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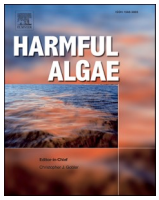

Molecular techniques for understanding harmful algal blooms: A review

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ABSTRACT

Harmful algal blooms (HABs) are intricate ecological events caused by diverse algal species and are influenced by a myriad of biotic and abiotic factors. The urgently needed development of effective prevention and control techniques face two primary challenges. The first challenge is the technical shortfalls in rapidly identifying and monitoring the causative species. The second challenge is the absence of research frameworks and technologies for accurately diagnosing the primary drivers of these blooms. Molecular techniques offer promising solutions to these issues, and research in this field has seen significant growth over the past two decades. Previous reviews have predominantly focused on species identification and monitoring, leaving the status of bloom driver studies less clear. This review provides a comprehensive overview of molecular techniques for HAB identification and driver analysis. HAB-specific use cases of techniques and comparison between them based on technical specifications are provided. Nucleic acid-based techniques presently dominate over antibody-based techniques due to their tunable taxon-specificity and ease to prepare probes. In situ applications and monitoring platforms still have a large room for improvement. The omics approach is the most promising choice for unraveling HAB drivers but requires a framework and a quantitative model for estimating the contribution of potential responsible factors. Future prospects relating to particular needs in HAB research and emerging technologies are also discussed.

1. Introduction

Harmful algal blooms (HABs) occur in aquatic ecosystems when algal species excessively proliferate due to specific environmental factors, leading to unusually high algal biomass and/or toxins that adversely affect the ecosystem or human health. According to the Global Ecology and Oceanography of Harmful Algal Blooms science plan (GEOHAB, 2001), HABs include outbreaks of photoautotrophic (algae in the strict sense) or heterotrophic protists (often harboring kleptoplastids or algal symbionts), both toxin-producing and non-toxin-producing species, as well as low-biomass but toxic and high-biomass species, regardless of toxin production. Blooms exert harmful effects on the ecosystem and human health in direct and indirect ways such as obstructing light for benthic algae and plants, clogging animal gills, creating hypoxia in the water, producing toxins, or emitting harmful gases (Backer and McGillicuddy, 2006; Hallegraeff, 1993). HABs cause devastating economic losses (Anderson et al., 2012; Hallegraeff, 1993).

Most microalgal phyla contain species capable of forming harmful algal blooms (HABs). The Dinoflagellata phylum is the largest contributor of HAB species in marine environments and Cyanobacteria in freshwater ecosystems (Anderson et al., 2021; Paerl et al., 2001). In addition to these two phyla, common HAB species also originate from haptophytes, raphidophytes, pelagophytes, and diatoms, which like dinoflagellates, mainly form blooms in the coastal ocean. All three phyla of macroalgae (rhodophytes, phaeophytes, and chlorophytes) have species that can form blooms, ranging from harmful to benign (Gao et al., 2010; Lapointe and Bedford, 2007; Rodríguez-Martínez et al., 2019). The most infamous macroalgal blooms are caused by the chlorophyte *Ulva* and the phaeophyte *Sargassum* (Robledo et al., 2021; Xiao et al., 2020). Species from these two groups of seaweed are either facultative (*Ulva*) or obligate (*Sargassum*) plankton, in contrast to most seaweed species, which are attached to benthic substrates (Xiao et al., 2020). Cyanobacterial and chlorophyte HABs may turn water green due to their high levels of chlorophyll and accessory pigments like phycocyanin (IOCCG, 2021). In contrast, HABs from other phyla may exhibit various colors such as a reddish tint due to abundant carotenoids (IOCCG, 2021). Notably, low biomass HABs typically do not alter water color, hence are not visually detectable, exemplified by that of some paralytic shellfish toxin producing HABs (Sidabutar et al., 2021).

Most HABs occur on the sea surface, but benthic HABs are not

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uncommon. Benthic HABs are primarily formed by toxic dinoflagellates such as *Gambiediscus* spp. and *Ostreopsis* spp. and cyanobacteria such as *Planktothrix agardhii* and *Microcoleus* spp. (Berdalet et al., 2017; Wood et al., 2020). Some HAB species are native, while others are invasive and introduced through natural processes (e.g. ocean currents) or human activities (e.g. ship ballast water discharge, or by hitchhiking on traded aquaculture stocks). The number of recognized HAB species has increased over time, with the IOC-UNESCO taxonomic reference list of harmful micro algae being among the most extensive records of HAB species (doi:10.14284/362) (Lundholm et al., 2009). Additionally a high level overview of HAB species is accessible in the "Harmful Algal Species Fact Sheets" in Shumway et al. (2018).

It is generally believed that HABs have increased in recent decades, due to climate change, rapidly expanding fertilizer use and cultural discharges resulting from population growth. Although advances in technology and increasing awareness have contributed to the increases in HAB documentation (Hallegraeff et al., 2021), an uptick of HABs seems true (Dai et al., 2023; Garcés and Camp, 2012; Gobler, 2020; Gobler et al., 2017). This apparent increase in HAB proliferation necessitates an enhanced need for understanding the occurrence and processes undertaken by these organisms.

For both understanding the physioecological drivers of HABs and informing HAB management, two aspects are critical. One is to rapidly diagnose the causative species, and the other is to understand the intracellular driver(s) and environmental trigger(s) of HAB events. The two objectives converge at the goal of preventing and predicting (forecasting) HABs. Diagnostic techniques have constantly been improved thanks to the rapid advancement of molecular biological technologies. Diagnostic techniques are not only crucial for microalgae, which often lack distinct morphologies for accurate microscopic identification, but also for macroalgae, which can be difficult to distinguish due to morphological similarities in vegetative or spore stages. Furthermore, unraveling the major metabolic processes and underlying molecular regulatory mechanisms during the development, maintenance, and demise of blooms is critical to the identification of environmental factors responsible for the outbreak. The advent of metatranscriptomics and metaproteomics has revolutionized this field of research.

Numerous reviews have been published on molecular techniques as applied to HAB research. They have mostly focused on the identification and monitoring of HABs. Efforts to develop and apply molecular techniques to understand the crucial metabolic processes and environmental drivers of HABs have been expanded exponentially in the last two decades, but have been rarely reviewed. This present review aims to provide an analysis of the molecular techniques pertaining to HABs, both those for species identification and abundance quantification and those for bloom driver diagnosis. Comparisons of technical specifications of these techniques, along with case studies, are presented in the hope of guiding method selection. Current research needs and future directions for the development of molecular techniques in the HAB field are also discussed.

2. Techniques to identify species and quantify abundance

Table 1 provides an overview of molecular techniques used to identify HAB species and quantify their abundance. These techniques can generally be grouped into two major categories, those that are antibody-based, such as ELISA and Immunofluorescence, and those that are nucleic acid-based such as: CRISPR-Cas, FISH, LAMP, Barcoding/Metabarcoding, Microarrays, NASBA, PCR, RCA, RPA and SHA. Antibody-based techniques target antigens of interest such as cell surface antigens, proteins, and occasionally nucleotide sequences when coupled with other techniques. Nucleic acid-based techniques by contrast, almost exclusively target oligonucleotide sequences of interest, particularly in genus and species conserved sequences (Table 1). Sample HAB species subjected to these methods are displayed in Table 1.

Table 1
Molecular techniques for the detection of HAB species with select species for which they are utilized.

