Biosensors for the monitoring of harmful algal blooms

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Harmful algal blooms (HABs) are a major global concern due to their propensity to cause environmental damage, healthcare issues and economic losses. In particular, the presence of toxic phytoplankton is a cause for concern. Current HAB monitoring programs often involve laborious laboratory-based analysis at a high cost and with long turnaround times. The latter also hampers the potential to develop accurate and reliable models that can predict HAB occurrence. However, a promising solution for this issue may be in the form of remotely deployed biosensors, which can rapidly and continuously measure algal and toxin levels at the point-of-need (PON), at a low cost. This review summarises the issues HABs present, how they are difficult to monitor and recently developed biosensors that may improve HAB-monitoring challenges.

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The current issue

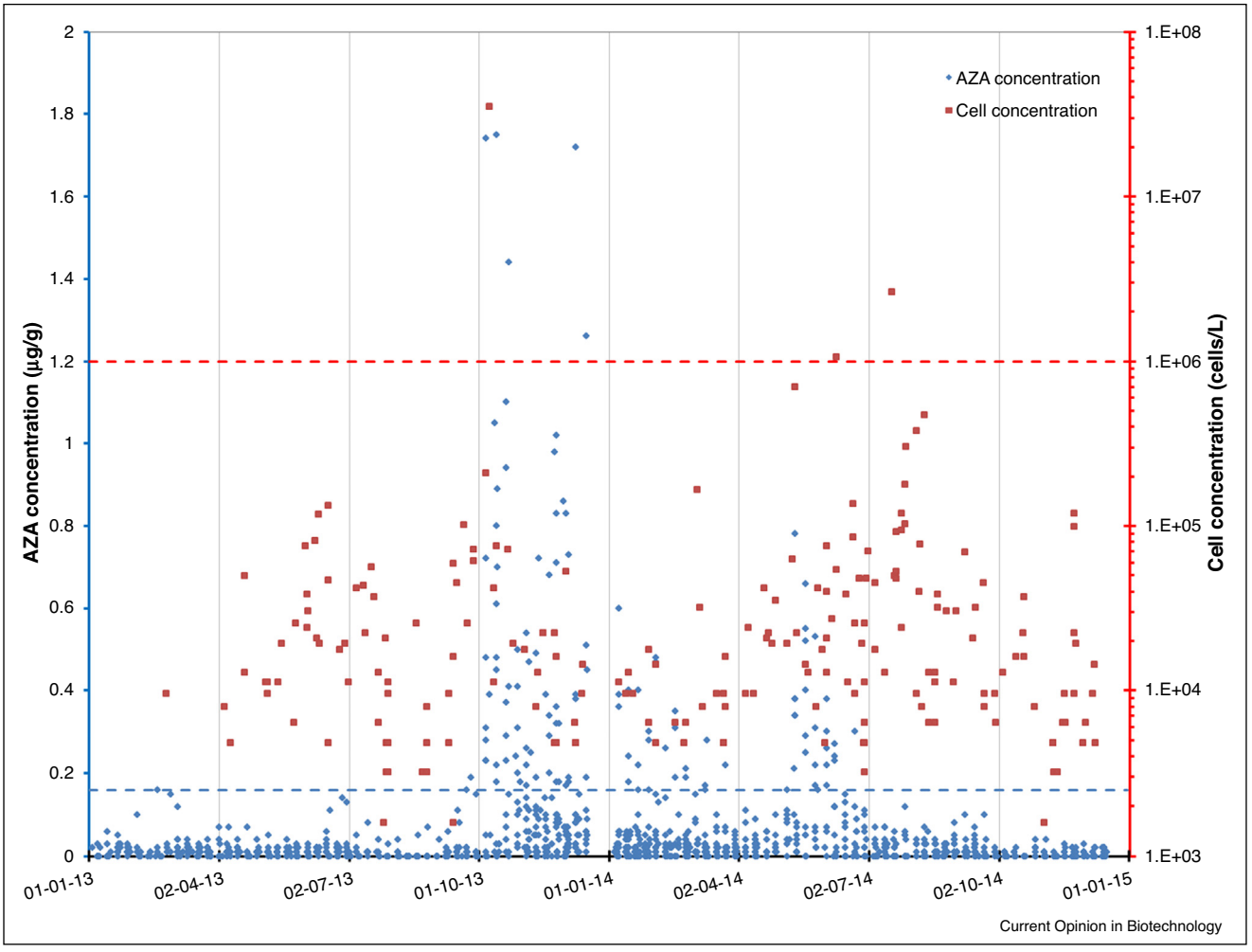
Algae are microscopic phototropic uni- or multi-cellular organisms. Some algal species can form harmful algal blooms (HABs), phenomena that occur throughout the world's oceans that have led to increasing concerns for human health and environmental preservation. Additionally, severe economic implications are associated with HABs, with estimates of losses of tens of billions of US dollars annually [1•]. These concerns arise from the increasing frequency and geographic distribution of a number of toxin-producing algal species. The prevalence of these species is not fully understood, though toxicity has been suggested as a form of defence from predatory organisms [2]. Algae act as primary food sources in the aquatic food web. They are predominantly consumed by bivalve shellfish such as mussels, clams, oysters and scallops. Phycotoxins and cyanotoxins are secondary metabolites produced by marine algae and blue-green algae (cyanobacteria), respectively. These algal toxins accumulate to highly significant levels in the digestive tracts and muscles of these shellfish. Subsequent consumption by humans often results in poisoning and severe illnesses. Their presence is responsible for numerous human intoxications yearly [3]. The toxins' associated potencies are intimately linked to their chemical structure and the organs they target. They are known to cause intoxications to humans, birds and farm animals, and impact negatively on tourism.

