide freely available regular and reliable measurements over synoptic scales which are simply not attainable using standard *in situ* sampling techniques. Remote sensing, including satellite and airplane as well as *in situ* devices, hold great promise for detecting selected taxa. All together, these techniques allow for the detection of the presence of the toxic blooms. These together with the continuous, long-term meteorological and oceanographic monitoring provides the information on the environmental conditions to understand the underlying mechanisms that lead to their formation and variability through time, especially in a Global Change context. The different techniques for using earth observation data to monitor HABs, as well as the limitations and opportunities, will be discussed in chapter 6 of this report.

Microscopic image-based approaches may provide key information for early detection of HAB within aquaculture vicinities and other applications. It allows the identification of individual organisms and

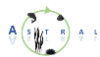


related species within the aquatic environment. HAB events occur at a range of biomass densities and the cell counting threshold for potential HAB events depends on involved species. Microscopic image-based solutions coupled with AI models for phytoplankton identification may be key for early warning and identification of HAB.

For phytoplankton-related studies a reliable automated analysis is fundamental for archive results in large-scale. For this matter, several commercial instruments based on Image Flow Cytometry (IFC) are available. The rich information captured using IFC makes it an ideal candidate for various and microscopic phytoplankton studies. Imaging Flow Cytometers are complex systems based on high-level technology that combine conventional flow cytometry's high-throughput speed and easy identification of individual particles with spatial image acquisition.

The FlowCAM is a bench-top integrated commercial system for particle analysis. The equipment combines fluorescence detection, light scattering detection and image processing capabilities. Combining it FlowCAM automatically counts and analyses the particles in a sample or a continuous flow, providing the user with the capability to evaluate biological particulates and organisms rapidly. This is a flexible and widespread instrument, and despite its high cost, it is highly used in the study and analysis of phytoplankton populations and HAB detection. FlowCytobot is a IFC device focused in situ marine applications, it can be submersed and operated at a maximum depth of 40 meters for up to 6 months to transmit acquired data to a remote facility in real-time. The functionality of the FlowCytobot is like that of the standard flow cytometer and relies on hydrodynamic focusing of a sample stream passing a laser interrogation point. The system is an advanced in-situ device deployed in a myriad of scenarios for marine experiments. Imagestream X Mark II, also known as FlowSight is a relatively new instrument. Unlike the other products cited the FlowSight is not developed for aquatic studies but is primarily used and within the biomedical industry for cellular analysis. The classical use of this instrument family includes cell signalling, cell cycle and mitosis, cell-to-cell interactions and cell death. However, the marine research community has already started to utilise it for phytoplankton related works.

In order to move beyond monitoring into the predictive space it is vital to have a comprehensive understanding of the environmental, physicochemical and biological parameters that lead to a particular observation. Models are often used to synthesize information and quantify relationships within a complex system; to strengthen predictive HAB models, it is not only important to monitor a bloom once it has formed but also to monitor the environmental conditions prior to the event to



identify trends, diagnose the conditions that lead to the formation of the HAB, and observe possible seeding stocks. Well-calibrated diagnostic models can be coupled with physical circulation models and/or dynamic biological models to capture the complex interactions between HAB species with their ecosystem (Franks 2018). Predictive management models generally use inputs of cell counts and environmental conditions (whether derived in situ or from remote sensing) coupled with regional circulation models in order to forecast HAB toxicity, intensity and location; these models are specifically aimed at minimizing the economic effect of HABs and the forecast information are made publicly available to interested parties such as the aquaculture industry (Franks 2018).

As mentioned above, gaining a better understanding of HAB dynamics and the conditions (meteorological, oceanographic) that lead to their formation and decay by means of integrated monitoring systems is of great importance not only in the actual scenario but also to model and forecast these blooms dynamics with the predicted climate change.

2.9 Description of this report

HABs present a significant challenge in terms of predicting when and where they will occur as well as the duration, size and impact it will have. A myriad of factors plays a role in bloom dynamics therefore a multifaceted approach is required. Collecting information from a variety of sources will help our understanding in risks associated with HABs. Identifying all the different causes involved in HAB formation can give a better understanding of IMTA sites vulnerability to HABs. This is of vital importance for the future of aquaculture.

One of the main objectives of this report is to identify potential environmental and climatic risks for ASTRAL IMTA labs affecting production sustainability. In order to do this, we first need to identify all potential biotic, abiotic, physical, chemical and environmental factors which are involved in HABs (chapter 3). Once these are identified a table (chapter 4) will be made to assess which of these factors potentially has the largest effect on the different IMTA's in this study. For these factors methods of monitoring and predicting them will be developed such as sensor development and software integration in WP3 and the earth observation data in chapter 6. Case studies of HAB occurrence and monitoring programmes at the IMTA sites (chapter 5) will be conducted and analysed for global trends. Then we will discuss how HABs might behave under predicted climate change (chapter 7) and how this will affect the IMTA sites in the various geographical regions. This will be concluded (chapter 8) with recommendation for future monitoring at (integrated multitrophic) aquaculture sites.

3 Biotic and abiotic factors

Factors affecting the formation, succession and, demise of an algal bloom can be split up into biotic and abiotic factors. Abiotic factors can be further split up into chemical (nutrients), hydro dynamic (currents, upwelling, stratification), meteorological (wind, rain) and pollution (microplastics). It is important to note that none of the factors responsible for the formation of HABs stand by themselves, however it is rather caused by a complex interaction of environmental parameters. For example, currents will transport HAB to new areas while increases in temperature and precipitation promotes stratification of the water column which provides an ideal environment for many harmful species to form blooms. The effect of those factors will also greatly vary between habitats such as open ocean, coastal areas, and estuaries.

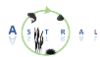
3.1 Abiotic factors

3.1.1 Nutrients, micro and macro, concentrations, and ratios

One of the most often proposed causes for the increase in frequency of algal blooms is eutrophication (Anderson et al., 2002; Glibert et al., 2010; Zhou et al., 2017). Eutrophication can come from a variety of sources, i.e. agriculture use of fertilizers, industry, farming, urban waste. It is not just directly linked to increases in N and/or P sources. The ratio, total concentration, and molecular form all play a role. In addition, the wide variety of feeding strategies as well as the absence or presence of silica, iron, trace metals and vitamins have large effects on the community composition and can favour certain HAB species. In the following sections we will discuss various factors and how they affect harmful algal species and bloom formation.

3.1.1.1 Nutrient sources

It was recognized at the end of the 20th century that there were increasing linkages between nutrient input from wastewater and HABs. Mounting evidence from different areas such as North European coastal waters, Hong Kong Harbour, and the Seto Inland Sea has shown that nutrients from domestic, industrial, and agricultural wastes stimulate HABs (Sha et al., 2021). Several blooms have been directly linked to eutrophication events such as blooms off the coast of China, involving *Prorocentrum sp.*, *Karenia mikimotoi* and other species. These have expanded during recent years in geographic extent and duration, related to the increase in fertilizer use (Anderson et al., 2002). A direct impact was seen in Sequim Bay, Washington, where an intense bloom of the toxigenic diatom *Pseudo-nitzschia*



pseudodelicatissima was found only a week after elevated ammonium levels were reported in these waters (Trainer et al., 2007).

However, it should be noted that although several blooms are associated with increased nutrient levels, not all harmful algal blooms are caused by eutrophication and some areas with high eutrophication levels do not harbour any HABs. This indicates that other factors need to be considered when assessing the likelihood of HABs to occur.

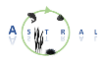
Nutrients can be introduced into the environment from a variety of sources ranging from cattle/pig farm waste ponds, inefficient use of manure/fertilizers to waste disposal/sewage waters of municipal and industrial origin. There is also the potential of increased storm events/flash floods causing wash-off of nutrients into coastal areas. Aquacultural production of shellfish and finfish generates large amounts of excretory products and feed waste, which are rich in N and P and may also include toxins from the medication of caged fish. Nutrients can also be reintroduced into the water column by being dragged up from bottom sediments either due to civil engineering (e.g., dredging) or storm-induced scavenging of bottom sediments in harbours and bays, coastal zones, and even in shallow non-coastal areas (Pettersson & Pozdnyakov, 2013a). These activities or weather occurrences can also resuspend dormant phytoplankton from the sediment back into the water column and act as a seed for blooms. This list of eutrophication sources is certainly not exhaustive and new pathways are constantly being studied.

3.1.1.2 Macro nutrient pool composition and ratios

The composition, not just the total quantity, of the nutrient pool impacts HABs. Various algal taxa display preferences for specific nutrient regimes, including nutrient ratio or form (Anderson et al., 2002; Glibert & Legrand, 2006; Smayda, 1997). For example, diatoms require silica for their growth, whereas certain dinoflagellates apparently have a higher phosphorus requirement than some other species groups. While certain cyanobacteria are able to fix N_2 as a nitrogen source but requires high levels of iron to accomplish this. An overview of the various nutrient ratios and the effect on the phytoplankton community can be found in *Table 2*.

Table 2 Effect of various nutrient ratios on phytoplankton community composition

Nutrient	ratio	Taxa or phytoplankton species
N:Si	increase	increase in flagellates
N:P	decreases	increased harmful dinoflagellate species
phosphorus	increase	shift from diatoms to dinoflagellates
N:P	decrease	shift from diatoms to dinoflagellates
N:P	drops	toxic dinoflagellates



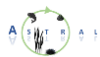
N:P	low dissolved N:P ratios from high phosphate loading	toxic dinoflagellates, <i>Pfiesteria piscicida</i> and <i>P. shumwayae</i>
N:P	lower dissolved inorganic N:P ratios	<i>Karenia brevis</i>
N:P	higher N:P ratios	diatoms

Nitrate loading into coastal waters has increased over recent decades while silicon loading has remained relatively constant or decreased. Seawater is undersaturated with silica and the silica cycle is controlled by diatoms and radiolaria (Smetacek, 1998). As the N:Si ratio in coastal waters shifts due to anthropogenic influences, silicate limitation in diatom biomass may become a feature in coastal waters especially in regions of reduced water exchange (Gilpin et al., 2004). Research into Norwegian fjord systems highlighted that silicate limitation of diatom biomass only occurred at the high N:Si ratio of 4:1 (Gilpin et al., 2004). It has long been established that nutrient stress influences the chemical composition of phytoplankton. Diatoms under N limitation can still accumulate carbon for a short period resulting in an increase in C:N ratio while under Si limiting conditions, both C and N accumulation can continue, resulting in the C:N ratio remaining approximately constant (Gilpin et al., 2004). Such differences in chemical composition under varying nutrient limitations influence the energetic value of an algal cell to predators, such as copepods and in turn their predators, causing changes in energy fluxes throughout the marine food web.

Changes in nutrients can have a multitude of effects, it can favour certain species over others (see *Table 2* for a complete overview), it can affect the chemical compositions of species, but it can also affect the toxin production. Concentration of domoic acid in *Pseudo-Nitzschia fraudulenta* cells increases significantly under Si(OH)_4 limitation (Tatters et al., 2012). This Si(OH)_4 effect on cellular toxin production rates is dramatically enhanced under increased CO_2 concentrations and a lower pH. Si(OH)_4 -limited diatom cells produce more than twice as much toxin when grown in acidified seawater (Tatters et al., 2012) which can have significant effects in the future under predicted climate change (Masson-Delmotte et al., 2021).

3.1.1.3 Macronutrient form

In addition to the composition of the nutrient pool, the chemical form the nutrients are presented in will affect their bioavailability. Nutrient substrates of a given element are not considered 'equal' by phytoplankton. For example, common nitrogen substrates (ammonium, nitrate, and urea) may differentially affect the growth and toxicity of HAB species. Waste excreted in finfish mariculture is mainly in the form of urea and NH_4^+ . This can both directly and indirectly stimulate HABs. Reduced N forms (urea and ammonium) are favoured by some types of HAB species (Anderson et al., 2002;

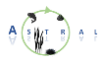


Glibert, Heil, et al., 2018), or may stimulate the growth of non-HAB species on which the phagotrophic HAB species feed (Heisler et al., 2008).

The type of N-source available can have a significant effect on the toxicity. For instance, intracellular toxic concentration in *A. tamarins* was the highest under ammonium enrichment compared to nitrate and urea (Leong et al., 2004). Cyanobacteria *Synechococcus* Clade VIII strains have been shown to be capable of utilizing urea and amino acids as nitrogen sources but are inhibited when inorganic nutrient sources are abundant (Berry et al., 2015). Thus, the ability to utilize urea and amino acids may provide bloom forming *Synechococcus* strains with a nutritional advantage over other phytoplankton.

3.1.1.4 Algal feeding strategies

Many harmful algal blooms have been associated with eutrophication and/or alterations in the concentration, chemical form and ratio of nutrients supplied (Glibert & Legrand, 2006). Linking HABs feeding strategies with specific nutrient forms is a key in understanding HAB development. Feeding strategy amongst algae differ widely as well as the form of nutrients that they can utilize. Many HABs rely strictly upon photosynthesis for their carbon, and use inorganic nutrients for their nutrition (NO_3 , PO_4 , NH_4^+ etc.): these are the *autotrophs*. Some phototrophic species, however, have alternative pathways for acquiring carbon or nutrients: these are the *mixotrophs*. They use dissolved or particulate organic substances to renew their cellular reserves of carbon, macronutrients, amino acids, trace elements or phospholipids. When carbon is incorporated, in this way rather than through photosynthesis the process is *heterotrophy*. Mixotrophic feeding strategies can be further sub-divided, this includes *osmotrophy*, nutrition by direct absorption and uptake of organic molecules, and *phagotrophy*, ingestion of prey or other food particles (Barsanti & Gualtieri, 2014, Richmond, 2003). Many mixotrophic species display a continuum of dependence on these alternative pathways. Some species only display osmotrophy or phagotrophy when cellular requirements can only be partially met by autotrophy. Other species have lost the ability for autotrophy and rely exclusively on phagotrophy and osmotrophy. Heterotrophic dinoflagellates are among the species that have lost the ability for autotrophy. In fact, roughly half of all dinoflagellates are obligate heterotrophs (Glibert & Legrand, 2006). Many flagellate species, including various harmful dinoflagellates, are mixotrophic or heterotrophic phagotrophs predominantly consuming particulate rather than dissolved nutrients (Stoecker et al., 2006a). Due to those various feeding strategies, it is important to not only assess total nutrients but also nutrient chemical composition and ratios.



3.1.1.5 Trace metals & vitamins

Whilst the availability of macro-nutrients (N, P, Si) is important, so too is the availability of micro-nutrients (trace metals, vitamins). Iron is needed for metabolic functions in photosynthetic electron transport, respiratory electron transport, nitrate and nitrite reduction, sulphate reduction, N₂-fixation (Andersen, 2005). Iron is highly insoluble in oxygenated water, hence upwelling waters are poor in iron (Smetacek, 1998). However, dust storms can introduce iron-rich particles from desert areas to the sea (Tan et al., 2011). These storms can often be linked to large algal blooms in coastal areas. This iron input is predicted to increase due to increases in desertification, storm intensity and frequency (Stocker et al., 2021).

Many HAB forming dinoflagellates have a high demand for B12 and to a lesser extent B7 and B1. HAB species such *Cochlodinium polykrikoides*, *Karlodinium veneficum*, and *Prorocentrum minimum* can reduce B12 levels by 90% during blooms and may exhaust standing stocks of vitamins in hours to days (Tang et al., 2010). In eutrophic regions, due to entry of contaminated river flow and organic wastewater, which include high levels of vitamin B12 a fast growth rate can be maintained.

3.1.1.6 Geography and retention time

Similar nutrient loads do not have the same impact in different environments or in the same environment at different points in time (Anderson et al., 2002). Geography and topography play a role in nitrification and retention time. In boundary upwelling systems, such those found in California, Canary and Benguela areas, nutrients are introduced to the surface by means of cold nutrient-rich water upwelling to the surface (Trainer et al., 2010). In open coastal regions retention of nutrients and phytoplankton is controlled by solar induced stratification, wind stress, and tidal-driven vertical mixing (Pettersson & Pozdnyakov, 2013a) While in urban coastal areas the water column nitrification and structure is more likely to be determined by run-off patterns (Anderson et al., 2002) as well as effecting turbidity levels (Phlips et al., 2020). Coastal topography features such as banks, canyons, and islands affect not only the retention of nutrients from land-based sources but also affect local circulation patterns that may transport cells or nutrients from offshore to inshore (Glibert, Beusen, et al., 2018). Coastal retention will also have important implications for HAB formations. In retentive regions, nutrients can be retained, recycled, and transformed, keeping the site in a nutrient-enriched condition. This can lead to enhanced phytoplankton accumulation, high-biomass HABs, and hypoxia. The retention of nutrients has been shown to vary greatly with coastal type and oceanographic conditions. In particular, fjords and estuaries are highly effective in retaining nutrients with characteristic long residence times (Glibert, Beusen, et al., 2018). Understanding the coastal topography, nutrient loading and retention times is critical to assess the impacts of aquaculture-derived nutrients when establishing



IMTA. When coastal retention is high, coupled with nutrification, the likelihood of HABs increases compared to a well-circulated system (Glibert, Beusen, et al., 2018). Retentive zones can also accumulate phytoplanktonic cysts resulting in reoccurring blooms once a population of harmful algal species becomes established (Glibert, Berdalet, et al., 2018).