Technique	Selected Target	Selected HAB Species	Reference
CRISPR-Cas	ITS	*Karenia mikimotoi* *Karlodinium veneficum*	Wang et al., 2023, 2025
Enzyme Linked Immunosorbant Assay (ELISA)	Cell surface antigen rDNA: ITS-5.8S	*Alexandrium minutum* *Alexandrium* spp. *Gymnodinium* sp.	Gas et al., 2009, Penna and Magnani, 2008, Xin et al., 2005
Fluorescent In Situ Hybridization (FISH)	rDNA: LSU (D1-D2 domain) rRNA: LSU	*Alexandrium* spp. *Alexandrium tamarense* *Cocholodinium polykrikoides* *Pseudo-nitzchia australis*	Eckford-Soper et al., 2013, Greenfield et al., 2006, Hattenrath-Lehmann et al., 2016, Kim et al., 2005
Immunofluorescence	Cell surface antigen Proteins: Flavodoxin, p34cdc2, PCNA, Rubisco, Tubulin	*Alexandrium* spp. *Aureococcus anophagefferens* *Emilania huxleyi* *Isochrysis galbana* *Prorocentrum minimum* *Skeletonema costatum* *Thalassiosira oceanica* *Thalassiosira weissflogii*	Carrera et al., 2010, Lin and Carpenter, 1996, Stauffer et al., 2008, Wang et al., 2007
Loop Mediated Isothermal Amplification (LAMP)	rDNA: 5.8S, LSU, LSU (D1-D2), ITS1-ITS2	*Alexandrium* spp. *Chattonella marina* *Cocholodinium polykrikoides* *Karenia mikimotoi* *Karlodinium veneficum* *Prorocentrum shikokuense**	Chen et al., 2013, H. 2020, Huang et al., 2017 Nagai et al., 2012, 2013, Qin et al., 2019, Trinh and Lee, 2018, Wang et al., 2019
Barcoding/ Metabarcoding	Amplicon sequence variants (ASVs) COI eDNA: 18S-V7, 18S-V9 rDNA: D1-D2 LSU, D2-D3 LSU, ITS, V4 SSU rRNA: 16S Toxin biosynthesis genes: *anaF, mcyE, sxtI*	Cyanobacteria *Fukuyoa* spp. *Gambierdiscus* spp. Macroalgal assemblage Phytoplankton assemblage	Casero et al., 2019 Huang et al., 2021 Jacobs-Palmer et al., 2021 Ørberg et al., 2021 Saunders and McDevit, 2012Smith et al., 2017
Microarray	Cell clones mRNA: rbcl rDNA: 18S, 28S rRNA: ITS1–2, LSU	*Alexandrium* spp. *Gyrodinium instriatum* *Heterosigma akashiwo* *Karenia mikimotoi* *Prorocentrium shikokuense** *Prorocentrium*	Ahn et al., 2006, Chen et al., 2016, Miranda, 2009

(*continued on next page*)

Table 1 (*continued*)

		minimum *Pseudo-nitzschia australis* *Ulva* spp.	
Nucleic Acid Sequence Based Amplification (NASBA)	mRNA: rbcl	*Karenia brevis* *Karenia mikimotoi*	Casper et al., 2007, Loukas et al., 2018, Ulrich et al., 2010
Polymerase Chain Reaction (PCR)	cDNA: *Ppcob*, sxtA4 domain mRNA: rbcl rDNA: 5S, ITS, LSU rRNA: ITS-1, 5.8S, ITS-2	*Alexandrium* spp. *Azadinium* spp. *Chattonella marina* *Heterosigma akashiwo* *Karenia brevis* *Pfiesteria piscicida* *Prorocentrum minimum* *Pseudo-nitzchia* spp. *Ulva* spp.	Andree et al., 2011, Duan et al., 2011, Gray et al., 2003, McGirr et al., 2021, Murray et al., 2011, Zhang and Lin, 2002, Zhang et al., 2024
Rolling Circle Amplification (RCA)	rDNA: ITS, LSU D1-D2	*Amphidinium carterae* *Chattonella marina* *Karenia mikimotoi* *Karlodinium veneficum* *Prorocentrum minimum*	Chen et al., 2015, F. 2020, Liu et al., 2019 Qin et al., 2020, Zhang et al., 2018
Recombinase Polymerase Amplification (RPA)	rDNA: 5.8S-ITS, ITS-1, LSU D1-D2	*Heterosigma akashiwo* *Karlodinium* spp. *Ostreopsis* cf. spp.	Fu et al., 2019, Toldrà et al., 2018, 2019, Wang et al., 2021
Sandwich Hybridization Assay (SHA)	rRNA: LSU, SSU	*Alexandrium catenella* *Akashiwo sanguineum* *Chaetoceros curvisetus* *Gymnodinium* sp. *Heterosigma akashiwo* *Microcystis* spp. *Phaeocystis globosa* *Pseudo-nitzchia australis* *Pseudo-nitzchia* spp. *Prorocentrium* spp. *Scrippsiella trochoidea* *Skeletonema costatum* *Thalassiosira rotula*	Dearth et al., 2022, Greenfield et al., 2006, 2008, Miller and Scholin, 1996, Scholin et al., 1997, Zhen et al., 2009, Zhu 2012a & b

* Formerly *Prorocentrum donghaiense*.

2.1. Antibody-based techniques

Antibody-based techniques rely on the specific binding of antibodies to unique molecular motifs (antigens) on target organisms. Antibodies can be monoclonal (mAb)—produced by clonal B cells in response to a HAB-associated antigen—and bind a single epitope, or polyclonal (pAb)—a mixture of antibodies from diverse B cells—binding multiple epitopes in the antigen. While pAbs are more sensitive, they are also more prone to cross-reactivity. However, their production is less labor-intensive than that of mAbs (Pohanka, 2009).

Antibodies are used in direct or indirect detection methods. In direct detection, the primary antibody is labeled with a reporter molecule (e.g., a fluorophore or enzyme) to generate fluorescent or colorimetric signals. In indirect detection, a labeled secondary antibody binds the primary antibody, amplifying the signal due to multiple secondary antibodies binding each primary, thus increasing sensitivity.

2.1.1. Enzyme linked immunosorbent assay (ELISA)

While antibodies can be applied in various ways, ELISA is the principal immunoassay for the detection of HABs. Furthermore, ELISA takes different forms, including direct, sandwich, competitive and indirect ELISA (Reen, 1994). The vast majority of HAB studies involving ELISA use either direct or competitive techniques (Fig. 1). A direct ELISA, the benchmark of all ELISAs, is completed in four major steps: coating, blocking, detection and signaling. Between each step, buffer is removed and washing step(s) are performed to remove unbound material. A standard direct ELISA begins with coating the solid phase (usually a 96 well plate) with the antigen (Reen, 1994). A blocking buffer is then added to block all remaining binding sites on the solid phase. The detection antibody conjugated to an enzyme is added and given the opportunity to bind to the antigen. Finally, a signal is achieved by adding a substrate which reacts with the enzyme conjugated to the detection antibody. The result is usually a colorimetric or fluorometric change that can be detected by a plate reader. The intensity of the colorimetric or fluorometric signal can be interpreted as a concentration of the antigen of interest.

An example of using ELISA for HAB detection is based on a monoclonal antibody targeting surface antigens of *Alexandrium minutum* (Gas et al., 2009). In another study, the ELISA technique was combined with PCR to target the internal transcribed spacer (ITS)-5.8S rDNA of the genus *Alexandrium* (Penna and Magnani, 2008). While standalone PCR can effectively detect *Alexandrium* species (Galluzzi et al., 2004; Penna and Magnani, 1999), including saxitoxin-producing species (Murray et al., 2011), its combination with ELISA enables high throughput quantification of *Alexandrium* cells (Penna and Magnani, 2008). However, with quantitative PCR being easily accessible now, the advantage of the PCR-ELISA technique is no longer so clear.

As direct ELISA requires the immobilization of samples (antigen) onto a solid substrate, its capacity is limited. To facilitate high-throughput analyses, competitive ELISA (cELISA) has been developed. In cELISA, the primary antibody is bound to multiwells of microplates, which can be stored for extended periods. In experiments, samples containing the antigen are added, allowing the antigen to bind the antibody. After the unbound samples are removed with washes, a signal-carrying secondary antibody is applied to bind the unbound primary antibody. The resulting signal intensity is inversely proportional to the abundance of the antigen in the sample. cELISA has frequently been used in toxin detection (Meyer, 2010). This assay principle was used by Xin et al. (2005) to create a competitive ELISA for the highly specific identification and quantification of *Gymnodinium* sp.

2.1.2. Immunofluorescence

Antibodies containing fluorophores can be used to stain the algal cells, which are then analyzed microscopically or using flow cytometry (Lin, 2008; Lin and Carpenter, 1996; Lin et al., 2003; Stauffer et al., 2008). Immunofluorescent labeling of cells allows for the in situ visualization of cells, discrimination between taxa, locating proteins of interest, and visualizing cell morphology (Lin and Carpenter, 1996; Stauffer et al., 2008; Wang et al., 2007).