Global populations are on the increase and so the demand for uncontaminated food sources has never been as urgent. Governments and food safety authorities now recognise the need to regulate and closely monitor toxin contamination in foods for human and animal consumption. The ability to detect, analyse and monitor these harmful algae and their associated toxins at the required limits necessary to meet legislative requirements to avoid consumer harm must be a worldwide priority.

Currently, the monitoring of HABs and their toxins relies on the use of laboratory-based methods. Light Microscopy (LM) is the principle method used for identification and enumeration of HABs species. However, accurate identification of some species can prove extremely difficult. For example, Figures 1 and 2 display data of occurrences of *Azadinium*/*Heterocapsa* spp. off the coasts of Cork and Galway, Ireland, respectively [4,5]. These algal genera are grouped together in this manner due to the difficulty in discerning these algae by LM. Therefore, the data shown may at times under- or over-estimate levels of harmful *Azadinium spinosum*. The use of further confirmatory methods, such as electron microscopy, is often required for further species identification. In regards to the associated algal toxins, the current detection methods involve the use of expensive chromatography-based separation methods coupled to sensitive detectors [6,7]. In addition to these issues, such laboratory-based methods require trained personnel and have an inherently long turnaround time due to sample transport and handling from the sampling site to the laboratory.

Figure 1 displays the occurrences of *Azadinium*/*Heterocapsa* spp. from water sample and AZA toxins extracted from shellfish from coast of Co. Cork, Ireland, from 2013 to 2014. The data show a high abundance of AZA