Though the majority of the nutrification will happen in the coastal regions, due to terrestrial run-off, the open ocean should not be ignored. The upper ocean is physically forced by a variety of interactions from air, land, rivers, and the ocean interior which affect nutrient retention time. Increased water temperature leads to increased stratification ($N\downarrow$), changing winds ($N\uparrow\downarrow$), altered current velocities ($N\downarrow\uparrow$) and eddy kinetic energy ($N\uparrow\downarrow$) (M. L. Wells et al., 2020). This example illustrates the difficulties in predicting future trends of harmful algal blooms and determining what effect nutrification has on algal blooms.

3.1.1.7 Conclusion of nutrification and HAB

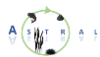
Nutrient loading, ratio, chemical form, and retention time are key to successful bloom formation. Experimental studies are critical to further the understanding about the role of nutrients in HABs expression and will strengthen prediction and mitigation of HABs. This needs to be done on local strain due to high strain variability. Field observations (Gilpin et al., 2004; Hardison et al., 2013; Larson et al., 2018) demonstrated that management and regulation of nutrient inputs to water masses can lead to a significant reduction in HABs.

Though more in depth understanding is needed in nutrification and HAB dynamics, there is consensus about causes and effects:

- Degraded water quality from increased nutrient pollution promotes the development and persistence of many HABs.
- The composition, not just the total quantity, of the nutrient pool impacts HABs.
- High-biomass blooms must have exogenous nutrients to be sustained.
- Both chronic and episodic nutrient delivery promote HAB development.

3.1.2 Hydro dynamic factors: Stratification, upwelling, currents, eddies, frontal jets

There are several different and sometimes competing physical factors that promote growth conditions for phytoplankton. These factors include the natural variability of hydrodynamics, light availability, nutrient loading, geographical features, and climate cycles. The various physical parameters work differently on different marine ecosystems such as open ocean, open coastal regions and (semi-)



enclosed coastal systems. Furthermore, coastal areas have a wide variety of features such as keys, islands, sandbanks, silt banks, tidal fronts, and others. It restricts hydrodynamic interaction with the open ocean, resulting in longer residence times not only in the inner but also outer parts of estuaries, fjords, or embayment. Depending on the marine system some physical parameters play a larger role in HAB formation than others. Several hydrodynamic features associated with an increased risk or occurrence of HABs are; upwellings, tidal fronts, formed at the abutment of stratified offshore water and tidally mixed waters, convergence at fronts associated with upward vertical movements of significant water masses or invasion of river plumes (Pettersson & Pozdnyakov, 2013a). The various hydrodynamic factors contributing to HAB formation, maintenance and demise are discussed below and summarized in Table 3.

3.1.2.1 Stratification

Water column vertical mixing and stratification are controlled by water temperature and salinity. This in turn is influenced by freshwater discharge from rivers, rain, offshore currents, and upwellings (Pettersson & Pozdnyakov, 2013a), solar radiation and water evaporation. Stratification of the water column can occur in a variety of ecosystems such as fjords, open and semi-enclosed coastal zones, shelf seas, and upwelling areas to varying degrees. Stratified water columns have a vertical structure typically composed of layers separated by pycnocline: a wind-mixed surface layer and a tidally mixed bottom layer. Stratification stability depends on local geography such as catchment characteristics and river, estuarine, bay, and coastal geomorphology local hydrography, depth, and intensity of the pycnocline while wind stress, weather events causing and magnitude of river flow effects duration (M. L. Wells et al., 2020). Environments where water residence time is increased creates a condition favourable to many HAB species. Inshore areas of coastal upwelling systems, in the lee of headlands, are one such retentive environment (Berdalet et al., 2014). Phytoplankton can increase to high numbers in these waters. The persistence and strength of stratification often appears as one of the most important physical conditions for phytoplankton development (Raine et al., 2018). Though it occurs in a wide range of systems it tends to be more pronounced the more isolated the bay is from the open coast. Any increase in stratification is expected to exacerbate the potential for HABs (M. L. Wells et al., 2020). It should be noted that although HABs occurrence is associated with stratification, the majority of stratified waters do not harbour HABs. Several other factors are required for successful bloom formation. Depth, timing, duration, and the type of stratification are important factors for blooms development, sustainability, and species type as well as nutrification regime.



Stratification effects in water columns where nitrification occurs will influence which taxa are dominant in that water column. Stratified waters have a warm, and possible low salinity, surface layer with limiting water exchange which tend to limit the delivery of nutrients (Pitcher et al., 2010). Many dinoflagellates, raphidophytes, and cyanobacteria HAB species have physiological ranges and life history traits that make them strong competitors under stratified conditions. These conditions favour flagellates, which have the ability to actively move between stratified surface waters and nutrient-rich deeper waters (Smayda, 1997), and cyanobacteria which prefer warmer water and can fix atmospheric nitrogen (O'Neil et al., 2012). There is a consistent pattern of lower affinity for nitrate and ammonium in dinoflagellates and raphidophytes compared to diatoms which is a strategic advantage in stratified waters (R. M. Kudela et al., 2010). Many HAB species are strong vertical migrators which enables them to access nutrients across pycnoclines (Smayda, 1997) or are phago-mixotrophic and adapted to assimilate alternative forms of nutrients when dissolved inorganic nutrients are low, a frequent occurrence in stratified water bodies (Flynn et al., 2018; R. M. Kudela et al., 2010; Stoecker et al., 2006a).

The timing of stratification can determine which taxa might become dominant in the water column. During winter in temperate regions and higher latitudes the day length is too short and the water too cold for most species to grow. However, at this time mixing of the water column returns nutrients to the surface layers. In spring, with increasing daylight and water temperatures, rapid phytoplankton growth begins which is characterised by diatom species. They are capable of utilising available nutrients and growing fast at a time on which the population of zooplankton grazers has not yet increased (Stubbs et al., 2016). During summer, when the water temperature is warmer with lower nutrient levels and increased stratification, the composition of the phytoplankton community contains a greater proportion of dinoflagellate species (Stubbs et al., 2016). While warmer water causes stratification which can benefit algal groups such as cyanobacteria and dinoflagellates, if stratification occurs early in the spring when nutrient levels are still high it can favour diatom blooms. With increased warming, and potentially earlier peak runoff, more rapid onset of stratification leads to earlier spring blooms and the opportunity for HAB species to be present for a greater portion of the year (Moore et al., 2011). However, intensified spring diatom blooms sometimes can lead to lower nutrient retention in subsequent summer stratified surface waters. Thereby, it reduces the potential for dinoflagellate HABs to develop later in summer (M. L. Wells et al., 2020).

3.1.2.2 Upwelling

Upwelling systems bring nutrient-rich deep water to the surface. As discussed earlier, nutrients play a vital role in achieving high phytoplankton biomass. HABs occurring in upwelling systems have



historically been dominated by flagellate species, in particular the proliferation of dinoflagellates (Pitcher et al., 2010). Open embayment's tend to be the most susceptible to bloom development in upwelling systems. These provide sufficiently high nutrients but remain stratified and accessible to the introduction, concentration, and retention of coastal blooms (Pitcher et al., 2010). In open coastal regions with shallow waters, algal cysts buried in bottom sediments can be brought back to surface layers and initiate a massive growth of the phytoplankton community. This process is regularly observed, for example, in connection with the winter rest of several algae cysts at the bottom and their activation during early spring blooms (Pettersson & Pozdnyakov, 2013a). This up- and downwelling can transport cysts over large areas. For open coastal regions, upwelling events are thus critically important for initiation and proliferation of HABs.

The timing and duration of upwelling is another physical feature that plays an important role in many blooms. For instance, in the case of the Benguela upwelling system HABs accumulate subsurface as stratification increases during the upwelling season and move onshore as upwelling relaxes (R. Kudela et al., 2005). These regions are subjected to strong seasonal cycling with intermittent infusions of nutrients from nutrient-rich deeper water layers (Pettersson & Pozdnyakov, 2013a).

3.1.2.3 Currents, eddies, frontal jets

Current, eddies and frontal jets are more often associated with spatial distribution of the HAB (Pettersson & Pozdnyakov, 2013b; Pitcher et al., 2010). They affect the alongshore transport of algal blooms and upward vertical movements or invasion of water. This may cause a spike of algal concentration enhancement or subduction of algal blooms beneath the surface (Pettersson & Pozdnyakov, 2013a). These hydrodynamic features are in turn affected by topography and shore geometry which affect further distribution of HABs (Pettersson & Pozdnyakov, 2013a).

Eddies promote a rapid increase of cell concentration due to the accumulation and retention of cells because of the circular movement of the water which traps the phytoplankton community (Pettersson & Pozdnyakov, 2013a). It leads to the formation of isolated water masses with specific chemical, biological, and thermo-hydrodynamic regimes. Such unique properties are associated with a relatively long residence time favouring rapid and massive development of algae blooms. At the same time, under such circumstances high algal biomass can lead to the rapid depletion of nutrients, extensive zooplankton grazing and, ultimately, a premature collapse of the population (Pettersson & Pozdnyakov, 2013a). The Oman Coastal Current (east Arabian Current) and the Sea of Oman currents form a frontal zone which generates well developed cyclonic and anti-cyclonic eddies of ~ 200 km in



diameter. Cyclonic eddies bring large amounts of nutrients up to the surface and stimulate phytoplankton growth. While anti-cyclonic eddies down well and are associated with lower levels of nutrients and phytoplankton. These eddies can transport blooms on and offshore with significant impacts to the aquaculture industry when these blooms occur (Harrison et al., 2017).

3.1.3 Meteorological abiotic factors

3.1.3.1 Wind

Wind stress mainly affects the surface boundary layer and phytoplankton assemblages therein by various physical processes, which are not mutually independent, such as waves, currents, and turbulence (Figueiras et al., 2006). This layer is also heavily influenced by run-off and solar radiation. Hence, the upper ocean is the site of a myriad of interacting dynamic physical processes all of which influence distribution of blooms both directly and indirectly.

Wind has been linked to transport of harmful algal blooms over long distances. It can also concentrate biomass when the wind direction is towards the shore. *Dinophysis* sp. blooms observed around the Shetland Islands in Scotland during the summer of 2006 and 2013 coincided with a change in the prevalent wind direction (Whyte et al., 2014). Although low levels of toxins and *Dynophysis* sp. were found in the preceding weeks, it was not until the wind direction changed that numbers increased to high levels. It dissipated to baseline levels after the wind direction changed back to normal (Whyte et al., 2014). Wind stress fluctuations vary at seasonal and interannual scales, and thereby clearly control the timing of blooms (Pitcher et al., 2010). Analysis of wind patterns therefore offers a potential method for early warning of harmful algal events (Whyte et al., 2014).

For example, wind direction and speed in the Northeast Atlantic and the North Sea is strongly influenced by the North Atlantic oscillation (NAO). It has been noted that a positive trend in the NAO suggests there will be an increasingly westward component to prevalent wind directions in the North Sea, which could lead to an increase in the occurrence of these harmful algal blooms (Whyte et al., 2014).

Excessive wind such as hurricanes can resuspend nutrient as well as sediment in shallow water environments such as estuaries. It enhances the potential for blooms by elevating nutrient load but may also provide increased turbidity levels. This can lead to a reduction in photosynthetic activity reducing the risk of HABs. Blooms tend not to form until the turbidity decreases (Phlips et al., 2020).



Hurricanes often go accompanied with excessive rainfall which will have their own influences on phytoplankton communities and bloom dynamics.

3.1.3.4 Precipitation

HAB events often follow a period of intense rainfall and increased runoff, both of which enhance stratification and increasing nutrient load, which is favourable to algal growth (Pettersson & Pozdnyakov, 2013a). Excessive rainfall can cause lakes to reach maximum water levels and flood mitigation might result in a quick discharge into nearby areas such as an estuary. These flood waters can be rich in nutrients and possible HAB seeding stock (Phlips et al., 2020). External nutrients enter marine systems through surface run-off, groundwater discharge, direct rainfall inputs, septic tank leakage and permitted and accidental releases from sewage treatment systems (Phlips et al., 2020). One of the important cyclical phenomena that affects rainfall are El Niño/La Niña periods. El Niño periods are often characterized by higher rainfall than La Niña periods which has a direct effect to the stratification of the water column.

3.1.4 Seasonal and climate cycles

HAB dynamics are influenced at all spatial-temporal scales. A variety meteorological and climate cycles affect the timing and intensity of HABs as well as the community composition. The main cycle is the seasonal cycle which affects light, and temperature. In subtropical ecosystems, relatively modest seasonal variability in temperature and irradiance can reduce the predictability of seasonal trends in phytoplankton biomass and composition (Bienfang et al., 1984). Other cycles which affect HAB likelihood are monsoon patterns which can bring excessive rain and the lunar cycle which causes tidal mixing. Tidal cycles affect the vertical mixing and stratification of the water which play major roles in bloom dynamics (R. M. Kudela et al., 2018; Smayda, 1997; M. L. Wells et al., 2020). Tide has the lowest amplitude around the summer solstice and the largest around the equinox. Lower tide amplitude results in less dilution of the water masses which favours bloom development. Ni Rathaille et al., (2006) showed that the potential for bloom development, in terms of tidal dilution, is greatest in June and July in Cork harbour, Ireland. Tidal cycles also influence abundance and community composition of phytoplankton. High abundances of diatoms were associated with the major spring tide of each month while dinoflagellates were more abundant during the intervening minor spring and neap tides for Monhegan Island, Maine U.S.A. (Balch, 1981). Since tides are predictable, they can aid in understanding when blooms might occur.

The intensity and the effect of seasonal occurrences such as stratification and upwelling can be further enhanced by climate cycles such as the NAO, the Pacific Decadal Oscillation (PDO) and El Niño/La Niña

which causes changes in currents, upwelling, wind, precipitation, and temperature patterns (GEOHAB, 2006). Climatic cycles can have a direct effect on phytoplankton communities. For instance, *Gymnodinium catenatum* tends to disappear during warm El Niño conditions in Mexico (Shumway et al., 2018).

Table 3 Various meteorological and climate cycles and their effect on other abiotic factors

Factor	Effect	References
Wind	Timing of bloom Transport of bloom Concentration of bloom Turbidity Mixing	(Pitcher et al., 2010; Whyte et al., 2014)
Precipitation	Nitrification Stratification	(Pettersson & Pozdnyakov, 2013a; Phlips et al., 2020)
Seasonal & tidal cycles	Predictable Effected by climatic cycles Mixing and stratification	(Balch, 1981; R. M. Kudela et al., 2010; Ni Rathaille et al., 2006; Smayda, 1997; M. Wells et al., n.d.)
Climatic cycles	Stratification, salinity, wind	(Glibert & Legrand, 2006; Shumway et al., 2018)
Upwelling	Provides nutrients	(R. Kudela et al., 2005; Trainer et al., 2010)
Currents	Transport blooms	(Pettersson & Pozdnyakov, 2013a; Pitcher et al., 2010)
Eddies	Concentrate blooms	(Pettersson & Pozdnyakov, 2013a; Pitcher et al., 2010)
Stratification	Nutrient distribution Community composition	(Flynn et al., 2018; R. M. Kudela et al., 2010; Pitcher et al., 2010; Smayda, 1997)

3.1.5 Microplastics

Plastic pollution in marine waters around the globe is increasing exponentially. Plastic can be classified in macro, micro and nanoplastic, according to the size. Microplastic pieces range in size from 100 nm to 0.5 cm and nanoplastics are smaller than 100 nm. Microplastics can be a vector for the dispersion of metals, antibiotics, toxic chemicals, and marine organisms across the continents through ballast water (Naik et al., 2019) or by means of currents, wind and waves. Various taxa have been found on microplastics. It ranges from pathogenic bacteria (*Vibrio cholerae*), phytoplankton such as diatoms, coccolithophores and cyanobacteria to bryzoans, marine worms and insect eggs. There are evidences that microplastics provide a unique substrate for attachment and growth of organisms. This has implications for the emergence of novel species of fouling organisms which have superior attachment/degradative abilities with respect to plastic (Naik et al., 2019).