A general protocol for immunofluorescent detection of phytoplankton as described by Lin and Carpenter (1996) is as follows. Samples are first collected and fixed in paraformaldehyde (4 % for 2 h at 4 °C), and transferred to −20 °C methanol for storage (between overnight to 9 months). Immunofluorescent staining then proceeds with incubations

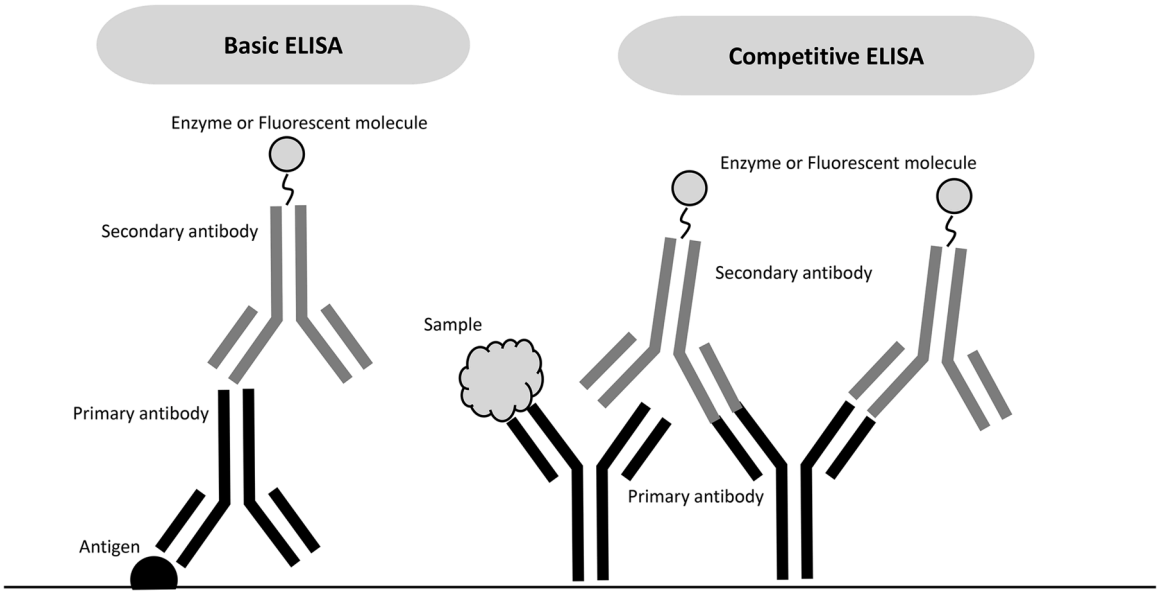

Fig. 1. Schematic of ELISA. Major types of enzyme-linked immunoassay (ELISA) used for HAB studies. A basic ELISA and competitive ELISA are differentiated anchoring of antigens to the test vessel and primary antibodies in the case of competitive ELISA.

with primary and then secondary antibodies. If the primary antibody targets intracellular molecules, cells need to be permeabilized using detergents for penetration by antibodies, a step not required for detecting cell surface antigens. Three washes with the buffered phosphate saline (PBS) are carried out in between steps. A counterstain is then applied to increase contrast. Cells are then mounted onto a slide (if not already) with a coverslip and examined under an epifluorescence microscope to visualize and quantify stained cells.

Immunofluorescence can also be coupled with flow cytometry, often referred to as immunologically based flow cytometry (IFCM), as a simpler and more rapid alternative to traditional immunofluorescence (Stauffer et al., 2008). To begin, algal cells are placed in a preservative until the time of analysis. Subsequent processing is similar to what is described above, but without mounting the cells onto slides. The resultant fluorescently labeled cells are analyzed by a flow cytometer. This method has been successfully applied to field samples using monoclonal antibodies specific to *Alexandrium minutum* (Carrera et al., 2010). These antibodies targeted intracellular antigens of *A. minutum* and allowed for the discrimination of this species from other *Alexandrium* species via flow cytometry.

A common challenge presented by techniques involving the use of fluorescence and phytoplankton is the autofluorescence from chloroplasts and some other molecules in many of these organisms (Wang et al., 2007). Techniques to mitigate this include ethanol washes to extract pigments and calibration to account for autofluorescence (Wang et al., 2007). An additional consideration in the design of immunofluorescence techniques is antiserum dilution. For example, at a 1:5 dilution, an antiserum specified for *Prorocentrum shikokuense* (formerly *P. donghaiense*) cross-reacted with three other members of the genus *Prorocentrum* while discriminating against other members of the genus as well as more distant genera (Wang et al., 2007). Diluting the antiserum to 1:100 preserved the fluorescent intensity of *P. shikokuense* while discriminating against all other tested species.

2.2. Nucleic acid-based techniques

Nucleic acid-based techniques offer several advantages over antibody-based approaches. First, the specificity of probes or primers required in the techniques is highly customizable, ranging from universal to strain-specific, depending on research needs. Second, probe and primer syntheses are rapid and cost-effective. Finally, nucleic acid-based methods are versatile and can be adapted to various formats and platforms.

2.2.1. CRISPR/CAS

Originally developed as a genome editing technique, CRISPR-CAS takes advantage of the prokaryotic immune system. CRISPR first employs CRISPR RNA (crRNA) or guide RNA (gRNA) to target a gene sequence (Pickar-Oliver and Gersbach, 2019). The spacer of the crRNA hybridizes to the target allowing for the protein Cas to selectively cleave the target oligonucleotide (Pickar-Oliver and Gersbach, 2019).

Progress in the CRISPR-Cas field has allowed for the detection of specific oligonucleotide sequences (Zhou et al., 2018). This has allowed for the application of the CRISPR-Cas technique to HAB detection. Researchers have developed a CRISPR-Cas based technique for detecting *Karenia mikimotoi* (Wang et al., 2023). Their technique utilized Cas12a from *Lachnospiraceae* bacterium ND2006 (LbCas12a) to target and cleave the internal transcribed spacer (ITS) of *K. mikimotoi*, guided by RNA (Fig. 2). They leveraged the target-activated non-specific single-stranded deoxyribonuclease cleavage activity of LbCas12a to generate signals detectable by a fluorescence detector or lateral flow devices (LFDs). By combining recombinase polymerase amplification (RPA) and LbCas12a with reporters, they significantly enhanced sensitivity, enabling the detection of ITS-harboring plasmids at concentrations as low as 9.8 aM and genomic DNA of *K. mikimotoi* at levels as low as 3.6×10^{-5} ng/μl. Additionally, they simplified the genomic DNA extraction technique using cellulose filter paper (CFP) by directly eluting the DNA into RPA reactions, reducing the extraction time to less than 30 s. The entire process, from genomic DNA extraction to result reporting, takes less than an hour, allowing for the identification of nearly a single cell. CRISPR also shows promise as a field deployable method, with a crude DNA extraction protocol having been shown to enable the detection of *Karlodinium veneficum* (Wang et al., 2025). These examples indicate that the CRISPR/Cas technique is promising to provide an easy, specific and sensitive approach for detecting HAB species, offering potential for efficient monitoring and management of harmful algal blooms.