Figure 1



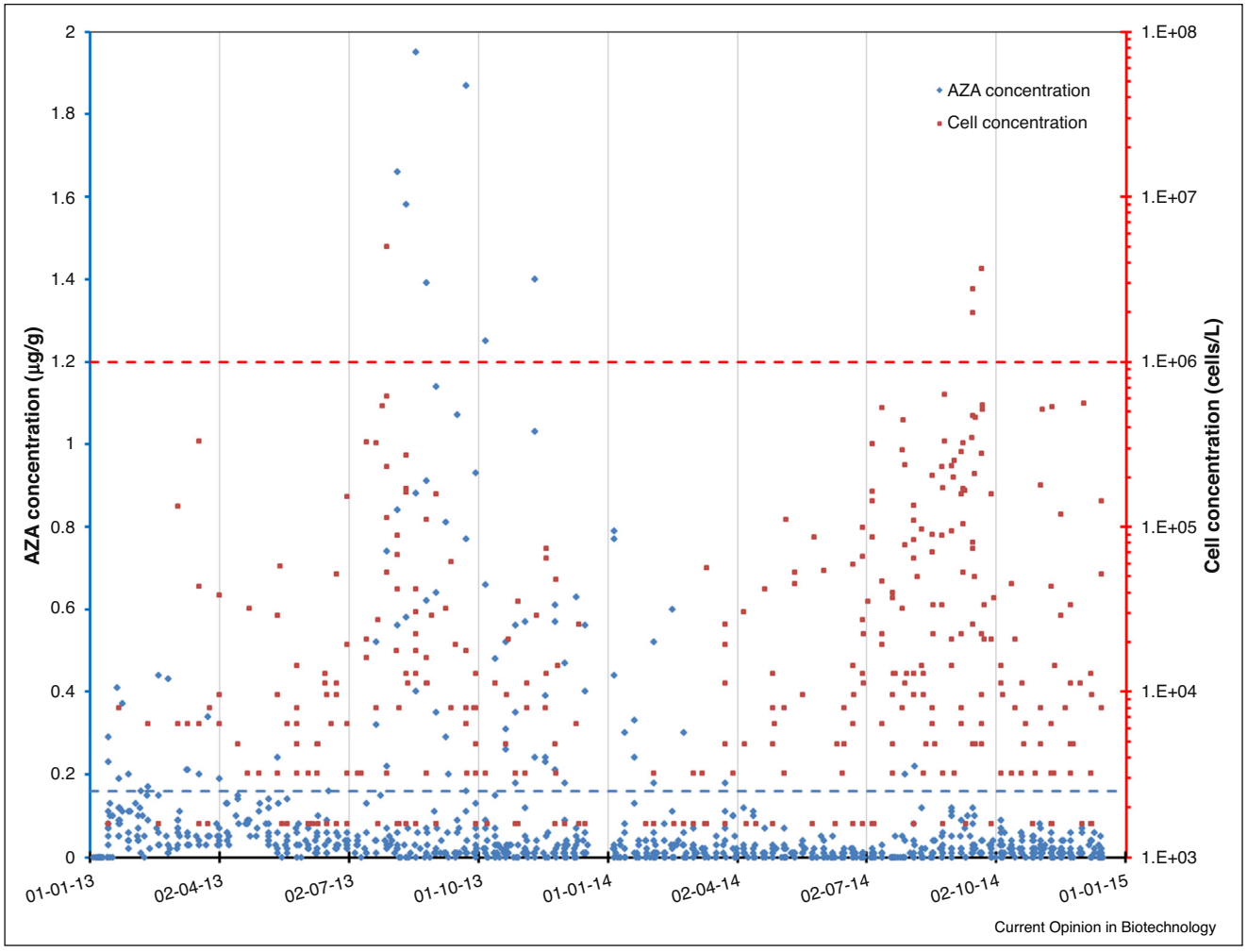
Occurrences of *Axadinium/Heterocapsa* spp. and AZA toxins (in shellfish) off the Cork coast from 2013 to 2014. Blue dashed line represents the current regulatory cut-off of AZA toxins in shellfish (0.16 µg/g). Red dashed line represents the level of a high volume algal bloom.

toxins in the Q3 of 2013. However, a very different trend was observed for 2014, with AZA toxin levels exceeding the regulatory cut-off throughout Q1 and Q2 of the year. Figure 2 displays a similar dataset but from samples acquired off the coast of Co. Galway, Ireland. Even with a geographical difference of only a few hundred kilometres, a very different trend for the occurrences of AZA toxins was observed. In 2013, Q1 experienced levels exceeding the cut-off limit, while Q3 and Q4 displayed levels up to 12-fold greater than the cut-off. In 2014, Q1 experienced AZA levels exceeding the cut-off, while the remainder of the year was largely below the cut-off. This comes despite the very high cell concentrations of *Azadinium/Heterocapsa* spp. that occurred at this time, with levels exceeding 10^6 cells/L. Such trends may give credence to the growth characteristics observed by Jauffrais *et al.*, [8], in which AZA cell quota was found to be antagonist to growth, that is cells that grew faster had lower intracellular AZA concentrations and vice versa. These data also highlight the significant variation of cell concentration and shellfish toxin levels observed annually.

HABs present a significant monitoring challenge

HABs present a significant challenge in terms of predicting when and where a bloom may occur and the scale of a bloom. A myriad of factors play a role in influencing the dynamics of bloom growth. Physical factors include temperature [9,10], salinity, current, water level [11], turbulence, shear [12], occurrence of upwelling or downwelling winds [10], light availability [9] and biological factors, such as excystment behaviour [13], tropism and cell–cell interactions [14], among others (see graphical abstract). Human activities such as sewage-dumping and agricultural run-off are also linked closely to the occurrences of HABs.