Microplastics collected during an active bloom of *Alexandrium taylori* were covered with dividing cells, vegetative cells and temporary cysts of this dinoflagellate. Cysts have the ability to survive long periods under unfavourable conditions. If they are attached to microplastic they can be dispersed to new areas which were previously unaffected by this species (Masó et al., 2003). Potential harmful benthic dinoflagellates are known to attach to several biological substrates such as patches of detritus where



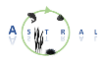
it contributes to the 'seed-banks' for blooms. Though these organisms can also occur on natural substrates (faecal pellets, algal aggregates, rocks, wood, etc) they might form a larger problem on microplastics due to the extremely low degradation rates of microplastics (Kettner et al., 2019). Masó et al., (2003) identified several potential harmful dinoflagellates such as *Ostreopsis* sp. and *Coolia* sp. on microplastics as well as several resting cysts of unidentified dinoflagellates. The ability of microplastics to remain in the water column rather than settling out implies attached organisms can persist longer. They can also be transported over larger distances by wind, wave and ocean currents. Microorganisms can be found on all types of plastics. Kettner et al., (2019) studied the eukaryotic composition on samples of polystyrene and polyethylene, some of the most commonly found plastics. Common organisms found were from the SAR supergroup (Stramenopiles + Alveolata + Rhizaria), fungi, Holozoa including Metazoa, and a variety of algae such as diatoms *Skeletonema* sp. and *Thalassiosira* sp. and chlorophyta such as *Scenedesmus* sp.

However, it should be noted that other studies indicate the harmful effects to the health of microalgae that are attached to plastics. It will affect organisms directly through harmful chemicals released from their surface (Naik et al., 2019). It is critical to look further into the interaction between HAB forming species, the dispersal by microplastics and the survivability/ health effects of this on those species. However early indication shows that microplastics play a very important role in disseminating HAB species (either vegetative cells or cysts), as part of their associated microfouling communities, to new areas, where they may then form blooms (Naik et al., 2019) and introduce previous unknown species.

To summarize, life stages that enhance species survival at the dispersal phase can greatly contribute to the success of invasion. Waters both affected by bloom events and susceptible to litter accumulation might become meaningful sources of the seed pool. In particular, species which are naturally attaching to substrates and/or have obligatory cysts stage life cycles. The increase in plastic debris in the marine environment could favour the probability of success in microalgae dispersal. Astral work package 3 deals with developing a microplastic sensor and it will be of great interest to monitor microplastics at IMTA sites in relation to harmful algal species occurrence.

3.2 Biotic factors

Biotic factors such as grazing, viruses and allelopathy can have positive and negative effects on the bloom initiation, duration, and termination. These biotic factors cause effects throughout the food chain. The effect of the biotic factors is further modified by the oceanographic and chemical



conditions present. In this chapter we discuss biotic factors and the role they play in promoting and/or mitigating harmful algal blooms.

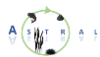
A variety of biotic and abiotic conditions favour HAB formation. However, it is also important to monitor factors which can mitigate, minimize, or destroy blooms because the presence or absence of these factors will influence the overall impact of the algal blooms. Understanding both the factors which are associated with increase of HABs as well as controlling HABs is important as a predictor for the impact of HABs.

3.2.1 Grazing

3.2.1.1 Grazers

Several taxa with a wide range of sizes, from micro-grazers (ciliates) to meso-grazers (rotifers) to macro-grazers (copepods), are involved in the grazing of phytoplankton. Grazing pressure is highly variable and depends on community composition and environmental conditions (mixing, turbulence, and season). Grazing may retard initial development of an algal bloom, but once developed, the bloom becomes immune to grazing pressure. Under other circumstances, grazing is incapable of preventing the development of algal blooms, but may contribute to their termination (Turner, 2006). For example, several species of ciliates ingest harmful phytoplankton incurring no adverse effect and play a role in controlling blooms (Strom, 2002). However, in other cases, toxic dinoflagellates or other harmful phytoplankton appear to have deleterious effects on ciliates such as changes in swimming behaviour, reduced ingestion, inability to support growth or even causing ciliate mortality summarized by (Turner, 2006).

Marine rotifers feed upon a variety of autotrophic and heterotrophic flagellate prey. Similar to previously discussed biotic factors, the response is species specific for the predator and the prey. For instance, the rotifer *Brachionus plicatilis* feeds upon the toxic dinoflagellate *Pfiesteria piscicida* (PFTx toxin) with no apparent adverse effects in terms of reduced fecundity or increased mortality. When fed a different species, *Aureoumbra lagunensis* (Texas brown tide alga), the same rotifers failed to grow. The same holds true for copepods. Various species of copepods feed on toxic phytoplankton which induced adverse effects, such as reduced ingestion, fecundity, egg hatching success delayed development and/or behavioural changes or survival (Turner, 2014). However, in other studies with different harmful algal diets or copepod species, there were no apparent adverse effects associated with eating toxic algae. Much of this variability likely relates to different concentrations of toxins



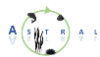
ingested, and different tolerance for various toxins by different copepod species from different locations (Turner, 2014). Generally, diets with more toxins cause greater effects.

Little is known about bioaccumulation of toxins in zooplankton, but some evidence suggests that PSP toxins such as those produced by *Alexandrium* sp. might accumulate in rotifers, mesoplankton and copepods to some extent (Turner, 2006). Accumulation can cause chronic problems which might not be obvious during short-term bloom studies. De-sensitivity to toxins can also play a role. There may be a biogeographic aspect to effects of harmful algal toxins on copepods. Colin and Dam (2002) found that copepods from areas where they are routinely exposed to HABs were less affected by ingesting these toxic phytoplankters than copepods of the same species that were from areas where they were not regularly exposed to algal toxins.

The toxins produced by microalgae do not necessarily repel grazers. The numerous documented cases where phycotoxins have minimal effects on grazers suggest that toxicity may be coincidental, and that these chemicals may have evolved for other reasons, such as nitrogen storage, chromosome structural organization, pheromones for induction of sexuality, or they may be vestigial remnants of archaic pathways for nucleic acid biosynthesis (Turner, 2006).

The most numerically dominant components of the macro-zooplankton are copepods. However, they seem to have less of an impact on grazing than the micro-zooplankton (i.e. ciliates) community. They are one of the dominant grazer groups of HAB species and while toxic HAB do not appear to kill micro-zooplankton, their chemical defences can impair grazer activity (Strom, 2002). Copepods do still play an important role in the grazing of HABs since they act as an entry point to bioaccumulation up the pelagic food web to species as fish and marine mammals (Turner, 2014).

However, one more group of grazers needs to be considered. Phagotrophic microalga, and this groups seem to have the largest impact on blooms. Phagotrophic algae obtains their nutrients and carbon by consuming other microalgae. This is a is common strategy among bloom forming dinoflagellates and haptophytes (Stoecker et al., 2006b). Many algal species can achieve fast growth rates under optimal light and nutrient conditions, but they do not form mono specific blooms. Phagotrophy can be an ecological strategy for eliminating or reducing predators and competitors (Stoecker et al., 2006b) and thus increasing mono specificity in communities. Phagotrophy might explain some aspect of bloom dynamics. By consuming their competitors, they utilize alternative nutrient resources, and this could explain why some blooms persists after inorganic nutrients have run out (Stoecker et al., 2006b). In



addition, phagotrophy aids species such as *Protoperdinium crassipes* to obtain toxins from other toxic dinoflagellates when feeding upon them (Jeongl & Latz, 1994).

3.2.1.2 Grazer-Grazing interactions

A complicated dynamics exist between grazing and toxin producing harmful algal blooms. Algae capable of producing toxins are mainly producing toxins when they are under stressful conditions such as deplete nutrients (Anderson et al., 2002; Granéli et al., 2008; Granéli & Johansson, 2003). So, not only do they provide grazers with a nutrient imbalance they can also be toxic to them (Flynn, 2008). This has consequences for health of the grazers and impacts to higher trophic levels.

One difficulty in assessing grazing effects is the wide array of conditions needed. It includes prey densities, predator-prey pairings, mixed prey opportunities, and strain differences among prey and predators as well as various abiotic factors (i.e., mixing stratification, turbulence). For instance, turbulence affects dinoflagellate predator-prey interactions by changing the swimming ability of dinoflagellates (Burkholder et al., 2006). Another difficulty in assessing the impact of grazing on the formation, duration and demise of harmful algal blooms is due to the spatial and temporal variability of the various species presents and their interactions. The varied response between harmful algal blooms and grazers requires a full understanding of which types of phytoplankton species and predator species are present in the area. Since zooplankton and phytoplankton communities change during the year, limited information is often available. Given the diversity of prey ecology and predator feeding strategies various defences and the influence of abiotic factors, it is unlikely that a general predator-prey interaction model will be sufficient to describe the effect of predations on the formation, duration, and demise on a harmful algal bloom.

3.2.2 Allelopathy

Several species of phytoplankton can produce and excrete allelopathic chemicals. For this report allelopathy is considered a process involving secondary metabolites produced by primary producers that influence the growth, development, and photosynthetic ability of any co-occurring phytoplankton or act as a grazer deterrent or predator avoidance.

While toxic HAB do not appear to kill micro-zooplankton, their chemical defences can impair grazer activity or affect their health. Though the function of some allelopathy metabolites is targeted against co-occurring species of phytoplankton or zooplankton grazers, the effects of these metabolites are not limited to those organisms and can affect all trophic levels of the food web. For example, the dominant



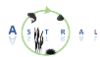
grazers on HAB species are micro-zooplankton (Turner, 2006), which in turn are preyed upon by macro zooplankton and fish.

The most well documented effects of allelopathic compounds are those that cause harm to humans through the ingestion of shellfish or fish. However, they are not the only victims of toxic algae. Brevetoxins produced by *Karenia brevis* for instance have been found throughout the food chain and are accumulated in or transferred by organisms at many trophic levels (Landsberg et al., 2009). An analysis on the impacts of various toxic algae on seabirds revealed an array of responses ranging from reduced feeding activity, inability to lay eggs, and loss of motor coordination to death (Shumway et al., 2003) whereas bioaccumulation of saxitoxin have been detected in common dolphins, seals, and whales (Fire et al., 2021).

Currently around 40 phytoplankton species, of which the majority are flagellates, have been identified with allelopathy toward algae. It should be noted that it is not always clear if it is true algal allelopathy or if it comes from associated bacteria (Granéli et al., 2008). Toxicity can be obtained from prey such as is the case for phagotrophic *Protoperdinium crassipes* when it feeds on toxic dinoflagellates (Jeongl & Latz, 1994). However, most mixotrophic toxic algae can be cultivated autotrophically and still produce toxins. Suggesting that they synthesize their own allelopathic factors.

The strongest allelochemicals known to date in aquatic ecosystems have haemolytic capacity which, by disrupting the cell membranes of other algal species, will kill the latter in the process. Several authors, beginning with Estep and MacIntyre (1989) have suggested that some algal toxins may immobilize, make “leaky” or kill other types of cells in the plankton as part of a nutritional strategy. Allelochemicals may reduce competition or predation, but they can also make resources available which can be utilized by phagotrophic or mixotrophic microalgae (Stoecker et al., 2006b). Allelopathy and phagotrophy are prevalent among bloom forming algal species which give them advantages over other species to dominate resources in the plankton community and form toxic or high biomass harmful blooms (Stoecker et al., 2006b).

Most algal allelochemicals, however, are not as damaging and might temporarily only inhibit some function in the target species’ ecophysiology. They do not cause death (Granéli et al., 2008). For example, induce cyst formation (Granéli et al., 2008), photosynthesis inhibition, decrease in growth rate, or grazing inhibition have all been shown to a certain degree as a reaction to allelopathic compounds. They can also aid in nutrient acquisition. For example, Prince et al. (2013) found that domoic acid can improve the competitive ability of *Pseudo-Nitzschia delicatissima* over the



cosmopolitan diatom *Skeletonema marinoi*. It is suggested that *Pseudo-Nitzschia* spp. may use domoic acid as part of an iron uptake system that might render iron unavailable for the competitor. By inhibiting the growth of other phytoplankton, albeit indirectly, through the production of domoic acid, *Pseudo-Nitzschia* spp. is likely to alter the composition of the phytoplankton community. In addition, domoic acid can directly alter ecosystem functioning due to its toxicity at higher trophic levels.

The timing of releasing allelopathy chemicals plays a significant role in bloom dynamics. With few exceptions, allelopathic effects are only significant at very high cell densities typical of blooms. Little experimental evidence exists that they play a role at pre-bloom densities and thus might not be as important for bloom formation as previously thought (Jonsson et al., 2009; Flynn, 2008). Allelopathic interactions can aid in prolonging blooms by lysing competitors and thus releasing assimilated nutrients back into the water (Jonsson et al., 2009). The general pattern is that higher cell numbers of the donor species result in more magnified detrimental effects. The intensity and effect differ not only by species but also by strain both for the producer and receiver of the chemicals (Granéli & Johansson, 2003).

The observed maximum allelopathic activity has been described in the exponential, late exponential and stationary phase depending on species (Granéli et al., 2008). Production can furthermore be affected by light, competition, grazing and pH. However, the most important contributor to the allelopathic concentration are nutrients especially, nutrient limitation (Granéli et al., 2008) or an unbalanced N: P ratio (Granéli & Johansson, 2003). *Pseudo-nitzschia cuspidate* production of domoic acid (DA) increases under elevated light conditions, while under low light conditions the N-substrate plays a role in determining the concentration of DA. Toxicity was increased with a NO_3 substrate followed by NH_4^+ and urea (Cuspidata et al., 2013). Nutrients play an important role in the allelopathy of microalgae and monitoring nutrient levels at IMTA sites is of great importance.

3.2.3 Bacteria

The biological factor potentially of greatest significance in regulating populations and even toxin dynamics of harmful algae are bacteria. They exist as free-living forms or attached to algal cells and can influence either positively or negatively algal growth rate and transition between life stages. Many bacteria have algicidal properties and have been responsible for the decimation of algal blooms (Barak-Gavish et al., 2018; Imai & Imai, 2015; F. Zhang et al., 2020), while others can aid algae by providing essential compounds such as vitamins needed to support their growth (Croft et al., 2005). There is a large diversity in bacteria and thus it can be expected that they play multiple roles during initiation,



progression, and degradation of an algal bloom with changes in bacterial communities occurring over the course of a bloom. Several of the function of bacteria in plankton dynamics are listed in *Table 4*.

Table 4 Various roles of bacterial communities during an algal bloom (list summarized from Kodama et al., 2006)

- Provide nutrients
- Produce siderophores which chelate iron
- Have algacidal properties
- re-mineralization dissolved organic matter excreted by algae
- specific bacterial taxa have been linked with growth stimulation of dinoflagellates
- Bacteria can act as a prey item for phagotrophic algae.
- Inhibition of a bacterial taxon by other bacteria may also indirectly modulate the population dynamics of HA species
- certain fraction of the naturally occurring
- bacteria can either promote or prevent the formation of cysts.

Phytoplankton bloom events result in distinct changes in the composition and availability of nutrients as well as physical conditions within aquatic ecosystems, resulting in significant effects on bacterial communities (Kamiyama et al., 2000; Matcher et al., 2021). Microbial communities play an important role in bloom formation, maintenance, and decline. There is a close correlation between the bacterial community profiles and the physiological state of the bloom, for example, as was observed with Flavobacteria increasing with increasing *Heterosigma akashiwo* abundance (Matcher et al., 2021). While during the decay of bacteria such as Synechococcales, Cryomorphaeaceae and Sporichthyaceae increased (Matcher et al., 2021). Changes in the microbial loop can be found during the initial stages of the bloom and during bloom decay. During the bloom bacterial abundance diversity decreased, while it increased during the late stages of the bloom. Suggesting that the temporally increase in the microbial loop was caused by dissolved organic matter produced from *Heterosigma akashiwo* (Kamiyama et al., 2000).

The interactions between bacteria and microalgae are species specific and even life stage specific. Removing the associated microbial community from *Pseudo-Nitzschia multiseriis* showed a decline in growth rate suggesting a dependence on them while introducing the bacteria strain Croceibacter strain



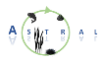
proved lethal (Amin et al., 2015). Sulfitobacter strains significantly enhanced specific *P. multiseriis* growth however a closely related *Phaeobacter* strain did not (Amin et al., 2015).

Algicidal bacteria in marine coastal ecosystems have received attention concerning the termination of toxic blooms (Imai et al., 2006). Algicidal bacteria induce rapid cell lysis in algae (Kodama et al., 2006). Most known algicidal bacteria belong either to the phyla Bacteroidetes or Gammaproteobacteria (such as *Alteromonas*, *Pseudomonas* and *Pseudoaltermonas*) but algicidal Alphaproteobacteria are also reported. Only a minority are Gram positive such as members of the phylum Actinobacteria and the genus *Bacillus* (Firmicutes) (Meyer et al., 2017). Due to the large diversity of bacteria and algae there is also variety in the algicidal specificity. Many algicidal bacteria show a broad host range and inhibit or lyse microalgae from different phyla. The algicidal bacterium *Cytophaga* sp. J18/M01 prey upon, harmful *Chattonella antiqua* microalgae, but also other common microalgae such as diatoms (Imai et al., 2001). Others are only active against specific groups or certain species. *Karenia brevis* C2 and *Karenia mikimotoi* G303ax-2 and CCMP429 are susceptible to the algicidal bacteria 41-DBG25 while 5 other *K. brevis* and 2 other *K. mikimotoi* were resistant to it (Roth et al., 2008). Bacterial community interactions play a role in their algicidal ability. *K. brevis* NOAA-1 was susceptible to the algicidal *Cytophaga*-Flavobacteria-Bacteroides (CFB)-bacterium S03 and 41DBG2 when no other bacteria were present but is resistant to it when other bacteria are present (Roth et al., 2008), suggesting some interaction between the various bacteria.