To assess the toxin production capacity of a HAB population, toxin production genes are promising candidates for CRISPR-Cas analysis (Durán-Vinet et al., 2021). Select target genes implicated in toxin production are summarized in Tables 1 and 2 of Durán-Vinet et al. (2021). These genes include *dabA* in *Pseudo-nitzschia* spp. implicated in domoic

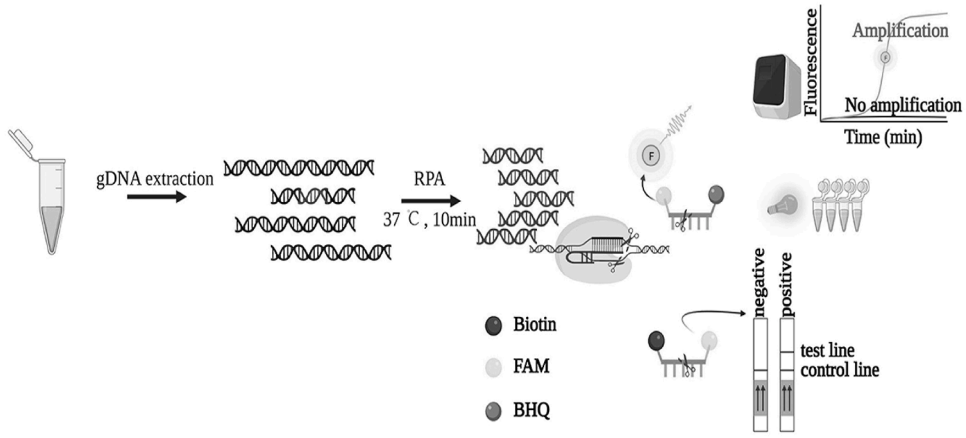

Fig. 2. Schematic of LbCas12a-based detection. The genomic DNA of K. mikimotoi is extracted, followed by RPA pre-amplification. By identifying and digesting specific target DNA, the "collateral activity" of LbCas12a is activated, which will digest bystander non-specific ssDNAs. The released signals are detected by lateral flow strips or fluorescence readers such as a real-time PCR machine or blue light transilluminator. Reproduced from Wang et al. (2023).

acid synthesis (Brunson et al., 2018) and *sxtA* in *Alexandrium* sp. implicated in saxitoxin synthesis (Murray et al., 2011). By targeting these genes for analysis, discrimination between toxic and nontoxic strains in a population could be achieved, enhancing predictive capacity of HAB impacts.

2.2.2. *Fluorescent in situ hybridization (FISH)*

Fluorescent in situ hybridization, FISH, utilizes fluorescently labeled oligonucleotides which hybridize to a target ribosomal nucleotide sequence in situ where the resultant fluorescence post-hybridization is detectable and, in some cases, quantifiable (e.g. % cells showing the hybridization signal). Like antibody-based techniques, FISH involves washing to remove unbound materials between steps. Traditional FISH techniques begin with the preparation of the hybridization target (a RNA or DNA sequence), resulting in the fixation and permeabilization of cells (Bari and Yeasmin, 2014). The fixed cells are then incubated with a fluorescently labeled oligonucleotide probe. Successful hybridization can then be viewed by fluorescence microscopy (Tönnies, 2010). A standard schematic of a FISH reaction is shown in Fig. 3.

The first application of FISH came in 1980, in which a rhodomine-bound RNA probe was used to visualize mitochondrial DNA of trypanosomes (Bauman et al., 1980). Improved probe specificity and labeling have advanced FISH to be applicable for the detection of diverse HAB species (Table 1). Several challenges had to be overcome to apply FISH to HAB species. First, the requirement of probes to traverse cell walls and membranes while preserving cellular integrity has proven challenging for some algal species (Medlin and Orozco, 2017), although nucleic acid probes are much smaller than antibodies and hence less difficult to enter cells. Techniques covering diverse phytoplankton groups have been developed (Groben and Medlin, 2005). Second, autofluorescence of HAB cells stemming from pigments or unknown molecules has also proven challenging for FISH visualization (Zeller et al., 2016), as in the case of immunofluorescence. Pretreatment of cell samples with methanol (−20 °C) removes chlorophylls and facilitates cell permeabilization (Lin and Carpenter, 1996), whereas pretreatment with H_2O_2, ethanol, or $CuSO_4$ suppresses autofluorescence (Cohen et al., 2021; Zeller et al., 2016). Additionally, tyramide signal amplification (TSA) can be used to amplify FISH signal, which can overcome autofluorescence, as demonstrated in cyanobacteria (Schönhuber et al., 1999).

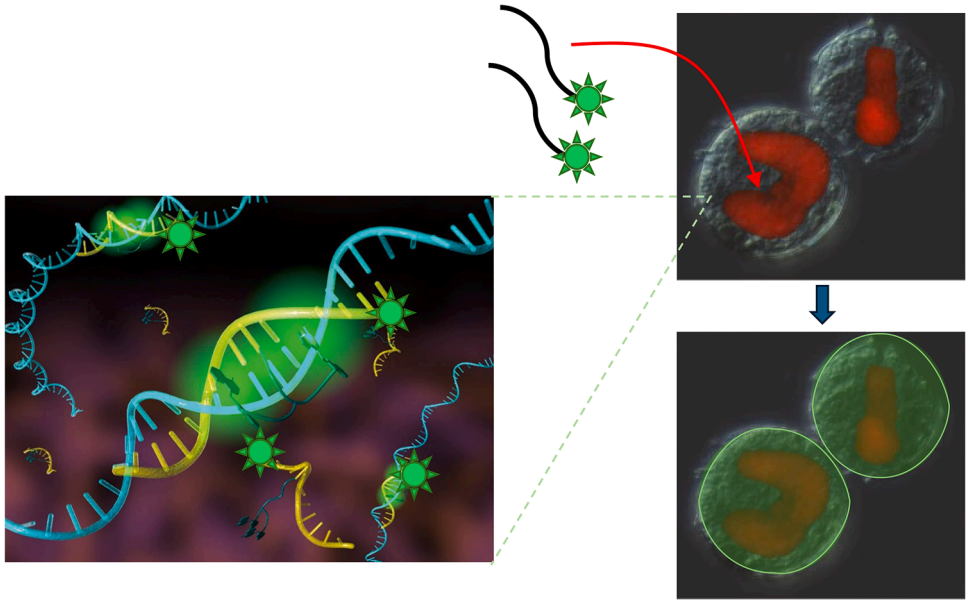

Fig. 3. Illustration of fluorescent in situ hybridization (FISH). A pair of Alexandrium catenella cells are used as an example. The fluorescent micrograph of their propidium iodide-stained horseshoe-shaped nuclei was superimposed on a brightfield micrograph.

FISH is used for a wide array of HAB detection techniques. A FISH assay for the detection of resting cysts of *Cochlodinium polykrikoides* facilitated the first identification and mapping of these cysts (Hattenrath-Lehmann et al., 2016). Effective discrimination between species of the ciguatoxin-producing dinoflagellate *Gambierdiscus* spp. allows for characterization of complex community samples via FISH (Pitz et al., 2021). FISH has also shown promise in combination with other analytical techniques. Traditional FISH results must be read via epifluorescence microscopy. When combined with an automated enumeration technique such as flow cytometry, greater testing efficiency can be achieved (Toebe, 2013). For example, the use of FISH and flow cytometry has proven effective at rapidly quantifying and discriminating between toxic and non-toxic *Microcystis* (Gan et al., 2010).

FISH for HABs is time-intensive, and probes need to be custom-designed as they are typically commercially unavailable (Huber et al., 2018). Time-to-result in FISH protocols for HAB detection often requires several hours (Gan et al., 2010; Hattenrath-Lehmann et al., 2016; Pitz et al., 2021). Outside of the HAB field, FISH time-to-result has been shown to be achievable in as little as 1-hour for bloodborne bacteria (Peters et al., 2006). These rapid FISH assays rely on commercially available assay kits, specialized lysis mixtures specific for the rapid lysis of bacterial cells and the feasibility of replacing the water bath incubation with a microwave oven. No such commercially available kit or microwave protocol currently exist for HABs.

2.2.3. Sandwich hybridization assay (SHA)

Sandwich Hybridization Assay (SHA) is a technique in which a species-specific probe immobilized on a membrane or glass slide binds to, and pulls down, rRNA of the target species, and a second species-specific probe that carries a signal molecule binds to the captured rRNA (Fig. 4). SHA has proven to be a robust technique for the detection and quantification of numerous HAB species (Table 1). The first probe, the capture probe, acts as a structural support for the target sequence and enables the subsequent binding of the second probe, the signal probe. The signal probe, usually consisting of fluorescent molecules (ex. Cy5-, Cy3-) or colorimetric compounds (ex. DIG-), can be used to detect and potentially quantify a target sequence (Gong et al., 2022). Since its initial uses in the 1970′s for the detection of viral DNA (Dunn and Hassell, 1977), SHA has since expanded to be applied to the detection and quantification of HAB-forming species. Extensive catalogues of probes for HAB- forming species have been developed, allowing for enhanced probe selection (Bowers et al., 2017; Diercks et al., 2008; Haywood et al., 2007).