Figure 2



Occurrences of *Axadinium/Heterocapsa* spp. and AZA toxins (in shellfish) off the Galway coast from 2013 to 2014. Blue dashed line represents the current regulatory cut-off of AZA toxins in shellfish (0.16 µg/g). Red dashed line represents the level of a high volume algal bloom.

Nutrient-loading into fresh and marine water bodies, especially from phosphorus- and nitrogen-containing sources, can lead to proliferation of HABs, leading to eutrophication [15–18]. In addition to effecting HABs and toxin-production, the above-mentioned factors also play a role in shellfish toxicity as a result of toxic algal blooms. However, shellfish toxicity exhibits no or only weak correlation between the individual factors described. It is more likely that shellfish toxicity occurs due to complex interactions between numerous factors [19], which creates a greater level of complexity to accurately predict shellfish toxicity.

An established means to predict the spatial and temporal occurrences of HABs is through the development of predictive models. However, the requirement of up-to-date and high resolution sampling data on the water's physical characteristics and the current algal and toxin levels is of paramount importance for predictive models to give a reliable forecast [20–22]. Real-time data on harmful algae and toxins is required for use of bloom forecasts models, and is analogous to the way that weather forecasts require up-to-date meteorological data. Therefore, the currently employed laboratory methods for monitoring algal species and their toxins are not sufficiently rapid to provide up-to-date data required for accurate modelling. However, a promising means to monitor HABs and marine toxins *in situ*, with a low cost, high sensitivity and rapid turnaround time is in the form of biosensors. Such systems incorporate biorecognition elements with a chemical or physical measurement element to allow for detection and quantification of a target analyte. The theory of biosensors has been discussed extensively in other review articles [23,24]. Additionally, biosensors designed to specifically measure marine toxins have been previously reviewed [25,26]. Therefore, the focus of this review will be on recently

developed biosensors that can detect, quantify and identify harmful algal species (HAS).

Biosensors to detect harmful algal species

In the previous 5 years, much effort in the area of algal biosensors has been focused on the development of nucleic acid-based methods for identification of HAS. This is due to the excellent sensitivity and specificity of nucleic acid probes to their complementary binding partners, the majority of which target ribosomal RNA (rRNA) or DNA (rDNA). In the authors' literature search, such nucleic-acid based methods have come in the form of those with quantitative polymerase chain reaction (qPCR) at the heart of the assays or those based on multiplexed microarray technology. qPCR has the distinct advantage of exquisite sensitivity, due to the ability of polymerase enzymes to amplify very small quantities of target RNA or DNA. Microarrays, on the other hand, utilise RNA or DNA probes printed or spotted onto a support matrix, with each spot dedicated to the detection of a different algal genus or species. Thus, microarrays have an excellent broad range of detection. In addition to nucleic-acid based methods, some research has focused on the development of antibodies to target distinct protein biomarkers that can be used to identify HAS. In the following section, these recently developed biosensors will be discussed, paying attention to their advantages and disadvantages, and how they will better serve the pressing need to improve HAS-monitoring.

The ALGADEC system, developed by Diercks-Horn *et al.* [27], is a semi-automated rRNA biosensor. This system was used to detect *Alexandium minutum*, with the potential to detect 14 different HAS. Sample-analysis took only 2 hours, in which the user need only carry out sample filtration and sample addition to the device. The analysis does not require specialised equipment or advanced user-training. However, the limit of detection of 6250 *A. minutum* cells may not be sensitive enough to detect low levels of this strain. A similar system was developed by Orozco *et al.* [28]. This genosensor array can detect the RNA of 13 HAS, with a limit of detection of 10^5 cells in spiked seawater samples. However, this system requires numerous sample-handling steps in terms of acquisition, concentration and subsequent extraction of RNA.

Chen *et al.* [29] have developed a reverse dot blot hybridisation (RDBH) array that can currently detect five HAS. This system makes use of PCR to detect as few as 10 cells. While the system is currently limited to detection of 5 target species, the nylon membrane array can be expanded to allow for detection of a greater number of target species and the array is easy to fabricate. The array signal can be visualised by naked eye or by specialised equipment, meaning the results are semi-quantitative. Thus, this system is suitable for use as early-warning of impending or on-going blooms, but further analysis would be required to determine definitive cell numbers.