Specificity of algicidal bacteria could not only be affected by community composition but also by compounds produced by the microalgae. A Roseobacter strain (Sulfitobacter D7) with algicidal effects against *Emiliania huxleyi* was isolated from a bloom. *E. huxleyi* is a bloom-forming microalga which produces large amounts of dimethylsulfoniopropionate (DMSP). Sulfitobacter D7 consumes and metabolizes algal DMSP with a strain-specific response and *E. huxleyi* strains that exuded higher amounts of DMSP were more susceptible to Sulfitobacter D7 infection (Barak-Gavish et al., 2018). However, lab experiments with exogenous application of DMSP enhanced bacterial virulence and induced susceptibility in an algal strain typically resistant to the bacterial pathogen (Barak-Gavish et al., 2018) suggesting that the specificity is based more on whether DMSP is present or not.

3.2.4 Virus

Viruses are important regulatory factors in marine ecosystems and play a direct role in controlling the dynamics of their host population (Baudoux & Brussaard, 2005; Brussaard & Martinez, 2008; Chattopadhyay et al., 2004). Viral infection has been implicated in the rapid termination of HABs (Imai



et al., 2006). Viral lysis of phytoplankton affects the structure and functioning of food-webs, especially when it involves bloom-forming algae. Since phytoplankton are unicellular and thus infection by a lytic virus will consequently result in rapid cell death of the organism and the release of more virus resulting in a rapid increase of virus abundance during an algal bloom (Brussaard & Martinez, 2008). Upon cell lysis cellular compounds and nutrients are released which are quickly utilized by bacteria and are in turn food for phagotrophic algae and protozoa causing changes in community composition.

The rapid decimation of algal blooms releases large amounts of cellular components into the water column which can still be harmful. For example, the genus *Phaeocystis* (Prymnesiophyceae) can generate high biomass spring blooms in temperate regions (Baudoux & Brussaard, 2005). Viral infection of these blooms with PgVs can cause rapid bloom degradation and release excessive production of foam, which becomes a nuisance for socio-economical activities like fisheries and tourism and cause the closure of beaches (Baudoux & Brussaard, 2005; Brussaard & Martinez, 2008). Viruses infecting microalgae are usually highly host specific or even strain specific (Baudoux & Brussaard, 2005; Brussaard & Martinez, 2008; Imai et al., 2006). This high degree of specificity prevents mortality of the total algal population and can cause a switch of phytoplankton community towards more virus resistant strains. For instance, Berry et al., (2015) demonstrated that during a bloom in Florida bay by the cyanobacteria *Synechococcus* the compositions changed from phycoerythrin Clade III to phycocyanin containing Clade VIII. Cells within this clade are coated with mucilage, and are known to grow rapidly, utilize organic nutrients, and resist top-down control by protozoan grazers and viruses.

3.2.5 Seaweeds

According to Imai et al. (2006) HABS can have a direct effect on seaweeds, for instance diatom blooms have a negative impact on “Nori” thalli (*Porphyra* spp., red alga) thalli (Imai et al., 2006). Harmful algal bloom cannot only effect mature seaweeds, but also macrophytes. Wang et al. (2021) showed that the HAB species *Prorocentrum donghaiense* could restrain the development and photosynthetic activities of the seaweed *Sargassum fusiformis* embryos, reduce the seedlings stock, and eventually hinder the development of *S. fusiformis* cultivation industry.

However, seaweeds such as *Ulva* sp. (Chlorophyta) and *Gelidium* sp. (Rhodophyta) appear to have algicidal bacteria which can mitigate the impact of HABs (Imai et al. 2002). While Benitt et al. (2022a) showed that the allelopathy of the seaweeds *Gracilaria tikvahiae* and *Dasysiphonia japonica* significantly reduced the concentrations the HAB forming *Aureococcus anophagefferens*. Sylvers and Gobler (2021a) demonstrated that macroalgae *Saccharina latissima* (phaeophytes), *Chondrus crispus* (rhodophyte), and *Ulva* spp (chlorophytes) can restrict the growth of, and in some cases, cause the



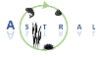
complete mortality of the HAB-forming dinoflagellate, *Alexandrium catenella*. In addition, *Saccharina latissima* significantly lessened saxitoxin (STX) accumulation in blue mussels (*Mytilus edulis*), keeping levels below US closure limits (Sylvers & Gobler, 2021a).

Not much is known currently about algacidal bacteria associated with seaweeds or about seaweeds with allelopathic compounds. Nevertheless, some studies suggest a positive impact on HAB mitigation (Benitt et al., 2022b; Imai et al., 2021; Sylvers & Gobler, 2021b). Macroalgae are considered a primary component of integrated multitrophic aquaculture (IMTA) systems, as many cultivated macroalgae have a high assimilative capacity for nitrogen and phosphorus, allowing for effective nutrient mitigation. Restoring or expanding seaweed beds can have a positive effect on the mitigation of HAB and could play an important role in IMTA. In contrast to chemical and physical mitigation of HABs this strategy of incorporating or expanding seaweed beds has no negative image for aquaculture fishermen and consumers. Moreover, species such as *Ulva* sp. and *Gracilariaria* sp. are being utilized as food source.

3.2.6 Fungi

The role of fungi in marine phytoplankton blooms are not well studied. In contrast much more is known about fungi and algal blooms in aquatic system due to their presence in drinking water reservoirs (H. Zhang et al., 2018) where fungi rapidly decline blooms. It has been suggested that fungi play a similar role in marine ecosystems, however, it is a much under studied area. Fungi have multiple roles in marine ecosystems, including decomposition of organic matter, denitrification, mediation of energy flow, and interfacing symbiotic process such as parasitism and mutualism. Relatively little is known about the composition and dynamics of eukaryotic fungal communities during HABs. Studies have shown that fungi community composition changes during a phytoplankton bloom and this could suggest that they play different roles during the various stages of the bloom. Fungal samples taken during a *Noctiluca scintillans* (dinoflagellate) bloom showed a fungal community composition change during the various bloom phases. *Basidiomycota* was strongly associated with the pre-bloom stage, *Ascomycota* with onset and *Neocallimastigomycota* with the peak-bloom stage. While during the decline and termination stages *Mucoromycotina*, *Glomeromycota*, *Blastocladiomycota*, *Mortierellomycotina*, *Chytridiomycota*, and *Cryptomycota* were more prevalent (Sun et al., 2017). During bloom decline, diversity was higher than the other phases of the bloom.

Future research might gain a better understanding at the interaction between phytoplankton blooms and fungi community composition and dynamics, but as of now it is unclear what role this biotic factor plays.



3.3 Conclusion & Summary Abiotic and Biotic factors

The effect of biotic factors on a harmful algal bloom are highly species or even strain specific for both the predator and prey. Furthermore, the abundance and community composition of all species involved changes dramatically during the succession of a bloom. Table 5 summarizes the effect of various biotic factors on bloom dynamics.

Table 5 Biotic effects on bloom dynamics

Algal bloom	Initiation	Growth	Maintenance	Late	Demise
Algal defence		Colony forming to minimize grazing due to size exclusion	Phagotrophic feeding Production of allelopathic factors	Production of allelopathic factors	Possible encystment
Grazer		Phagotrophic algae		Macro zooplankton	Bacteria
Impact of grazer on bloom species		Mono specific blooms	Utilizing alternative nutrient sources		Decomposing organic material
Allelopathy	Minimal allelopathic compounds produced		Increasing production of allelopathic compounds	High levels present	Could potentially be released into the environment
Bacteria abundance	+/-				
Bacteria diversity			+/-	++	++
Viral abundance		+	+	++	
Fungal diversity	<i>Ascomycota</i>		<i>Neocallimastigomycota</i>		<i>Mucoromycotina,</i> <i>Glomeromycota</i> <i>Blastocladiomycota</i> <i>Mortierellomycotina</i> <i>Chytridiomycota</i> <i>Cryptomycota</i>

4 Monitoring variables and parameters recommended for use at IMTA sites

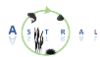
Understanding the causes of HABs requires a multifaceted approach by integrating data from chemical, physical and biological parameters. Identifying all the different causes involved in HAB formation can provide a better understanding of the IMTA site vulnerability to HABs such as proximity to nutrient loading, coastal upwelling, and stratification. For example, chlorophyll measurements provide phytoplankton biomass estimates which are taxonomically non-specific. An increase or decrease of biomass can be observed, but it gives no indication if it is a toxic or otherwise harmful algal species responsible for the bloom, nor does it provide any information about the habitat which promotes high biomass. Integrating measurements of water currents and stratification can provide information on the blooms origin and if it is likely to remain in the area while nutrient measurements can give an indication on bloom maintenance and stability.

The objective of this chapter was to develop a list of HAB-related variables and parameters to be monitored at IMTA sites based on expert opinion. Three IMTA sites with well-known environmental conditions and a historical HAB history were asked to make suggestions on what parameters to monitor at their site specifically in relation to HABs. The most requested parameters to measure were temperature, nutrients and chlorophyll-a (Chl a) (Table 66). These parameters have been widely used for years for monitoring HABs as well as to monitor water quality, environmental conditions, and biomass abundance.

Table 6 Recommended parameters for the monitoring of harmful algal blooms

IMTA	Parameter
Argentina	Temperature, NO ₃ , PO ₄ , NH ₄ , current/flow, wind speed and direction, Chl a , DOM, silicate, cell count
Ireland	Temperature, pH, NO ₃ , PO ₄ , NH ₄ , current/flow, salinity, DO, PAR, Chl a , turbidity
South Africa	Temperature, NO ₃ , current/flow, wind, Chl a , cell count

A comprehensive list of all environmental factors which are associated with HAB formation and maintenance, as well as parameters used for monitoring harmful algal species was produced (Table 7). The list of parameters to monitor covers a variety of environmental conditions which are associated with the different aspects of bloom dynamics. From chapter 2 (abiotic and biotic factors) we know that understanding the oceanography and seasonal cycles of the site is very important in predicting HABs. Measuring current regimes, temperature, up- and downwelling, salinity and stratification long term is very important because it can be the change in these factors that can initiate harmful algal blooms. Due to the wide variety of feeding strategies and nutrient requirements it is important to not only



understand the total nutrient loading, but also nutrient ratios and chemical form, as well as the concentration of dissolved organic matter (DOM), dissolved organic nitrogen (DON) and carbon (DOC). High nutrient levels are required to maintain a bloom and sudden influxes of nutrified water increases the risk of HABs. Furthermore, we also need to monitor the early stages of HAB developments by conducting algal cells counts, species determination and monitor the total biomass using *Chla* measurements as an indicator. When a bloom dies, microbes quickly utilize the DOM using up oxygen in the process and causing hypoxic conditions. As such, monitoring of dissolved oxygen levels is of great importance at the later stages of the bloom especially when the bloom occurs near fish farms.

After identifying relevant parameters, a score was assigned to each by the IMTA specialists with '5' being highly relevant to HABs and '0' not relevant at all for their site. Scores were added up to determine the overall importance of each variable. This value was used to determine the importance for developing a sensor and/or if the sensor needed to be integrated with other sensors into a single software package. For instance, temperature is seen as of great importance, however, there are many temperature sensors already commercially available, they are cheap and reliable therefore there is no need to re-invent the wheel and develop a temperature sensor. However, integrating these sensors on the same software as other sensors can make it more user friendly as well as improving data interpretation and data manipulation.

Table 7 shows that the most relevant parameters, and thus required monitoring, are temperature and nutrients which is similar to the requested parameters from the earlier assessment of 3 IMTA sites (Table 6). Water chemistry and light levels vary greatly throughout the water column and these parameters are measured at several different depths at the IMTA site in Scotland corresponding with the various cultivation depths. In addition, importance is given to some oceanographic features such as current/flow and stratification. We know from chapter 3 that water currents and upwelling play a major role in HAB distribution and stratification are a favourable habitat for many HAB species. All aforementioned factors are intrinsically linked and being able to integrate these sensors over the same software platform can help our understanding of algal bloom initiation, development, stability, and decline.

Table 7 Importance matrix for parameters to monitor in relation to harmful algal blooms

Variable	SAMS	CONICET	MI	CSIR/UCT/DAFF	TOTAL
	Scotland	Argentina	Ireland	South Africa	
HAB cell counts	yes	yes	yes	yes	yes
Temperature	5	3	3	5	16
pH/pCO ₂	4	4	5	2/4	16
NO ₃ - nitrate	5	5	5	4	19



PO4 - phosphate	4	4	5	1	15
NH4 - ammonium	5	5	5	1	16
SiO4 - silicate				4	
Urea/DON		4		1	
Current/flow	5	5	5	5	20
Wind (speed & direction)	5	4	1	5	15
Rainfall	1	3	1	0	5
Sea level	1	0	0	0	1
Salinity	3	3	5	1	12
Dissolved oxygen	3	3	4	4	12
PAR	4	3	5	1	13
Chl α	4	4	3	4	15
Non-HAB cell counts				yes	
Turbidity	4	3	3	2	12
DIC				1	
Alkalinity				3	
Ocean colour				5	
DOM				1	
Plastic	?	?	?	?	

An additional sensor which can be of value are plastic sensors. As discussed in chapter 3 plastic can be a seeding source for HABs as well as transporting seeding stock to new locations. Not much is known about the relationships and interactions between micro- and macroplastics, HAB occurrence, and IMTA operations. Increasing this knowledge will be of great value for understanding the cause and origin of bloom seedings.

Several of the parameters discussed will be monitored at the various IMTA labs. Case studies from this will be discussed in chapter 5.

5 HAB occurrence at IMTA

A survey was conducted among the 5 IMTA sites as part of the ASTRAL project to gain a better understanding of HAB occurrence, abundances and parameters used to monitor HAB. These case studies increase our understanding of factors controlling HAB, their likelihood and their possible impact on the various IMTA facilities. A full description of the survey conducted can be found in appendix A.

5.1 IMTA operational conditions

There is a large diversity between production systems used within the various IMTA (Figure 1, Table 8). The IMTA's in Ireland and Scotland are sea based while the ones in South Africa and Brazil are land based. The type of operating system has a large impact on the control of the environmental factors, with land based operating systems having additional protocols in place to minimize HAB impact and reduce water variability. Such as done in South Africa where all water, pumped from the sea, that comes into the system are treated to avoid the growth of HAB or other undesired organism addition they have the ability to go onto full recirculation mode for short periods of time when toxic dinoflagellates in the surrounding ocean exceeds a certain threshold.



Figure 1 The various cultivation methods used in IMTA facilities. Left Aerial overview of Buffeljags abalone farm, South Africa. Right Port-a-Bhuiltin, Scotland.

Table 8 Various operating systems used, and environmental conditions present at the IMTA facilities

Country	Operation type	Environmental conditions	Cultivation conditions
South Africa	Land based open raceway system with partial recirculating integrated aquaculture systems	Seawater from the adjacent ocean with control over temperature, aeration, mixing	Near shore water used for cultivation
Scotland	Coastal open-ocean aquaculture. Site is located near shore, 250m away from the mainland coast, in the Firth		

	of Lorn -Loch Linnhe estuarine system.		
Ireland	Pen grid for fish culture with attached low trophic grid for suspended longlines. Close to shore (0.25km)	Low current, sheltered, with little flow to disperse fish cage waste.	Bertraghboy Bay is a semi enclosed bay on the West coast of Ireland. subjected to runoff from rainfall reducing salinities and increasing turbidity with high particulate loading.
Brazil	Land based, 300m away from shore, bioflocs, ponds, tanks systems	Heavily influenced by the salinity of the estuary from which it received its water.	Estuary water used for cultivation
Argentina	Currently no IMTA facilities		The Beagle Channel is a semi-estuarine environment, with a seasonal dynamic tightly related to freshwater input from precipitation and glacial melt.

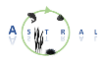
Argentina has currently no IMTAs facilities. However, the Austral Center for Scientific Research (CADIC), a research center belonging to the National Council for Scientific and Technical Research (CONICET), is working on a prospective IMTA lab that could eventually develop in an IMTA facility. The prospective IMTA Lab is intended to be designed around Port Almanza (54°52'15"S; 67°33'50"W, Figure 2). This area was selected because it is the only zone throughout the marine coast of Tierra del Fuego Province that has been enabled for aquaculture projects. Extensive monitoring and research have been conducted in this area and data obtained will help in understanding HAB and the risk factors IMTA face. Therefore, this potential future IMTA lab has been incorporated in the case study.