Traditional SHA has targeted genus or species specific rRNA (Gong et al., 2022). Advancements in understanding the gene clusters implicated in biotoxin synthesis in HAB species has allowed for more effective targeting of these species. Zhu et al. (2012a) employed an SHA targeting *mcyj*, a gene cluster implicated in the production of microcystin by members of the genus *Microcystis*, allowing for discrimination between toxin-producing and non-toxin producing species of *Microcystis*. Other genes implicated in toxin production such as *sxtA* found in several dinoflagellate species could also be candidates for SHA detection of toxin producing HAB species (Stüken et al., 2011).

Typical SHAs are read via a plate reader that interprets colorimetric or fluorometric signals. Developments outside the HAB field have shown the capacity of a variety of instrument types to be used for the detection of SHA. Surface enhanced resonance and Raman scattering has been combined with sandwich hybridized target sequences to detect DNA concentrations as low as 10^{-10} M (Feuillie et al., 2011). Additional alternative detection instrumentation applicable to SHA include microarray scanners, liquid scintillation counters, capillary electrophoresis (Clancy et al., 2017; Goldman et al., 2013; Zammatteo et al., 1997). These alternative techniques are discussed at length in Gong et al. (2022).

2.2.4. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) uses a heat-stable polymerase enzyme (e.g., Taq) to extend oligonucleotide primers (specific to species) based on the species' sequence as the template. The result of this process is the amplification of the template DNA by extending primers bound on templates (Bartlett and Stirling, 2003; Wood et al., 2013). A pair of primers is designed such that they have similar annealing temperatures (typically 55–60 °C), and will produce appropriate lengths of amplicons (~100–300 bp for quantitative PCR and up to ~3 kbp for non-quantitative PCR). Generally, three temperature-defined steps are undertaken in a PCR protocol (Khehra et al., 2023). First, in the denaturation step, hydrogen bonds between base pairs in double-stranded template DNA are denatured at 95 °C. In the subsequent annealing step, typically at between 50 °C-60 °C, the denatured template DNA is cooled to allow oligonucleotide primers to anneal to their complementary template DNA strands forming a double-stranded, template/primer complex. Finally, elongation occurs at an optimum activity temperature for the polymerase enzyme (typically 72 °C). The result is an exponential growth of the initial template DNA sequence.

Generally, PCR analyses should always include a positive and negative control. The positive control uses a template known to be amplified by the experiment's primers. Failure to generate a detectable amplicon suggests an issue with the chemical system or the PCR machine. The negative control contains all PCR components except the template and should produce no amplicon; if it does, the system is contaminated with DNA from the study organism or other sources (e.g., human, bacteria, fungi). If the positive control generates an amplicon correctly and both the negative control and experimental samples do not, the experimental samples might be truly negative, or the template DNA might be too impure for amplification. Diluting the DNA template with pure water by 10- or 100-fold can reduce inhibitory impurities and often results in more efficient amplification. Usually, amplification failure is due to poor quality rather than absence or low quantity of the DNA template.

PCR can be subdivided into two major categories: end-point PCR and real-time quantitative PCR (qPCR). The more traditional technique, end-point PCR, permits the PCR reaction to continue to saturation. Because amplification efficiency decreases as polymerase loses activity or free

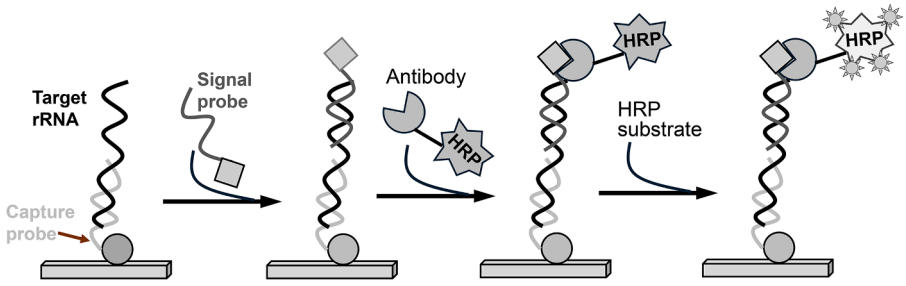

Fig. 4. Schematic of sandwich hybridization assay (SHA). Capture probe is immobilized to substrate by biotin. HRP, horseradish peroxidase. The final signal can be fluorescent or colorimetric depending on the technique of detection.

nucleotide runs low, the end-point PCR technique is suitable for determining presence or absence of, but not for quantifying, the target species or DNA molecule. In contrast, qPCR continuously monitors the amplification product (amplicon) as the reaction proceeds, and the increase in amplificon abundance over time is mapped. Absolute quantification is achieved based on a standard curve constructed with a dilution series of a known number of cells equivalent DNA (Lin et al., 2006; Zhang and Lin, 2005) or a known quantity of extracted DNA subjected to the same PCR run as the experimental samples (Antonella and Luca, 2013). It is preferable to use known number of cells for the standard because gene copy number within algal cells varies considerably between species and between growth stages (Galluzzi et al., 2009). Given this variation, the use of DNA for the standard can potentially lead to overestimations or under-estimations (Galluzzi et al., 2009). Therefore, when extracted DNA is used as the standard, qPCR results must be interpreted in light of species-specific gene copy number variations.

Considering the importance of quantification, multiple techniques are available to quantify the starting templates (Fig. 5). SYBR-Green relies on a DNA binding dye that emits green fluorescence under blue light excitation when bound to nucleic acids. The Taqman technique is based on a fluorescent probe added to PCR reaction to allow specific binding to the amplicon. The third technique is molecular beacon, which utilizes a probe with a signal molecular on the one end and a signal quencher on the other end. Without binding to DNA, the quencher is physically close to the signal molecule and prevents fluorescence. When the probe binds to DNA, the two ends are separated, relieving the inhibitory effect of the quencher, and the signal molecule emits fluorescence under blue light excitation (Lin, 2008).

As portable PCR machines are becoming available, HAB species in situ detection via PCR will be a reality. For instance, a fast, battery-powered, portable device for PCR amplification and end-point detection was developed (Jie et al., 2020). The device comprised a PCR thermal control system, a PCR reaction chip, and a fluorescence detection system. The thermal control system included a thermal control chip with thin-film heaters and resistance temperature detectors (RTDs), providing an average heating rate of 32 °C/s and a cooling rate of 7.5 °C/s. Such devices are promising for the future of HAB research.

2.2.5. Loop-Mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is an isothermal DNA amplification technique in which *Bst* DNA polymerase reacts with a series of 4 to 6 primers to amplify 6–8 distinct DNA sequences from the target (Toldrà et al., 2020). First described by Notomi et al. (2000), LAMP relies on the strand-displacing action of *Bst* DNA polymerase in concert with forward inner primers (FIP) and backward inner primers (BIP) to synthesize a DNA "loop" structure. This process results in an amplified oligonucleotide mixture containing stems and loops of DNA. Detection of amplification is generally conducted by one of two distinct techniques. The first technique is based on a perceptible change in color or fluorescence via the addition of a dye or a reaction byproduct such as magnesium pyrophosphate (white). Alternatively, detection can be achieved by the use of antibody-based lateral flow tests. Both colorimetric/fluorometric and lateral flow LAMP techniques have been applied to many HAB species. These include *Alexandrium* spp. (Wang et al., 2008, 2019) and *Karenia mikimotoi* (Huang et al., 2020; Zhang et al., 2009). Regardless of detection technique, the internal transcribed spacer (ITS) of ribosomal DNA is often targeted for amplification.