McCoy *et al.* [30•,31] have described a multiplex RNA microarray platform (MIDTAL, currently available commercially from Microbia Environnement) that can detect and quantify numerous *Alexandrium* species. This system would be suitable for improved lab-based detection and identification of HAS. It is not yet amenable for point-of-need (PON) monitoring, as it requires a number of sample-preparation steps. This system is further described by Dittami *et al.* [32]. The microarray contains 140 different probes for detection of various HAS, including numerous toxin-producing algae. A disadvantage of this system is due to variability depending on the extraction efficiency, labelling and quality of RNA in the sample. However, this system shows great promise for the high-throughput species-level identification of HAS. From our literature search, it has become apparent that a potential issue with RNA biosensors may be due to discrepancies between cell numbers determined by rRNA measurement and direct cell counting, which is due to varying amounts of RNA per cell, a factor that is dependent of cell size and growth rate [33]. Thus, this factor would need thorough investigation and correlation to current techniques prior to the deployment of such RNA biosensors.

Gas *et al.* [34] developed monoclonal antibodies (mAbs) that specifically recognise surface markers of *A. minutum* and that showed low cross-reactivity to other HAS. These mAbs were incorporated into a magnetic lateral flow immunoassay (LFIA) that could measure *A. minutum* cells in 30 min with a limit of quantification (LOQ) of 10^5 cells/L [35•]. It also showed good correlation of results obtained by LM but without the subjective user-interpretation.

A notable sensor developed in recent years is the Environmental Sample Processor (ESP). This is among the first *in-situ*, autonomous systems for the monitoring of HABs and their toxins [36–38]. Recently, it features an *in situ* sandwich hybridisation assay (SHA) and qPCR for the detection and quantitation of *Pseudo-nitzschia* RNA and DNA, respectively, to a species level [39••]. The system was capable of detecting *Pseudo-nitzschia* spp. ~100 cells/L or ~10 DNA copies/L by SHA and qPCR, respectively. The incorporation of two assay formats also allows for flexibility of detection. The ESP also autonomously carries out sample acquisition, nucleic acid extraction and sample-partitioning for multiple assays. Additional advantages of the ESP include the ability to be deployed at sea for 45 days and to report results wirelessly within hours of acquisition. However, a limitation of the qPCR assay would be the number of potential algal species that could be detected. This would be limited by the number of distinct fluorescent probes that could be used in

tandem, due to overlap of fluorescent signals. However, the SHA utilises an array format, which has greater potential for scaling up to accommodate the analysis of a greater number of HAS. The ESP was reviewed in greater detail by Ottesen [40•].

With the exception of the ESP system, none of the above mentioned biosensors are quite ready to be deployed at the PON. However, these systems show promise in terms of reducing the cost of sample analysis and reduction of analysis times. The microarray based systems, in particular the MIDTAL system, show great promise to allow for detection of a high number of HAS in-tandem. The main disadvantage of such microarrays is detection sensitivity, however, a number of strategies may be employed to increase such sensitivity [41,42]. Other disadvantages, such as sample extraction and handling, may be addressed through the use of automated microfluidics [43].

Conclusion

In the realm of HAB research and monitoring, the identification of causative species and associated toxins is paramount to understanding bloom dynamics and toxicity and to mitigating their impact. A key strategy for predicting HABs is the development of predictive models, but the reliability of such models requires up-to-date, high resolution data on HABs occurrences. Biosensors present an attractive means to establish such up-to-date, high resolution data, due to their low cost, excellent performance and their ability to be deployed at the PON. This review summarised a number of recently developed biosensors designed to detect and identify HAS. Much focus has been on the development of nucleic acid-based biosensors, such as qPCR and microarray formats. The advantages and disadvantages of these biosensors were discussed. Currently, the technology is not quite at the stage of PON deployment, but with incorporation of new technologies, such as microfluidics and nanomaterials, these biosensors may be deployable in the marine environment within the next five years. Once there, such systems should help to improve HAB monitoring, which will allow for improved prediction and mitigation of their harmful effects.

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- •• of outstanding interest

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