Figure 2 Proposed location of the future IMTA lab in the Beagle channel, Argentina

5.2 HAB risk factors for IMTA operation

Each IMTA site identified risk factors associated with HAB abundance (Table 9). Despite the large geographical range as well as various operating types used, we see some risk factors shared by most IMTA labs. There does not seem to be a clear separation between the risk factors associated with land-



based operation and off-shore operations. Some risk factors are shared universally such as salinity changes, severe weather events, and run-off events. Though salinity changes are found at most IMTA facilities the source of the salinity change varies. In addition to some unique type of risk factors for each IMTA such as glacier melt in Argentina agricultural run-off in Ireland and upwelling in South-Africa.

Table 9 Risk factors associated with harmful bloom events per IMTA region

Risk factor	Argentina (Future off-shore)	Brazil (Land-based)	Ireland (Off-shore)	Scotland Off-shore)	South Africa (Land-based)
River run-off	X	X	X		
Agricultural run-off			X		
Urban wastewater		X	X		X
Glacier run-off	X				
Upwelling					X
Stratification	X			X	
Current changes		X		X	
Salinity changes	X	X	X	X	
Severe rainfall		X	X		
Severe weather events (Storms, etc.)		X	X	X	X
Nutrification	X				X
Changes in light levels	X		X		
High shipping area		X		X	
Pollution (chemicals, plastic, etc.)		X			X
HABs, Pathogens					X

Sever weather events seem to be a common issue among IMTA independent if the operation is on-shore or off-shore and with predicted increase in several weather events in the future (Stocker et al., 2021) it will be critical to have plans in place to mitigate the effect it has on IMTA operation. Urban waste-water runoff, but also river and agricultural run-off, seems to be another universal problem independent if the facility is land based or off-shore.

Several IMTA lab have very specific risk factors such as the highly productive Benguela upwelling system in South Africa or salinity fluctuations in Brazil. Upwellings have been associated in many areas with an increased risk of HAB occurring by bring cold nutrient-rich water to the surface (Pitcher et al., 2010; Trainer et al., 2010).

The IMTA in Brazil is located close to a great estuarine region, The Patos Lagoon estuary (Figure 3), and the discharge of fresh water from the estuary has great influence in the water used in the IMTA system. In periods where large rainfall is recorded in the estuary or extensive drought periods, salinities fluctuations are common ranging from 16-36‰. When the discharge of estuary is reduced, salinities increase, and values closed to 36 are observed. The dynamics of the water masses varies by season. In the summer the Brazil’s current, brings warm water of high salinity to the area, while in winter, the

current of Malvinas, with cold high salinity water reaches the coast driven by winds from the south. Despite these water mass, the estuary discharge is the main controller of salinity in the Brazil IMTA lab. In Scotland variations in salinity, current speeds and stratification are linked to tidal cycles which causes daily fluctuations. Salinity changes in Brazil are linked with high rainfall which effects the salinity of the estuary from which they obtain their water for cultivation, while Argentina sees changes in salinity due to glacial and river run-off. This illustrates the importance of not only measuring the various parameters but also understanding what causes these changes. Salinity changes in Scotland which are linked to the tides are therefore regular and predictable while, salinity changes for Brazil, Argentina and South Africa are much more linked to weather events (i.e. rain, high temperature) which can be irregular.

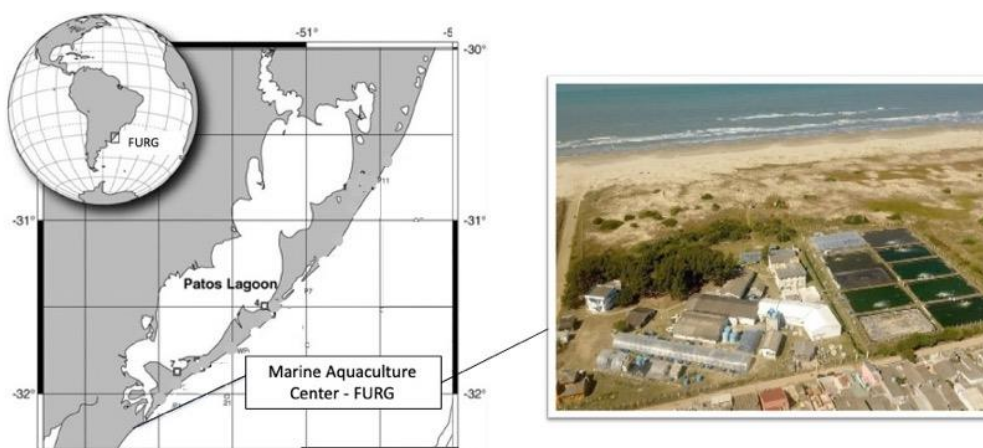


Figure 3: Location the Marine Aquaculture Center in Brazil is close to the Patos lagoon which has a large impact on water quality such as salinity ($32^{\circ}12'16S$, $52^{\circ}10'38W$).

5.3 HAB occurrences in IMTA regions

Harmful algal blooms are common across all regions (Table 10) and can reoccur for several years in a row or show large variability between years. This can be due to HAB species which are known to form cyst during adverse condition and can quickly excyst and reseed a bloom when conditions are favourable (Anderson & Rengefors, 2006). Several IMTA sites have reported recurring HAB in the vicinity, though not at the site itself, with extensive impact on the ecosystem. IMTA labs can harbour harmful algal species which are very specific for that area such as *Anabeana* in Brazil and *Protoceratium reticulatum* in South Africa as well as genera which are universally found such as *Alexandrium* spp. and *Pseudo-Nitzschia* spp. (Table 10). *Alexandrium* spp. which can be found in Argentina, Scotland, and Ireland has a life cycle which includes a resting stage (cyst) in which cells sink to the benthos and can be resuspended into the water column where it seeds a bloom (Azanza et al., 2018; Steidinger &

Garccés, 2006). Once a species with such a life cycle is established in the region it can cause recurring blooms for many years (Anderson & Rengefors, 2006). Due to the reoccurring nature and large impact HAB can have several IMTA sites have implemented strategies to mitigate or prevent the harmful effects such as temporarily going into full recirculation mode, using alternative water sources or using water filtering systems.

Table 10 Toxin producing phytoplankton species in ASTRAL IMTA Labs (Picture sources: <https://www.algaebase.org/> and <https://www.habreports.org/gallery.php>)

Genus & Species	IMTA	Class	Illness and Toxins	Picture
<i>Alexandrium</i> spp. <i>Alexandrium catenella</i> <i>Alexandrium ostenfeldii</i>		Dinoflagellate	PSP, saxitoxin etc.	
<i>Dinophysis</i> spp. <i>Dinophysis acuminata</i> <i>Dinophysis fortii</i>		Dinoflagellate	DSP, okadaic acid	
<i>Prorocentrum cordatum</i> <i>Prorocentrum lima</i> <i>Prorocentrum micans</i>		Dinoflagellate	DSP	
<i>Karenia mikimotoi</i> <i>Karenia cristata</i>		Dinoflagellate	Ichthyo- toxins, gymnocins etc.	
<i>Azadinium</i>		Dinoflagellate	Azspiracid toxins	
<i>Gonyaulax spinifera</i>		Dinoflagellate	PSP, yessotoxin	
<i>Protoceratium reticulatum</i>		Dinoflagellate	Yessotoxin	
<i>Lingulodinium polyedrum</i>		Dinoflagellate	Yessotoxin	

<p><i>Pseudo-nitzschia</i> spp. <i>Pseudo-nitzschia delicatissima</i> <i>Pseudo-nitzschia seriata</i> <i>Pseudo-nitzschia seriata multiseriata</i></p>		Diatom	Domoic acid	
<p><i>Nodularia</i> spp.</p>		Cyanobacteria	Nodularin	
<p><i>Anabaena</i> spp.</p>		Cyanobacteria	Anatoxin and microcystin	

South Africa: HABs are common in South Africa region near the IMTA site primarily during the austral summer months between January and April. Since HAB impacts to the Buffeljags abalone farm has been negligible, the HAB events listed below are those that have impacted the farmed abalone industry within 50 kilometres of the South African IMTA lab. Two major HAB events have occurred in the area. The first one in the Walker Bay area occurred from Late December 2016 to mid-February 2017 and lasted 1.5 months. It spanned several kilometres of coastline. The main HAB species were *Gonyaulax spinifera*, *Lingulodinium polyedrum* which are known to produce yessotoxin. The bloom in the Walker Bay area resulted in the mortality of over 250 tons (several million animals) of farmed abalone (see Pitcher et al 2019 for reference). The second bloom occurred from February to May 2019 and remained in the vicinity of Walker Bay on and off for several months. This bloom was patchier and spanned several kilometres of coastline. The primarily species in this bloom was *Lingulodinium polyedrum*.

Brazil: Although the presence of HABs is extremely rare, cyanobacteria from genus *Nodularia* and *Pseudoanabaena* are commonly observed in the area. Cyanobacteria are associated with warm stratified water such as found in estuaries and lagoons where they can form dense mats (O’Neil et al., 2012; Paerl & Fulton, 2006; Paerl & Paul, 2012; Visser et al., 2016). Although the environmental conditions in the Patos Lagoon estuary - adjacent to the IMTA Lab in Brazil - are ideal for the growth of these cyanobacteria, the water treatment in the greenhouses has been effective in preventing the entry of cyanobacteria into IMTA systems. The open ponds are more exposed to the potential entry of cyanobacteria, as these structures receive water which is pumped directly from the sea/estuary, while also being exposed to the entry of microorganisms through external sources such as birds. If present, very large cyanobacteria blooms in the ponds can lead to gill occlusion. However, despite the recurring presence of cyanobacteria in these open ponds, mortality from toxins have not been reported.

Ireland: A finfish site at Lehanagh pool near the Irish IMTA has been monitored in the past for HABs and the 2020 spring bloom was found to contain *Pseudo-nitzschia* and the late summer bloom consisted out of *Azadinium/Heterocapsa* while in 2021 spring showed species such as *Alexandrium*

spp. and *Prorocentrum micans* while autumn showed again an increased abundance of *Pseudo-nitzschia*. Indicating the present of multiple species each year Figure 4 which can be potentially harmful by forming blooms and or toxin production there is a lot of variety between seasonal abundance patters.

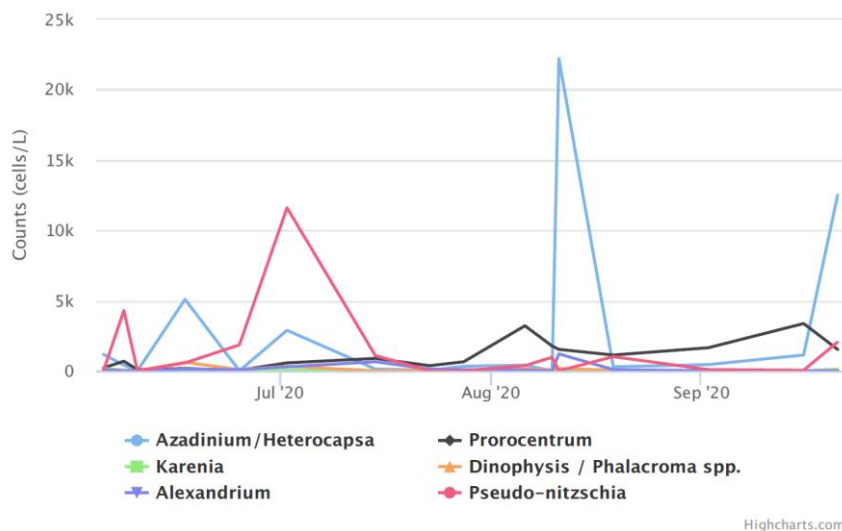
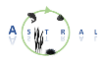


Figure 4 Toxic species by genus for Lehanagh pool during 2020.

Scotland: Two locations within a 50km radius which are most representative of the IMTA site are monitored on a weekly basis during March-September. Phytoplankton blooms in the region are most commonly from *Dinophysis* spp followed by *Alexandrium* spp. and low levels of *Prorocentrum lima* and *Pseudo-nitzschia* spp. *Dinophysis* spp. bloom are most common from late May to late August which can last several weeks. In contrast *Alexandrium* spp. from April to late September and are short lived (around 1 week). These sites compared to other sites around Scotland have smaller and less frequent blooms. *P. lima* occur later in the year around June-July.

Argentina: Toxigenic algal blooms have been reported since 1886, when the native population near Ushuaia (Argentina) was severely intoxicated, and some people died after the ingestion of bivalves (Segers, 1908). The Beagle Channel was thereafter considered- free of phycotoxins until 1992, favouring the commercial harvesting of molluscs with harvest volumes exceeding coastal fish catches (Goya and Maldonado, 2014). Then, in summer 1992 a major PSP outbreak occurred, in which PSP toxicity reached the highest values ever recorded worldwide, 127 000 µg saxitoxin equivalent per 100 g of tissue (µg STX eq 100 g⁻¹ tissue), 160x the allowable regulatory level of 80 µg STX eq 100g⁻¹ tissue (Benavides et al., 1995). This exceptional PSP event, associated with a bloom of the dinoflagellate *Alexandrium catenella*, had serious health consequences, with several cases of human intoxication (some of them fatal), and detrimental impacts on marine wildlife and ecosystem services (Carreto and Benavides, 1993, Vinuesa, 1993). From that moment on and up to 2011, detectable PSP toxin levels have been recorded repeatedly in molluscs, often exceeding the safety limit for human



consumption for extended periods mostly during summer seasons (Goya and Maldonado, 2014). *A. catenella* is considered the principal PSP toxin-producing dinoflagellate in the Beagle Channel (Carreto et al., 1985, 1986, 1990; Almandoz et al., 2011, 2019; Benavides et al., 2019). *Alexandrium catenella* blooms have been recorded in the austral spring-summings of 2009-2010, 2010-2011, 2012-2013 with a duration of 200 days, 150 days, 180 days respectively. These blooms caused toxins levels well above what is considered safe. In 2009-2010 toxin levels reached as high as 5601 µg eq STX/ 100 g which resulted in closing of the area. In adverse conditions *Alexandrium* species form a resting cyst and sink to the benthos. When conditions are favourable it can excysts and quickly seed a bloom (Granéli & Turner, 2008; Pančić & Kjørboe, 2018), which can result in reoccurring blooms.

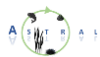
5.4 HAB occurrences during IMTA operation

Except for IMTA lab South Africa no HAB were observed during the IMTA operation. This can be due to the lack of regular monitoring at the other sites. For instance, IMTA lab Scotland does not have an actively monitoring directly at their site, but rather uses government data from sites nearby. Blooms in this area can be highly spatial and temporal (Coates et al., 2020) and thus it can be potentially missed.

HABs have been observed in the vicinity of the South Africa IMTA lab, with minor impacts to the IMTA during the 2017 and 2019 bloom events. The oceanography and the location of the Bufflejags Abalone farm, in addition to the ability of the farm to go onto full recirculation for short periods of time (12-48 hours) when toxic dinoflagellates exceed a certain concentration (e.g. 400 000 cells per litre), helps to mitigate HAB impacts. The location of the seawater intake pipe also makes it possible to draw water underneath the bloom during high tide, if necessary, preventing any harmful algal species from entering the system. As a result, there have thus far been no direct negative impact from HABs to the farmed abalone at the South African IMTA lab.

5.5 Environmental monitoring at the IMTA sites

Many of the IMTA sites have already a robust monitoring program in place to monitor for the health and environmental factors related to their cultivation species (Table 11). Several of these variables monitored are of particular importance as an indicator for HAB likely hood. Such as water temperature and precipitation, which are an indicator of coastal water stratification, and provides an indication of the stage of bloom succession and will influence the community composition. As discussed in chapter 2 increased stratification will favour dinoflagellates. Large injections of nitrate can lead to high biomass



blooms whereas the silicate/nitrate ratio plays a role in phytoplankton community composition with a reduction in the silicate ratio favouring flagellate species. Water current speed and direction is important in terms of providing an indication of water movement and potential area of impact of a bloom. Chlorophyll-*a* can be an indicator for high biomass density HABs but does not identify the species and thus is critical to supplement this data with phytoplankton enumeration.

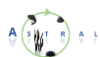
Brazil: In the IMTA system the regular measurements are salinity, temperature, dissolved oxygen, pH, turbidity, alkalinity, TSS, and nutrients such as ammonium, nitrate, nitrite, and phosphate. In the adjacent environment just salinity and temperature are measured. As discussed earlier the salinity in the estuary, from which they obtain their cultivation water, can fluctuate and therefore it is critical to include these measurements in the adjacent environment.

Ireland: Temperature, current, secchi depth and dissolved oxygen levels are all monitored daily. Monthly water sampling is analysed for nutrients and chlorophyll-*a* and phytoplankton composition and abundance is monitored through the Marine Institute's HABs monitoring programme.