LAMP provides a faster, less technically complex alternative to traditional PCR. The most significant technical advantage is that it does not require temperature modulations. Furthermore, as the technology improves, LAMP can operate at room temperature and a diverse array of DNA extraction methods for use in LAMP are available (Lee et al., 2021; Nagai et al., 2012). This renders LAMP suitable for field-based deployment. LAMP field techniques exist for *Alexandrium catenella* (Fujiyoshi et al., 2020; Wang et al., 2019), *Chattonella marina* (Qin et al., 2019) *Cochlodinium polykrikoides* (Trinh and Lee, 2018), *Karlodinium veneficum* (Huang et al., 2017), and *Prorocentrum shikokuense* (Chen et al., 2013). A LAMP technique was also developed to detect and discriminate between six species of *Alexandrium* (Nagai, 2013). Potential drawbacks of LAMP include the elevated risk of primer-primer hybridizations (leading to false positives) and LAMP products having less secondary uses as opposed to PCR amplification (Wong et al., 2018). False-positive results have been reported from LAMP assays due to the increased number of primers elevating the risk of primer-primer hybridizations. This process is known as template-free amplification (Wong et al., 2018). Unlike PCR products which can form singular bands for examination in gel electrophoresis, LAMP products in contrast form a ladder-banding pattern

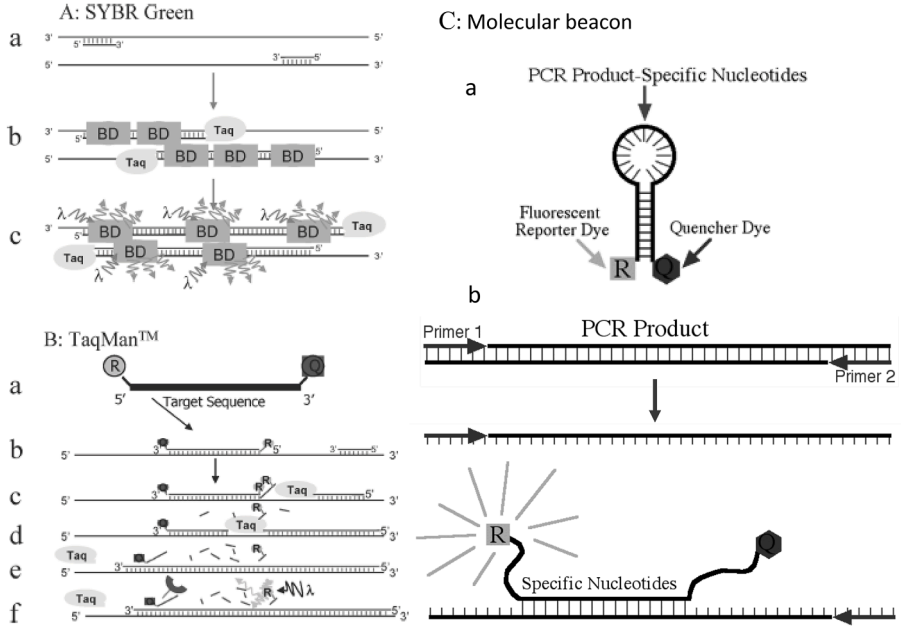

Fig. 5. Three techniques to quantify starting templates in qPCR. Quantification of PCR product is show to be achievable via the A) SYBR Green technique, B)TaqMan technique, or C) Molecular beacon technique.

which is less commonly used for definitive identification (Sahoo et al., 2016). Additionally, LAMP products cannot be used for cloning like PCR products (Sahoo et al., 2016).

2.2.6. Nucleic acid sequence-based amplification (NASBA)

Nucleic acid sequence-based amplification (NASBA) is used to amplify single-stranded RNA or ssRNA (Fig. 6). A typical NASBA protocol begins with the binding of the first primer to the template RNA. AMV (avian myeloblastosis virus) reverse transcriptase catalyzes the synthesis of a complementary DNA (cDNA) strand, resulting in an RNA-cDNA hybrid (Toldrà et al., 2020). RNase-H then digests the template RNA from the cDNA, leaving behind a single stranded cDNA to which primer 2 binds. AMV reverse transcriptase catalyzes the synthesis of a new DNA strand complementary to the cDNA, resulting in the creation of double-stranded DNA (dsDNA). From this dsDNA, T7 RNA polymerase synthesizes new copies of the original template RNA and starts the next cycle of producing dsDNA from ssRNA. The procedure leads to exponential growth of the original template RNA. NASBA has been used for field detection and quantification of *Karenia brevis* and *Karenia mikimotoi* (Casper et al., 2007; Ulrich et al., 2010). The field deployability of NASBA has been highlighted as being of particular interest to areas frequently affected by blooms of *Karenia* such as the state of Florida (Ulrich et al., 2010).

2.2.7. Rolling circle amplification (RCA)

Similar to LAMP, rolling circle amplification (RCA) is an isothermal oligonucleotide amplification technique reliant on polymerases with strand-displacement activity (Fig. 7). RCA begins with the hybridization of a target sequence to the 5′ and 3′ segments of a C-probe (padlock probe) sequence, typically 40–60 nt linear DNAs or RNAs. The hybridization triggers the ligase-based circularization of the padlock probe. A polymerase with strand displacement activity (e.g., Bst, Bsm, Vent, Phi29, and T7 RNA polymerase) catalyzes the synthesis of a new strand by displacing the old strand while moving along the padlock probe as the template. The long complementary ssDNA or RNA strands, i.e., tandem repeats of the target, resulting from the synthesis, can be detected using a detection probe. RCA can be adapted to various detection platforms (liquid, solid substrate) for various molecules (Garafutdinov et al., 2021). RCA has been used to detect a variety of HAB species including *Amphidinium carterae, Karenia mikimotoi, Karlodinium veneficum* and *Prorocentrum minimum* (Chen et al., 2015; 2020; Liu et al., 2019; Zhang et al., 2018). Lateral-flow dipstick technology, similar to LAMP has been applied to RCA to simplify detection (Liu et al., 2020; Qin et al., 2020; Zhang et al., 2019a).

Like LAMP and other isothermal amplification techniques, RCA is free from the requirement of thermocyclers and target-specific primer development as in PCR. Furthermore, the RCA technique is particularly useful for amplifying short targets such as microRNA (Xu et al., 2023). The other advantage of RCA is its single-nucleotide specificity, because only exact complementarity between the target and the complementary 5′ and 3′ segments of the padlock sequence induces the circularization of the padlock sequence. Potential limitations of RCA include relatively lower sensitivity associated with the linear amplification instead of exponential amplification in PCR. A major consideration with RCA is the impact of template length on amplification efficiency bias in RCA reactions (Joffroy et al., 2018). To avoid this impact, the same length of template should considered when the assay is designed, particularly when multiple targets are to be compared.

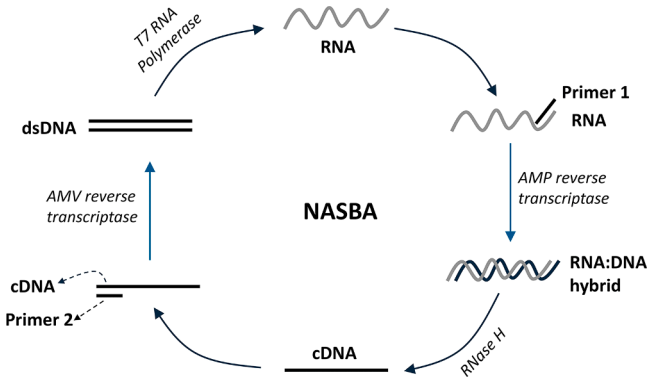

Fig. 6. Schematic of Nucleic acid sequence-based amplification (NASBA). The enzymatic process by which NASBA occurs results in the exponential amplification of a target sequence, allowing for its detection.

2.2.8. Recombinase polymerase amplification (RPA)

A relative of the RCA, RPA (recombinase polymerase amplification) requires a forward and reverse primer, utilizing the strand-displacement activity of a polymerase in combination with single-stranded DNA-binding protein (SBB) and a recombinase to amplify target DNA (Daher et al., 2016). First, the recombinase binds to the primers to form a recombinase-primer complex (Lobato and O'Sullivan, 2018). This recombinase-primer complex searches for homologous sequences. Upon locating its homologous sequence, the strand-displacing polymerase inserts the primers at the cognate site which is then stabilized by SBB. Following this insertion, the recombinase dissociates, permitting the polymerase to elongate the primer, ultimately resulting in amplification. RCA has been applied to HAB species including *Heterosigma akashiwo, Karlodinium* spp. and *Osteopisis* cf. spp. (Fu et al., 2019; Toldrà et al., 2018, 2019; Wang et al., 2021). RPA, like LAMP and RCA, has also proven amenable to lateral-flow dip stick detection (Fu et al., 2019).