Scotland: IMTA Scotland deployed various sensors which monitor the environment continuously such as temperature, light intensity, PAR, while at the nearby L1 buoy location (SAMS Site ID: LY1) additional continuous (every 30-60min) measurements are done on water current speed & -direction, temperature, conductivity, chlorophyll-*a*, turbidity, pH, dissolved oxygen, fluorescent dissolved organic matter (fDOM). Additionally, every 4-6 weeks samples are taken at the IMTA site for chlorophyll-*a*, particulate organic carbon and nitrogen (POC, PON), dissolved inorganic nutrients (nitrate, nitrite, phosphate, silicate). Since there is variation in water chemistry and light levels throughout the water column these parameters are measured at several different depths at the IMTA site corresponding with the various cultivation depths.

South Africa: At the Abalone-Ulva IMTA daily measurements of temperature, dissolved oxygen, and pH and monthly measurements of ammonia, nitrite, nitrate, turbidity, and total suspended solids. At the Urchin hatchery additional daily measurements of salinity are done and monthly Light.

Argentina: As part of the oceanographic study mentioned, water salinity, temperature, dissolved oxygen, and fluorescence are determined. In addition, atmospheric and underwater PAR are measured by means of a Li-Cor 192 and 193 as well as meteorological variables such as wind speed and direction, air temperature and precipitation. Water collected with 5-L Niskin bottles further allow for the quantification of phyto- and protozooplankton species, and net samples allow for the qualitative characterisation of plankton. During high toxin concentration moments, water is additionally filtered for its determination by HPLC (in Mar del Plata, Buenos Aires Province, Argentina). Furthermore, lipophilic toxins presence will be determined as for their absorption in SPATs (to be analysed in collaboration with Alfred-Wegener Institute researchers in Bremerhaven, Germany). Inorganic



nutrients will be determined at CESIMAR (Puerto Madryn, Chubut, Argentina). All this information will be accompanied with the data produced by the monitoring program, on the concentration of PSP toxin in mussels.

Table 11 Current monitoring regime done by the various IMTA labs.

measurements	Country	Frequency
Temperature (air)		Continuous; every 4h
Temperature (water)		Continuous; every 30min (1.5m)
		Regular
		Weekly
		Daily (At 4 different depths)
		Continuous; every 4h
		Continuous; every 15min
Light Intensity (lux)	 	Continuous; every 30min (1.5m) monthly
PAR		Continuous; every 30min (every 0.5m from 0-4m)
		Continuous; every 15min
Chlorophyll-a		Continuous; every 30min (1.5m) & every 4-6 weeks (1.5m)
		Monthly
		Regular with increasing frequency during bloom times
Particulate organic carbon and nitrogen (POC, PON)		every 4-6 weeks (1.5m)
Nitrate / nitrite		every 4-6 weeks (1.5m)
		Regular
		Monthly
		Monthly
Phosphate		every 4-6 weeks (1.5m)
		regular
		Monthly
Silicate		every 4-6 weeks (1.5m)
		Monthly
Ammonium		Regular
		Monthly
Water current speed & -direction		Continuous; every 60min (1.5m)

Conductivity		Continuous; every 30min (1.5, 3m)
Turbidity total suspended solids	 	Continuous; every 30min (1.5, 3m) monthly
pH	 	Continuous; every 30min (1.5, 3m) Weekly Daily
Dissolved Oxygen	 	Continuous; every 30min (1.5m) Regular Weekly Monthly Daily
Fluorescent dissolved organic matter (fDOM)	 	Continuous; every 30min (1.5m)
Salinity	 	Regular Weekly Continuous; every 4h Daily Continuous; every 15 min
Phytoplankton enumeration	 	Weekly from nearby area Regular with increasing frequency during bloom times monthly
Proto zooplankton enumeration		monthly
Wind speed and direction		Continuous, every 4 hs
Precipitation (rain)		Continuous, every 4 hs
Lipophilic toxins		During bloom periods
Secchi disk		Daily

5.6 HAB monitoring strategies at IMTA facilities

Many countries have already a robust phytoplankton monitoring program which can be of great benefit to research carried out at the IMTA labs. Though not all locations are currently actively monitoring for HABs there are regional or governmental programs in place such as in Scotland, Argentina and, Ireland which monitor for HAB by means of phytoplankton screening and counts while other IMTA labs have chosen to implement a preventative program rather than a monitoring program.



Brazil: The Brazil IMTA uses a preventative program rather than a monitoring program. They use a closed system, with total water reuse. The water, pumped from the sea, that comes into the system are treated to avoid the growth of HAB or other undesired organisms.

Ireland: The marine institute monitors for toxic HAB species (<http://webapps.marine.ie/HABs/>) in Ireland at the Lehanagh Pool where the IMTA is located as well as nearby sites are monitored such as a finfish site located on the outer side of the bay.

Scotland: IMTA Scotland has no early warning or active monitoring of HABs at Port-a-Bhuiltin. However, SAMS is performing HAB monitoring at a regularly visited monitoring site located ~1km south-east to our site in Loch Linnhe (SAMS Site ID: LY1). However, regular water sampling for chlorophyll-a has been conducted at the main seaweed growing depth (1.5m depth). Regarding chlorophyll-a, concentration vary following a seasonal cycle and in relation to the onset of the phytoplankton spring, and to a lesser extent summer blooms. However, figures are depicting overall chlorophyll-a concentrations without ways to distinguish between HAB and non-HAB produced chlorophyll.

In Scotland, HAB risk bulletins are prepared weekly, based on expert interpretation of the various data streams, for use by the aquaculture industry (Coates et al., 2020). Scottish Official Scotland has a robust phytoplankton and Monitoring Programmes delivered by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) in place. The programmes are delivered on behalf of Food Standards Scotland (FSS) and the phytoplankton analyses are performed by SAMS Research Services Ltd. (SRSL) in Oban. Results of the FSS toxin and phytoplankton monitoring programmes are available on the FSS website ([HAB Reports](#)).

Argentina: In Argentina a Shellfish Toxicity Monitoring Program is carried out by the Dirección de Laboratorio Ambiental (Environmental Laboratory of the Fisheries and Aquaculture Secretary of the Tierra del Fuego Province). Recently, an oceanographic and biological weekly-sampling program performed in the frame of a PhD thesis by MSc Andreana Cadaillon, a student of the Biological Oceanography Laboratory of CADIC under the direction of Dr. Irene Schloss. This monitoring is additionally at the heart of ASTRAL activities. To ensure the safety of bivalves for consumption, since 1985 a Shellfish Toxicity Monitoring Program is carried out in Beagle Channel coastal waters by the Dirección de Laboratorio Ambiental (Environmental Laboratory of the Fisheries and Aquaculture Secretary of the Tierra del Fuego Province). A detailed analysis of shellfish toxicity is further performed around Port Almanza zones.

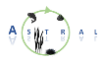


5.7 Environmental changes prior to bloom events

The majority of the IMTA did not have a HAB occurrence during their operation. However, some past observations from Ireland showed changes in dissolved oxygen, chlorophyll-*a* and turbidity prior to a full-scale bloom event. While anecdotal evidence from South Africa shows a concern during the summer months when upwelling favourable winds decrease and the wind turns onshore, or when the SST increases. Potentially indicating calmer ocean conditions favouring stratification and dinoflagellate bloom development. Argentina noticed the appearance of *Alexandrium catenella* in the phytoplankton community early on before it grew to a full-scale bloom.

5.8 Case study conclusion

Regular harmful bloom events have been documented in all IMTA areas, though not yet on the IMTA sites itself. Extensive environmental monitoring is in place as well as monitoring for harmful phytoplankton often in collaboration with government agencies. Land-based IMTA systems have protocols in place to mitigate the impact of any potential bloom by means of water treatment. Regardless of geographical location or operating system common issues increasing the risk of HABS are run-off (though causes of run-off varies between locations) and sever weather events. At this point in time no clear indicator has been observed to predict the likelihood of a harmful bloom to occur and thus it is vital to continue monitoring the various environmental conditions.



6 Earth Observation data

Earth observation (EO) uses remote-sensing technologies to monitor and assess the changes of the biological, chemical, and physical systems of the planet. These technologies can include satellite-based, airborne, as well as ground-based or in situ methods. A comprehensive overview of the sensors and technology that are available to monitor phytoplankton and HABs can be found in the ASTRAL D3.6 Technology User Guide. This section, although touching on a variety of methodologies for the remote sensing of HABs, will focus primarily on those involving satellite-based remote sensing.

Satellite-based remote sensing provides routine synoptic-scale measurements of the earth's surface across time-scales simply not achievable by in situ sampling techniques; it also allows the collection of measurements over inaccessible areas, or over regions where the costs associated with in situ sampling may be prohibitive.

Marine and aquatic remote sensing techniques utilise measurements of electromagnetic radiation that have interacted with a water body; the origin of this radiation can either be from the sun (passive remote sensing) or from pulses produced by the satellite sensors (active remote sensing). Different wavelengths of the electromagnetic spectrum are useful for measuring different physical or biological parameters. EO satellite sensors can be used to measure the surface temperature of water bodies, often referred to as sea surface temperature (SST): infrared radiation can detect the temperature of the top ten microns of the ocean surface, whereas microwave radiation is emitted from the top one millimetre of the ocean surface. Organic and inorganic substances (optically active constituents) in a water body absorb and scatter light differently depending on their size, shape and/or concentration; these constituents have a measurable and often predictable effect on the colour of the water (i.e. the water-leaving reflectance), which is why the radiometric measurements of the visible and near infrared part of the spectrum is most frequently utilised for their detection and quantification.

Phytoplankton and some bacteria (e.g. blue-green algae or cyanobacteria) absorb, attenuate, and scatter sunlight differently depending on their shape, size, photosynthetic pigments, and cellular structure; together with the abundance of the cells present in the water column, these characteristics determine how they affect the light field, and the resulting water-leaving reflectance. A variety of methods exist to detect phytoplankton and HABs using satellite sensors that make use of ocean colour radiometry – while a summary is provided in section 6.2 of this report, a more comprehensive overview can be found in IOCCG report 20 (IOCCG, 2021).

6.1 Opportunities and limitations of EO data

Ocean colour radiometry makes use of passive detection techniques, i.e. relying on the energy from the sun that is naturally reflected/emitted from the earth’s surface; as a result it can only provide measurements obtained during daylight, or when there are no atmospheric disturbances (e.g. clouds, dust or smoke) obstructing the sensor’s view of the earth’s surface. Even on a relatively clear day the satellite measurements still need to be corrected for possible sun glint, as well as the contributions of signals from the atmosphere (e.g. gasses, molecules and aerosols) which can often constitute up to 90% of the signal received by the satellite sensor. Water is a relatively dark target in comparison to bright land surfaces or sea ice, which means that the sensors with the capability of deriving spectral information over aquatic targets need to have sufficiently high sensitivity and signal-to-noise ratios, which often require a larger footprint (or pixel size) in the order of 300 – 1000 m (Figure 5); while multispectral earth-orbiting sensors can provide near daily coverage at these medium spatial resolutions, higher spatial resolution (e.g. 10-60 m from land-imaging sensors like Landsat or Sentinel 2) usually involve either decreased spectral resolution and lower revisit times (e.g. weekly or more), or obtaining data from costly specialised satellite missions. A combination of different sensors and approaches may be required to provide the appropriate spatial and temporal resolutions required for detecting and monitoring HABs.

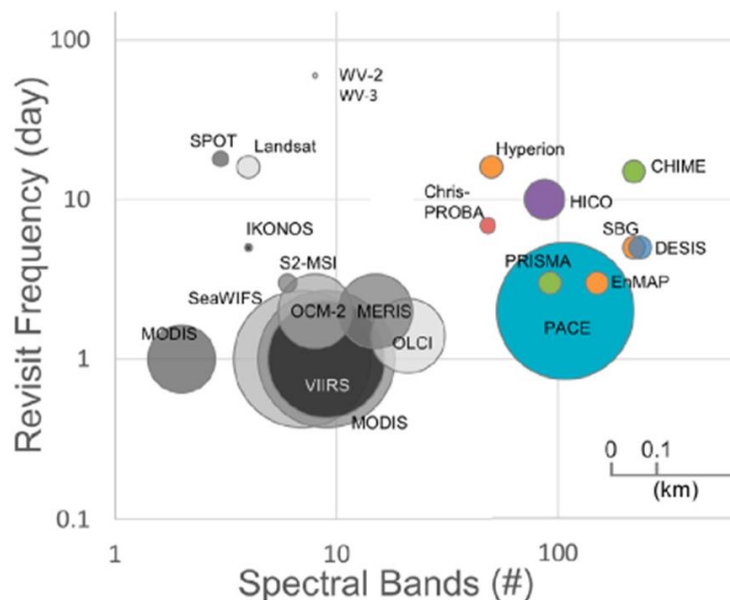


Figure 5 The trade-offs between spectral bands (x-axis), revisit frequency (y-axis) and pixel size (circle size) that exists between different international missions; coloured and gray circles represent hyperspectral and multi-spectral missions, respectively. Fi obtained from Dierssen et al (2021).

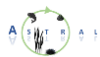


In certain cases, such as with *Dinophysis* species, toxin producing phytoplankton occur at low biomass which makes detection via ocean colour radiometry unsuitable; in these cases predictive capabilities featuring satellite data might focus on the use of meteorological and oceanographic information of favourable conditions for their proliferation or onshore advection (Davidson et al 2021). Satellite based radiometry also only provides information of the upper layer of the ocean and is considered generally unsuitable for monitoring sub-surface HABs often found in coastal regions with complex hydrodynamic processes or thermal stratification (Berdalet et al 2017).

The spectral resolution of the current suite of multi-spectral earth observation sensors are limited in their ability to fully characterise phytoplankton community composition or identify different phytoplankton types (Dierssen et al 2020). Several new global mapping hyperspectral sensors, consisting of multiple narrow waveband channels with the potential to capture the diagnostic accessory pigments and fluorescence signals associated with different phytoplankton groups, are under development, e.g. NASA's Plankton Aerosol Cloud and ocean Ecosystem (PACE) and Surface Biology and Geology (SBG) missions, and the European Space Agency's Copernicus Hyperspectral Imaging Mission for the Environment (CHIME) (Dierssen et al 2021).

Radiometric sensors deployed on small aircraft and unoccupied aerial systems (UAS) offer a valuable method for HAB detection, bypassing the usual constraints (e.g. fixed orbits and cloud-cover) imposed by satellite-based monitoring, enabling sampling at appropriate geometries and high temporal and spatial scales. The portability, flexibility, and fine spatial measurements achievable by UAS such as drones make them a particularly attractive technology for HAB monitoring over inland and coastal waters (e.g. Becker et al 2019, Wu et al 2019), while continuous technological advances are making these systems more user-friendly and affordable.

Permanent in situ sensor installations onboard buoys and moorings can offer valuable near-continuous measurements relating either directly or indirectly to HABs; these might include sensors that measure wind, temperature and/or current speed and direction, providing information relating to the environmental conditions favouring HAB formation, while further sensors might include above- or in-water radiometers and in situ fluorometers. Other moored or in situ methodology include the use of automated submersible imaging flow cytometers such as the FlowCytobot and FlowCam which generates images of particles and automatically classifies phytoplankton images into genus-specific cell abundance estimates; however, these are highly specialised and expensive instruments and may require expert training. ASTRAL will develop a prototype low-cost vision-based sensor with built-in



Artificial Intelligence Deep Learning models for phytoplankton identification, representing a significant advance in the accessibility of phytoplankton identification technology. It should be noted that all of the above-mentioned methodology are limited in the very small spatial scales that can be measured at any given time.

6.2 Satellite-based HAB detection techniques

Traditionally, satellite-based HAB detection methods have focussed on biomass-based techniques, using the concentration of chlorophyll a (Chl-*a*) as a proxy for phytoplankton biomass. Detection methods based on Chl-*a* thresholds or anomalies are largely appropriate for specific regions where a bloom of known species regularly dominates the optical signal. A Chl-*a* anomaly approach (Stumpf et al 2003) forms part of the current NOAA operational HAB products for the Gulf of Mexico; the method raises alerts for *Karenia brevis* when daily chlorophyll anomalies reach concentrations of more than 1 mg m⁻³ above a 60-day running mean. Standard satellite Chl-*a* products are generally based on empirically derived blue-green band-ratio algorithms, which are inherently sensitive to the effects of (re)suspended sediments, bottom reflection, and increased concentrations of coloured dissolved organic matter, while often producing inaccurate results in eutrophic conditions (IOCCG 2021).

HAB detection techniques based on wavebands in the red to near infrared (NIR) spectral regions are generally considered to be more reliable under conditions of elevated phytoplankton biomass and optical complexity (e.g. coastal regions with high sediment load and coloured dissolved organic material). A popular method focusses on the quantification of the solar-stimulated chlorophyll fluorescence signal (Letelier and Abbott, 1996), an emission of energy which is closely related to phytoplankton biomass and results in a reflectance peak near 685 nm. Several past and present ocean colour sensors (e.g. MERIS, MODIS, OLCI) have wavebands situated at and around this peak to enable the detection of this fluorescence signal; the signal is quantified by measuring the height of the reflectance peak above a baseline formed by the adjacent wavebands, a technique known as the fluorescence line height (FLH; Letelier and Abbott, 1996; Gower et al., 1999). Similar high-biomass baseline approaches include the maximum chlorophyll index (MCI; Gower et al 2005), which quantifies the height of the phytoplankton-related backscattering peak in the NIR, or the maximum peak height (MPH; Matthews et al 2012) algorithm which quantifies the fluorescence or phytoplankton backscattering peaks and discriminates between eukaryote and cyanobacteria dominated blooms. Several studies have used the abovementioned red-NIR baseline approaches in combination with novel products such as colour composites and other optically-derived indices to detect HABs (e.g. Hu et al 2005, Cannizzaro et al 2008, Zhao et al 2015, Ghanea et al 2016).

While the above-mentioned approaches distinguish HABs based on biomass and intensity, they do not carry any information about the phytoplankton species or associated level of harm of the identified blooms. Further approaches focus on the identification of unique spectral features associated with species specific backscattering properties and pigment assemblages through spectral classification techniques (e.g. Kurekin et al. 2014; Gokul and Shanmugam 2016) and spectral shape algorithms (e.g. Dierssen et al 2015). There is no single method that can work globally for the detection of HABs, and regional knowledge of species composition, bio-optical and environmental conditions are required to ensure that the appropriate detection techniques are employed.

6.2.1 Satellite-based HAB time series: a South African case study

The vast majority of the South African land-based abalone industry, including the Buffeljags IMTA lab, is located on the south-west coast of South Africa, in the vicinity of Walker Bay (Figure 6). For this abalone industry the primary concern in terms of HABs are phytoplankton that produce yessotoxins, which cause disruption of the gill epithelium, e.g. degeneration and necrosis of epithelial cells and modest inflammatory response in abalone (details in Pitcher et al 2019). Dinoflagellate blooms generally occur during the months from January to April during Austral summer and autumn in the southern Benguela, particularly when upwelling-favourable winds are less frequent, or during shoreward movement of meanders or eddies of warm oligotrophic water from the Agulhas current.

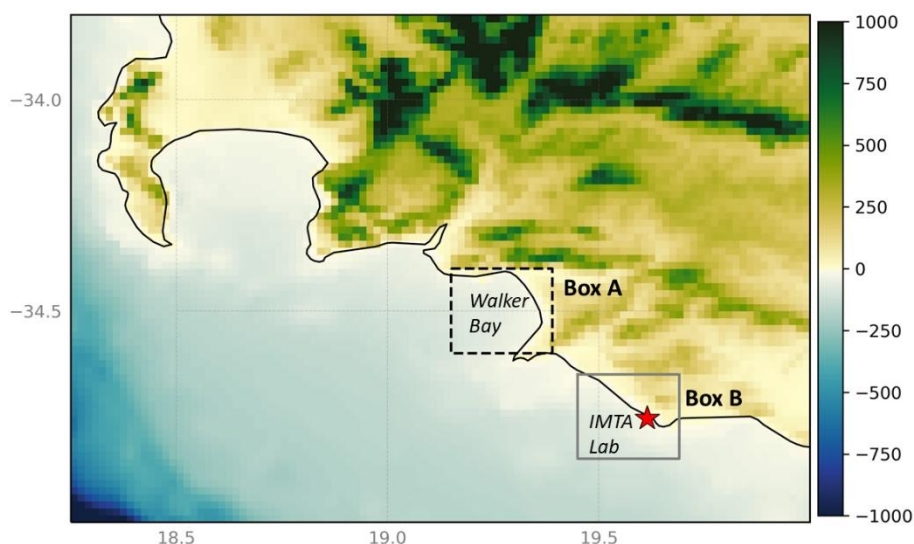


Figure 6 Map of the south-west coast of South Africa. The red star shows the location of the Buffeljags abalone farm and IMTA lab.

During 2017 a bloom of *Gonyaulax spinifera* and *Lingulodinium polyedrum* resulted in the mortality of >250 tons of farmed abalone in the Walker Bay region (Pitcher et al 2019). These are dinoflagellate blooms that generally occur at high biomass concentrations, which make them readily discernible in satellite ocean colour imagery. Dedicated phytoplankton type detection algorithms have been developed for the coastal waters of South Africa, capable of differentiating these high biomass dinoflagellate blooms from background phytoplankton (Smith and Bernard 2020) and for resolving the very high Chl-*a* concentrations that can be present in these blooms (Smith et al 2018). Figure 7 shows a time series of the satellite derived ocean colour and physical oceanographic conditions of a *Lingulodinium polyedrum* bloom that occurred and persisted for three months in the region during 2019.

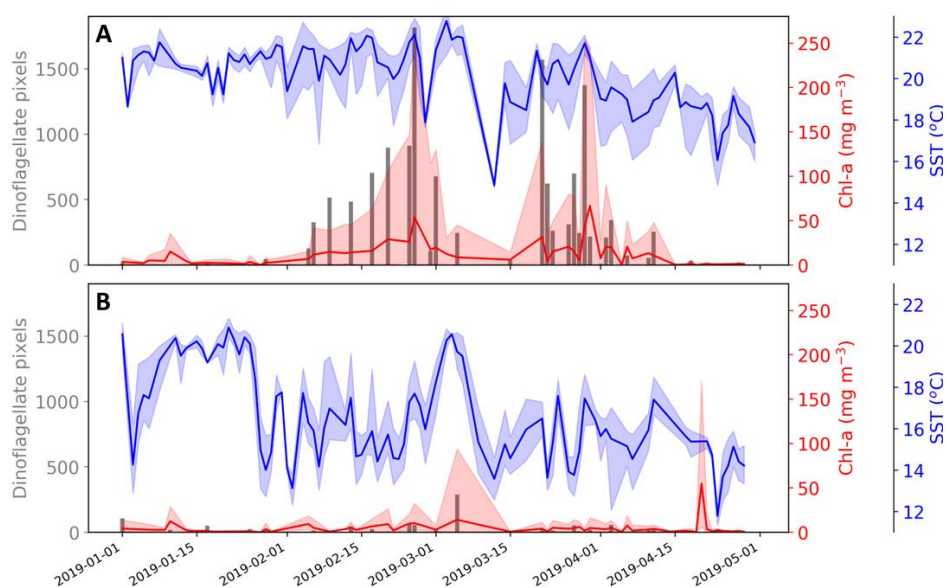


Figure 7 A time series of EO data over the Walker Bay (A) and Buffeljags IMTA Lab (B) areas from January to May 2019. The spatial extent of these boxes are shown in figure x. Red lines represent Chl-*a* concentrations derived from Sentinel 3 OLCI full resolution (300m) data, whereas the blue lines represent Sea Surface Temperature data derived from the VIIRS satellites. The shaded areas represent the 5th (lower extent) and 95th (upper extent) percentiles of each box, while the solid lines represent the box mean. The grey bars represent the number of pixels within the box that was classified as “high biomass dinoflagellate bloom” (as per Smith and Bernard, 2020).

The exposed coastal region around the IMTA Lab experienced more frequent upwelling events, as represented in the lower temperatures (mean ~16.5°C), as well as larger range and variability of SST for the period in figure 7b; this resulted in lower mean Chl-*a* concentrations (~4.4 mg m⁻³) and lower occurrence/presence of pixels classified as high biomass dinoflagellate blooms. During the same period the Walker Bay region, a mere 50km away, experienced warmer mean temperatures (~20.5 °C) and more stable water column conditions, favouring the formation of high biomass dinoflagellate blooms (Figure 7a). Bloom concentrations and spatial extent peaked at the end of February; the bloom was dispersed by upwelling conditions during the first two weeks of March but reformed again at the end



of March and beginning of April when the waters of the bay warmed again. This case-study demonstrates the important role that the farm location and oceanographic conditions play in mitigating the presence and impacts of HABs near the South African IMTA Lab.

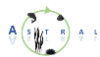
6.3 Operational EO for aquaculture support

Aquaculture support tools use a variety of data sources which may include in situ regulatory phytoplankton monitoring and biotoxin data, satellite information, and predictive models. These tools are used by aquaculture operations to not only inform on the presence of HAB species, but also on their spatial scales, intensities, and movement/trajectories; the information can be used in daily operational decision-making and could effect mitigation strategies, e.g. pre-emptive harvesting or farm closures.

6.3.1 Remote sensing

Satellite earth observation offers by far the most efficient and cost-effective monitoring option for phytoplankton types that are detectible via in ocean colour. If products can be made available to downstream users at sufficiently low latency (i.e. within a few hours of the satellite overpass) and at sufficiently high spatial resolution, they provide a powerful decision support tool. Several operational systems have been developed that either directly or indirectly support HAB-related risk mitigation in the aquaculture industry:

- [S-3 EUROHAB](#) (Sentinel-3 satellite products for detecting Eutrophication and Harmful Algal Bloom events in the French-English channel) project [2017-2022] has developed a live web-based [portal](#) and HAB and Water Quality alert system that uses Copernicus Sentinel-3 satellite products to detect *Karenia*, *Phaeocystis* and *Pseudo-nitzschia* events.
- The Harmful Algal Bloom Forecasting Branch (HAB FB) of the National Centers for Coastal Ocean Science (NCCOS) supports routine [HAB monitoring](#) through the provision of near-real time satellite-derived HAB products for a selection of high-risk lakes and coastal regions in the United States of America.
- A [Fisheries and Aquaculture decision support tool](#) was developed as part of the South African National Oceans and Coastal Information System ([OCIMS](#)); the service primarily provides relevant and regionally appropriate near-real time EO products regarding SST and phytoplankton blooms in coastal waters. The spatial extent of this service was expanded within the [GMES&Africa programme](#) under the Marine and Coastal Operations for Southern Africa



(MarCOSouth) project; this [aquaculture support service](#) includes southern African waters from Angola to Kenya.

- The algae bloom monitoring application ([AlgaeMAp](#)) for inland waters in Latin America is a tool using Sentinel 2 images at 30 m spatial resolution deployed on the Google Earth Engine (GEE) cloud computing interface, which provides products of Chl-*a* and Trophic State Index for reservoirs in Brazil and Latin America from August 2015 to present (Lobo et al 2021). Although not specifically designed for the aquaculture industry, it has potential to contribute to future aquaculture planning efforts.

6.3.2 Predictive modelling

In Europe several projects have built on the capabilities developed within previous programs such as the FP7 funded [ASIMUTH](#) (Applied simulations and Integrated Modeling for the Understanding of Toxic and Harmful algal blooms) project [2010-2013]. One of the outputs for the H2020 [AtlantOS](#) (Optimising and Enhancing the Integrated Atlantic Ocean Observing Systems) [2015 - 2019] research and innovation project was to assess the evolution of HABs on Atlantic shelf seas using a combination of in situ and satellite-derived information, forced by three dimensional numerical models; these information were synthesized and disseminated to downstream users as weekly HAB bulletins for Ireland, Norway and Spain to guide aquaculture-related decision-making and risk mitigation. The [PRIMROSE](#) (Predicting the Impact of Regional Scale events on the Aquaculture Sector) project [2017-2020] built upon these by further improving the forecasts, content and readability of HAB bulletins for the aquaculture industry. The resulting operational systems include:

- Weekly [Irish HAB bulletins](#) provided by the Marine Institute in pdf format; these bulletins provide a summary of the current status of harmful and toxic algae, summaries of satellite-derived properties (e.g. Chl-*a*, Chl-*a* anomaly and SST) and forecasts of water pathways (Leadbetter et al 2018).
- [HABreports](#) is an operational online early warning system for HABs in Scottish coastal waters, where mathematical modelling of cell advection is used in combination with satellite remote sensing to produce a weekly forecast and traffic light index of locations with HAB related risk (Davidson et al 2021).
- The Shetland Islands hosts Scotland's greatest density of shellfish aquaculture (Davidson et al 2021) and has their own Bulletin for HAB status; it includes preceding 3 weeks of wind, 6 days of satellite SST and 5 days of satellite chl_a as well as wind forecasts.



In the United States the HAB Forecasting Branch of the NCCOS also provide [HAB forecasts](#) for Lake Erie, Gulf of Maine, Gulf of Mexico and Florida, with more regions under development; these forecasts often use satellite imagery to obtain bloom location and spatial extent, and predict toxicity, mixing, or movement using different models. Furthermore, the California-Harmful Algae Risk Mapping (C-HARM) Model combines physical ocean circulation models, satellite-derived ocean colour information, and statistical models related to HABs and their toxicity, in order to predict *Pseudo-nitzschia* and/or domoic acid related risk mapping along the California coast (Anderson et al 2016).

7 Future algal bloom risk associated with climate change

7.1 Predicted climate change and effects on marine environments

The United Nations' Intergovernmental Panel on Climate Change's (IPCC) Special Report on the Ocean and Cryosphere in a Changing Climate (SROCC; 2019) has shown evidence that mean temperature all around the world has increased in the last century (Figure 8).

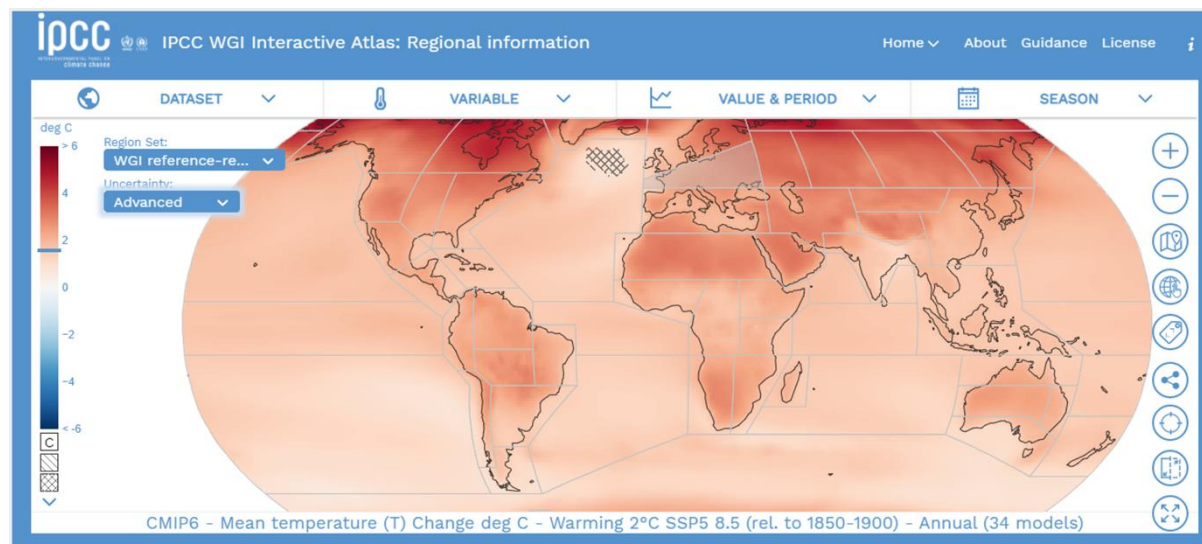


Figure 8 Map showing an increase of mean temperature (Gutierrez et al, 2021; Iturbide et al 2021).

Moreover, it is the first IPCC report to directly relate HABs to climate change. There is reasonable expectation that future climate scenarios will lead to changes in the spatial and temporal ranges of HAB species (Wells & Karlson, 2018). They further report (with “high confidence”) that HABs are displaying an expansion in range and frequency since the 1980s in response to climate and increased nutrients run-off. The concern is that these changes may increase the frequency and severity of harmful algal blooms (HABs). Relation with climate can be attributed to ocean warming, marine heatwaves, oxygen loss, eutrophication and pollution. It is further important not considering only the global trends, but also features related to local and regional drivers, so that causes and responses can be evaluated in the appropriate scales (Gobler 2020).

The 4 most discussed effects of climate change on HABs are changes in toxicity, timing and duration of the HAB and geographical distributions of HABs. However, it should be noted that the increasing number of toxic events might be additionally associated with a major monitoring effort that results from the increase in aquaculture initiatives (Hallegraef et al. 2021). Long-term data series are therefore essential to test whether a changing climate influences the frequency and intensity of the toxic events.

7.2 The effect of climate on HAB dynamics

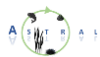
7.2.1. Changes in toxicity

Iron-Trace metals-nutrients: There is evidence that trace metal availability may change with pH, decreasing in some places and increasing in others. However, if changes in pH render the iron biological unavailable this in turn can affect algae taxa in different ways. Diatoms, which are responsible for nearly 20% of photosynthesis on Earth, are often found in the most iron-limited regions of the ocean. Domoic acid produced by some diatoms of the *Pseudo-nitzschia* genus play a role in the acquisition of iron under iron depleted conditions (Rue & Bruland, 2001). Furthermore, pH-driven decrease in iron availability can increase the production of this toxin (Wells et al., 2020), as well as under elevated copper conditions, where the process acts as detoxification mechanism (Rue & Bruland, 2001). Under high iron availability cyanobacteria blooms can become more common and these organisms are well known for their toxin-producing abilities. Many cyanobacteria which form toxic blooms also have the ability to fix atmospheric nitrogen. Synthesizing nitrogenase, the enzyme responsible for nitrogen fixation, is energetically expensive and requires substantial concentrations of iron (Larson et al., 2018). Although iron and other trace metals are likely to influence the growth and toxin production in other HAB species, there is little firm evidence for such effects (Sunda, 2006) and currently only some limited evidence exists on changes of toxicity due to iron and other trace metals. More is known about the effect of macronutrients on toxicity such as the increased toxicity of *Karenia brevis* under phosphate limitations (Hardison et al., 2013).