2.2.9. DNA barcoding/ metabarcoding

DNA barcoding is a diagnostic technique for species identification that uses a short, standardized DNA region known as a barcode (Lahaye et al., 2008). To qualify as a barcode, the DNA sequence must exhibit significantly greater divergence between species than within species, be easy to PCR-amplify, sequence, and analyze, and be "universal" (Lin et al., 2014). Practically, the inter-specific genetic distances based on the barcode should be markedly greater than the intra-specific distances, with a detectable gap. The sequence should be relatively short so one sequencing read can cover it completely, and it should be flanked by highly conserved sequences, allowing the design of a "universal" set of primers to amplify as many taxa as possible. This universal attribute of the primers is highly desirable for analyzing microalgal communities (metabarcoding). A practical application can be seen in the use of metabarcoding for the detection of microalgal resting cysts. By using either a single PCR amplification with genus- or species- specific primers or a "nested" PCR reaction using eukaryotic "universal" primers followed by the use of genus- or species- specific primers, a great diversity of resting cysts could be detected (Penna et al., 2010).

In practice, DNA is extracted from species or environmental samples (eDNA), subjected to PCR to amplify the barcode, and then sequenced. A phylogenetic tree is used to compare the genetic distance between taxa. According to the "easy" requirement of DNA barcoding, the simplest evolutionary model, UPGMA (Unweighted Pair Group Method with Arithmetic Mean) should be used. In contrast, if the barcode is used to address evolutionary questions, more sophisticated models are needed to account for uneven mutation rates of different nucleotides. Furthermore, a highly variable DNA region is required if barcoding is aimed to resolve intra-specific strains. The internal transcribed spacer (ITS) of the rRNA cistrom and some functional genes are suitable. For instance, intra-specific genetic diversity based on the functional genes for saxitoxin production (*sxt*) within *Alexandrium minutum* are believed to underpin the variability in toxicity and toxin composition (Mary et al., 2022).

Currently, no fully universal barcodes are available for all algae, let alone all eukaryotes. Multiple gene regions have been explored and found to provide different resolving power. Overall, nuclear ITS, the

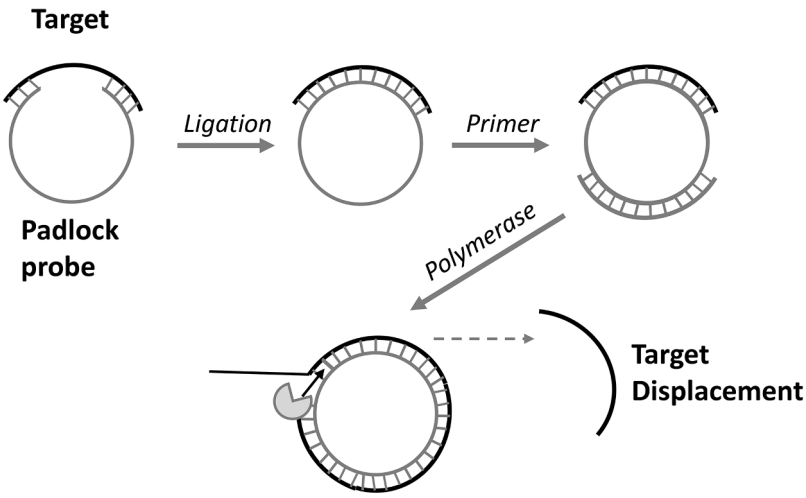

Fig. 7. Schematic of rolling circle amplification (RCA). A padlock probe allows for the circularization of a target oligonucleotide sequence. The combination of a polymerase and a primer allows for the creation of ssDNA. Based on Giuffrida and Spoto (2017).

large subunit of rubisco (rbcL), and mitochondrial cytochrome C subunit 1 (COI) are most commonly used, with COI originally proposed as the model barcode (Hebert et al., 2003). The use of dual barcodes has been suggested for macroalgae (Saunders and McDevit, 2012), with the 28S D2/3 region barcode serving as the common secondary barcode alongside the more group-specific primary barcode (e.g., ITS for dinoflagellates). For more technical details and precautions about DNA barcoding and metabarcoding, readers are referred to existing reviews (Bartolo et al., 2020; Burki et al., 2021).

2.2.10. Microarray

Microarrays are immobilized oligonucleotide sequences (e.g. probes printed on microscopic glass slides) that hybridize to target gene (DNA or RNA) sequences in the samples (Heller, 2002). In essence, microarrays are large-scale probe hybridizations on solid substrates. The hybridized probe-cDNA complex can be detected and quantified with the inclusion of a reporter molecule such as a chemiluminescent or fluorescent molecule.

To apply the technique to HAB research, when the genome or transcriptome is available for a HAB species, probes of genes are designed and printed in an array on a glass slide or membrane. This places the microarray method at the intersection of techniques to detect HAB species and techniques to characterize bloom drivers. A microscope slide can carry multiple arrays, and each array can consist of tens of thousands of probes. Arrays can be made in large batches and stored for future use. Microarrays can be applied for biodiversity research, in which probes are designed for many microbial organisms and hybridized to eDNA samples. When applied to RNA samples, the method reveals gene expression profile. In this case, samples from experiments or bloom events are subject to RNA extraction. mRNA will be collected and reverse-transcribed to cDNA libraries. CDNAs in the libraries will be transcribed, in vitro, to cmRNA, with labeled nucleotides. This labeled cmRNA will be hybridized to the immobilized probes. Signals from a specific probe indicates expression of the gene from which the probe is designed for. The intensity of the signal is proportional to the expression level of the gene.

A noteworthy application of microarray has been its use with *Alexandrium catenella* (Miranda, 2009). In this work, a custom cDNA microarray with 1512 features was developed to investigate gene expression at various growth stages and during cell cycle progression of strain CCMP1719. Approximately 2 % of the array showed differential expression between cultures in the exponential and stationary phases. Through time-series analysis of samples over a 25-hour period, 7 % of the array exhibited differential expression during cell cycle progression. Three genes, including actin, a putative "toxin-related" gene, and a functionally known gene (clone 8), showed a positive correlation in expression levels with the percentage of the cell population in the G2+M stage. This suggests their involvement in cell cycle progression and indicates that these genes could serve as potential cell cycle markers for in situ growth rate studies. Furthermore, the probe array was used to analyze a diel transcriptional profile for a natural bloom of *A. catenella*. Results showed that 10 % of the array features were differentially expressed during the light-dark and dark-light transitions, coinciding with the S and G2+M phases of the cell cycle, indicating that the expression of these genes was entrained by these transitions. Some of these genes (DNA damage protein, histone-like protein, and major basic nuclear protein) showed up-regulation only in the G2+M phase, indicative of potentials as cell cycle markers.

2.3. Comparison of techniques to detect species and quantify abundance

Table 2 compares molecular techniques to detect HAB species and quantify their abundance based on lowest reported limit of detection, time to result (TOR) and applicable use cases. This review will not discuss costs in detail due to the highly variable nature of pricing reagents, labor, and set up costs. Overall, DNA sequencing is more expensive than nucleic acid-based techniques, but the difference has been decreasing, and in some cases where a high number of samples are involved, may reverse.