Temperature: There is little evidence to date that changing temperatures directly affect toxin production in HAB species although these findings are by no means comprehensive. It is unlikely that temperature alone will drive competitive selection of HAB species over non-HAB species, but it is probable that temperature effects on metabolic rates will magnify or lessen the influence of other climatic pressures on HAB prevalence (Wells et al., 2015). It is also highly probable that the response to increasing temperature is species- and sometimes strain-specific.

7.2.2 Increase in temperature and changes in HABs timing and duration

There are indications that HABs are occurring earlier in the year due to warmer temperatures. The intensity of these blooms can be further exacerbated by increased stratification and a predator-prey mismatch at this time of year. Blooms earlier in the year, when day length is shorter, and less light is available will also favour certain species over others.



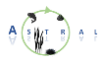
Of great significance is how temperature may expand the “windows of opportunity” for existing HAB regions assuming that physiological limits are not exceeded during peak temperatures (i.e., late summer).

Temperature: Temperature has strong influence on phytoplankton community compositions. While increasing annual temperatures should broaden the windows of some HAB activity it will not affect others or even lessen other harmful algal blooms. Ecosystem interactions, strain variability within HAB and non-HAB species, and concurrent hydrographic or oceanographic changes all complicate even this seemingly straightforward expectation (Wells et al., 2015).

Stratification: While higher temperatures will warm surface water and thus increases stratification, water column stratification will also be enhanced by greater precipitation and river runoff (Wells et al., 2020), and increase in storm intensity and/or frequency. This will have large effects on stratification patterns, and thus complicating HAB predictions. Adding other physical factors such as tidal mixing forces, increasing sea level, precipitation changes, etc. it can result in larger variability of temporal and spatial patterns of stratification in the future (Wells et al., 2020), which will greatly affect the duration of HAB either negatively or positively. Evidence on the effect of stratification on HABs have been reported in a high latitude environment, such as Beagle Channel (Southeast South America), where marked summer stratification favours *Alexandrium* sp. and other toxic dinoflagellates blooms (Cadaillon et al, 2022 and references therein) in BC (Almandoz et al., 2011), as well as in other regions (Tasmania; Condie et al., 2019, Trainer et al., 2020).

7.2.3 Climate change and effects on species distribution

Temperature has a strong influence on phytoplankton community compositions. Projections suggest that the optimal habitat of most species will shift poleward this century, with suitability increasing in the central and northern North Sea (Townhill et al., 2018). While increasing annual temperatures should broaden the windows of some HAB activity it will not affect others or even lessen other harmful algal blooms and other environmental factors such as salinity, turbidity and stratification, eutrophication should also be taken into account. Ecosystem interactions, strain variability within HAB and non-HAB species, and concurrent hydrographic or oceanographic changes all complicate even this seemingly straightforward expectation (Wells et al., 2015). This provides CyanoHABs a distinct advantage under nutrient-enriched conditions, when competition with eukaryotic primary producers, including diatoms, chlorophytes, cryptophytes and dinoflagellates can be intense (Paerl & Paul, 2012). Increasing atmospheric pCO₂ decreases the pH of surface waters in offshore, coastal and upwelling marine regions, as well as in freshwater environments (Wells et al., 2020), with most of this change occurring in high latitude regions (Stocker et al., 2013). Increase in pCO₂ results in increasing carbon



availability for photosynthesis. Under current CO₂ conditions many algae species use carbon concentrating mechanisms (CCM) which are metabolically expensive (Beardall et al., 2009) in order to satisfy their CO₂ requirements. However, not all phytoplankton groups have this mechanism, or some might have a less efficient CCM (WEHR, 2007). So, changing CO₂ values could affect phytoplankton communities and distribution, especially in the higher latitudes where CO₂ dissolution is enhanced by low temperatures. This mechanistic-based scenario, however, has not yet been demonstrated to occur (Wells et al., 2020).

Dinoflagellates such as the HAB forming *Akashiwo sanguinea* and *Prorocentrum micans* have the ability to migrate through the water column. However, lowered pCO₂ can affect the swimming ability of at least one HAB species, the raphidophyte *Heterosigma akashiwo*. It changes its swimming behaviour at lower pH in ways that may impede the formation of surface harmful blooms (Kim et al., 2013). This would limit where in the water column a bloom can form because they will no longer be able to migrate between high light- nutrient poor areas and nutrients rich-low light areas. Not only phytoplankton is affected by a change of pH, but also their grazers and the wider food web both directly by changes in pH, but also indirectly due to changes in available food.

7.3 Beagle channel: high latitude case study

In the Argentinean Patagonia, as in the rest of the world, climate change is also evident. Temperature has increased over 0.5 °C in the last 60 years (Figure 9) and all models predict it will continue to increase reaching *ca.*+3°C for 2100. Nevertheless, according to the last IPCC (2021) report there is limited data or literature on the Southern Patagonia region and therefore observed changes, modelling or trend estimates are of low confidence. This highlights the need of long-term monitoring programs and data acquisition in the region and, at the same time, the short time span of in situ data collection might result in insufficient for climate trends analysis.

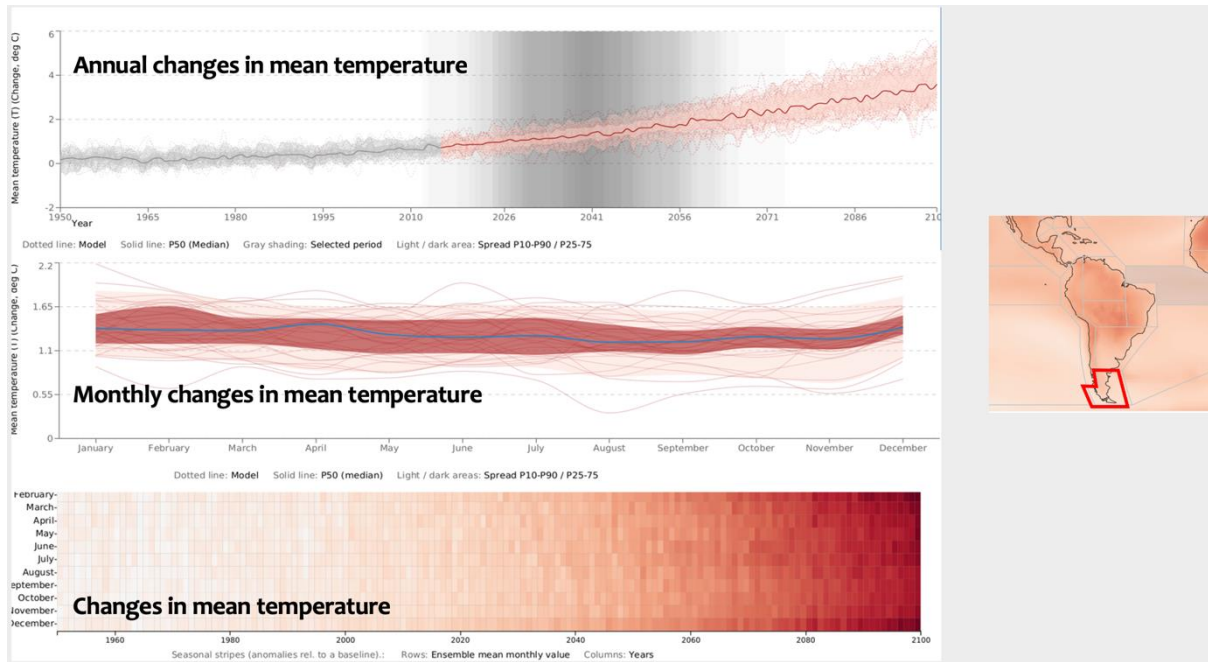


Figure 9 Observed and predicted changes in mean temperature for the Patagonia (Gutierrez et al., 2021; Iturbide et al. 2021).

In Beagle Channel, minimum air temperature has also increased *ca.* 2°C in the last 40 years (Argentinian National Meteorological Service). As evidenced in Figure 10, there is a lack of continuity in the environmental-meteorological data which renders the estimation of trends difficult.

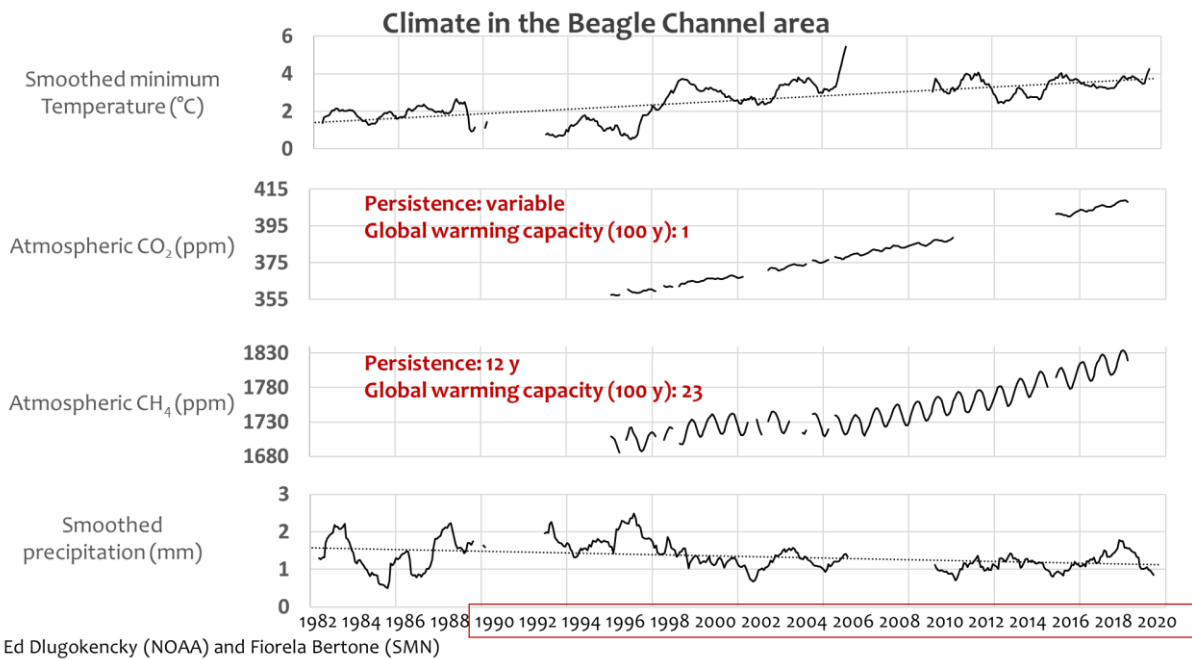


Figure 10 Changes in minimum air temperature (°C), atmospheric CO₂ and CH₄ and precipitation (mm) for the Beagle Channel area in the last 40 years. Ed Dlugokencky (NOAA) y Fiorela Bertone (SMN)

Cadaillon *et al.* (2022) have analysed the dynamics of 12 years PSP outbreaks in the Beagle Channel, based on data of the presence, spatial distribution, and temporal patterns, as well as peak evolution of PSP toxins in two mussel species (*A. ater* and *M. edulis*) during the period 2005–2017. Results have shown a marked interannual variation in PSP outbreaks. This variability could not be associated with long-term climate indexes such as the El Niño Southern Oscillation or the Southern Annular mode indexes (Figure 11). Unfortunately, no environmental nor hydrographic data are available for the complete series of toxin data. However, for some periods information exists and is being recopilated for further (future) analysis.

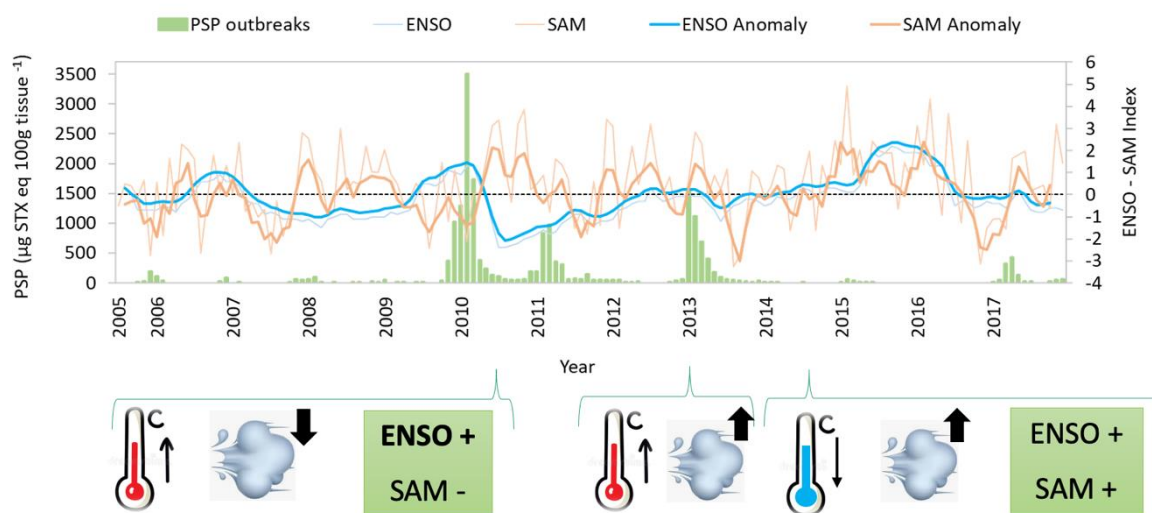
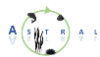


Figure 11 Preliminary interannual variability analysis. PSP toxin concentration and climatcal-oceanographical phenomena variation along the years for west Beagle Channel (Cadaillon, pers. com.). Southern Annular Mode (SAM) Marshall (2003) <http://www.nerc-bas.ac>.

7.4 HABs and climate change are results conclusive?

Recently, an unprecedented analysis on global HABs has been launched by the Intergovernmental Oceanographic Commission of UNESCO. The study found that reported HAB events have increased in some regions and decreased or held steady in others, sustaining that the widely spread view on the rise of HABs throughout the world due to climate change, could not be confirmed.

For the Beagle Channel area, more data is needed to draw any conclusions. Higher PSP toxicity values were observed in the mussel culture areas compared with the natural mussel beds, possibly associated with aquaculture activities (Cadaillon et al. 2022). In fact, even at the small spatial scale of that study, results suggests that local environmental features can lead to different HAB dynamics. This further stresses the importance of not only continuing monitoring but also doing so in several locations in an area.



8 Summary and Conclusions

Harmful algal blooms occur across the world where they cause significant economic, environmental and health problems. This review identified the abiotic and biotic factors contributing to harmful algal blooms (chapter 3) the methods of monitoring recommended (chapter 4, 6) and how the IMTA facilities are currently impacted by harmful blooms (chapter 5) followed by how climate change will impact the future of harmful blooms (chapter 7).

One of the largest contributors to the increase in frequency of harmful algal blooms is eutrophication. Eutrophication occurs from a variety of sources such as from agricultural and urban waste and the type, ratio and organic form all affect which algal species can become dominant and form large detrimental blooms. This can be further exacerbated by wind, waves and currents which transport blooms to new areas followed by stratification which is a favourable environment for many toxic dinoflagellates which have the potential to form HAB. A harmful algal bloom is a highly dynamic environment with many communities composition changes in viruses, grazers and bacteria during the course of the bloom. Furthermore, the effect of bacteria, virus and grazers on a harmful algal bloom are highly species or even strain-specific for both the predator and prey. This makes studying the microbial environment of a bloom complex.

Regardless of the system used (on-shore, off-shore, open-, closed system) all IMTA facilities have a robust monitoring system in place for detecting harmful algal species with nutrient, salinity and temperature being the most important environmental measurements and Chl-*a* and algal cell counts the most commonly biological parameters used. Other techniques used can include remote sensing, satellite data and predictive modelling. Increasing emphasis is given to predictive modelling based on remote sensing, with several projects across Europe and North America.

The literature and case studies suggest that blooms are highly spatially variable and even at the small spatial scale of that study, results suggest that local environmental features can lead to different HAB dynamics. These further stress the importance of not only continuing monitoring but also doing so in several locations with a variety of methods. Specially in an area such as the Beagle Channel, where no IMTA are yet in place, monitoring HAB and the environment provides the unique opportunity to study conditions before the presence of this aquaculture initiative, against which future comparisons could be done, once the IMTA is installed in the area.



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