A challenge in quantifying HAB cells using nucleic acid-based techniques involves the copy number variability of the commonly used gene marker rDNA. rDNA copy numbers vary greatly between algal genera, species, population (strain) and life stage (Kawaguchi et al., 2023; Ruvindy et al., 2023). Tables of rDNA copy numbers have been reported for selected HAB species (Casabianca et al., 2017; Yarimizu et al., 2021). It is therefore preferable to use known numbers of cells for standards, as mentioned earlier. For instance, consider two populations of *Alexandrium catenella*, one strain, AC—Chile has an average copy number of 46,719 copies cell^{-1} while another strain of the same species, AC02-Japan, has an average copy number of 73,100 copes cell^{-1} (Yarimizu et al., 2021). The same signal intensity of detection for the two strains represents a higher abundance for AC—Chile than AC02-Japan. Result interpretation without considering the difference in copy number between these two populations would underestimate the abundance of AC—Chile or overestimate that of AC02-Japan, depending which strain's DNA is used as the standard. For this reason, limits of detection (LOD) reported in this review should only be interpreted as being pertinent to the species examined in that publication. Alternatively, it

Table 2
Comparison of technical specifications of techniques to identify HAB species. Limit of detection based on lowest reported LOD pertinent to HABs.

Detection technique	Limit of detection (LOD)	Time to result (TOR)	Applicability	Source
CRISPR-Cas12a	3.6×10^{-5} ng gDNA μl^{-1}	<1 h-1.5 h	Species detection (in vitro)	Wang et al., 2023, 2025
ELISA	3 cells	>10.5 h* - >12.2 h*	Species detection and quantification Detection of PCR products	Gas et al., 2009, Penna and Magnani, 2008, Xin et al., 2005
FISH	1 cell	<1 h - >17.5 h*	Species detection and quantification Onboard environmental sample processor	Eckford-Soper et al., 2013, Greenfield et al., 2006, Hattenrath-Lehmann et al., 2016, Kim et al., 2005
Barcoding/ Metabarcoding	N/A	Highly variable due to bioinformatic processing needs	Species detection Determination of community composition	Casero et al., 2019 Huang et al., 2021 Jacobs-Palmer et al., 2021 Ørberg et al., 2021Smith et al., 2017
Microarray	0.5 ng gDNA	0.75 h - >9 h*	Species detection Detection of differential gene expression	Ahn et al., 2006, Chen et al., 2016, Miranda, 2009
Immunofluorescence	1 cell	>1 h - >6 h	Species detection and quantification Detection of antigens of interest (e.g. proteins)	Carrera et al., 2010, Lin and Carpenter, 1996, Stauffer et al., 2008, Wang et al., 2007
LAMP	3.4×10^{-4} ng gDNA μL^{-1}	<1 h - 2 h	Species detection Amplification of gene of interest	Chen et al., 2013, Huang et al., 2017, 2020, Nagai et al., 2012, 2013, Qin et al., 2019, Trinh and Lee, 2018, Wang et al., 2019
NASBA	0.01 cells mL^{-1}	0.33 h - 1.5 h	Species detection and quantification	Casper et al., 2007, Loukas et al., 2018, Ulrich et al., 2010
PCR	$1.0 * 10^{-3}$ ng gDNA μL^{-1}	<1 h - >2.5 h	Species detection and quantification Amplification of gene of interest	Andree et al., 2011, Duan et al., 2011, Gray et al., 2003, McGirr et al., 2021, Murray et al., 2011, Zhang and Lin, 2002, Zhang et al., 2024
RCA	$8.0 * 10^{-8}$ ng gDNA μL^{-1}	>0.75 h - 3 h	Species detection Amplification of gene of interest	Chen et al., 2015, Liu et al., 2019, 2020, Qin et al., 2020, Zhang et al., 2018
RPA	$3.37 * 10^{-4}$ ng gDNA μL^{-1}	0.75 h - >9 h*	Species detection and quantification Amplification of gene of interest	Fu et al., 2019, Toldrà et al., 2018, 2020, Wang et al., 2021
SHA	0.47 cells mL^{-1}	~1 h - 4 h	Species detection and quantification	Dearth et al., 2022, Greenfield et al., 2006, 2008, Miller and Scholin, 1996, Scholin et al., 1997, Zhen et al., 2009, Zhu 2012a & b

* Indicates an overnight incubation. Assumed to be 8 h.

has recently been shown that rRNA gene copy number is significantly correlated with cellular carbon content, suggesting a potential solution to circumvent gene copy variation effects when carbon is used as the proxy of cell abundance (Zhou et al., 2025). RNA-based applications of the nucleic acid-based methods face the same challenges as DNA-applications, because rRNA abundance varies with physiological conditions of the cell. In addition, RNA molecules are highly liable to degradation. However, if applied in whole-cell formats (e.g., FISH), the variability of rDNA copy number or rRNA abundance is less problematic because quantification will be based on the number of cells stained by the probes rather than the signal intensity.

While all methods are shown to be capable of species identification, other factors vary considerably. These factors include limit of detection (LOD), time to result (TOR) and applicability as displayed in Tables 1 and 2. One other factor is cost, which is highly variable and goal dependent, as mentioned earlier. Detection sensitivity and specificity are crucial consideration when a technique is considered. Clearly, methods targeting abundant molecules in a cell are more sensitive than those targeting single copy genes or low-abundant molecules. Specificity depends on how the methodology is developed. These differences highlight the need to consider a multitude of factors when selecting a method to detect and quantify HABs.

Perhaps the most amenable to field usage, techniques employing lateral-flow dip sticks (LAMP, RCA, RPA) are all isothermal nucleic acid amplification techniques. A major difference between these techniques is the number of primers required, with 4–6 for LAMP, 1 for RCA and 2 for RPA (Lobato and O'Sullivan, 2018). While increasing primer requirements adds additional layers of technical complexity, specificity is likewise enhanced. Additionally, while RCA and RPA can detect both DNA and RNA, LAMP can only be used to detect DNA (Lobato and O'Sullivan, 2018).

As opposed to nucleic acid-based techniques, a major constraint on the use of antibody-based techniques is the necessity of using animals to produce antibodies. This presents ethical and replicability concerns. The use of nanobodies and antibody expression systems present alternatives to traditional antibody production techniques and serve to alleviate these concerns. Nanobodies, fragments of antibodies, comprising a singular monomeric variable antibody domain, hold promising applications for HAB research (Harmsen and De Haard, 2007). Similar to antibodies used in ELISA, nanobodies can specifically target antigens of interest, allowing them to be used to detect HABs. At the time of writing, nanobody usage in immunoassays for HABs has yet to become a mainstay of detection techniques, however, current efforts to screen for candidate nanobodies for HAB detection have shown promise (Zu et al., 2022). A second alternative is the use of expression systems to generate antibodies. By cloning antibody coding gene regions into expression vectors, such as plasmids in *Escherichia coli*., antibodies targeting algal species have been created (Jiang et al., 2014; Mazzega et al., 2019). These expression systems would remove the requirement for the use of animals to generate antibodies, potentially breathing new life into the field of antibody-based techniques.

3. Techniques to characterize metabolic processes and identify bloom drivers

Understanding of HAB species at the physiological level has been dramatically enhanced over the past decades thanks to extensive research with rapidly improving technologies. Ecological insights have also constantly emerged from increasing temporal and spatial coverage of field studies and remote sensing. Gaps exist, however, in understanding the connection between the physiologies documented in laboratory studies and the phenomics from field observations. This connection is crucial for identifying the critical metabolic processes and environmental factors driving HAB blooms.

Metaomics, including metatranscriptomics, metaproteomics and metagenomics can not only provide an effective avenue to make the afore mentioned connection, but also allows for holistic searches in the metabolic landscape to identify all potentially important metabolic processes. This avoids framing the analysis within a "box" that has the potential to miss previously unrecognized critical metabolic processes. This advantage of metaomics is it can be incubation-free (truly in situ) and complements physiological and ecological measurements. The greatest advantage of the metaomics approach lies in the ability to characterize the metabolic profiles on individual species coexisting in the sampled community. This ability is crucial for unraveling how a HAB microalgal species outperforms other species in the assemblage to form a bloom or how grazers and microbes interact with the HAB species influencing HAB dynamics. The metaomics approach mainly involves metatranscriptomics and metaproteomics. They deserve some elaborate discussion.

3.1. Metatranscriptomics

Metatranscriptomics refers to the study of gene expression (via mRNA transcripts) in microbiota (Cooper et al., 2014; Yu et al., 2023; Zhi et al., 2014). The metatranscriptome is a rich repository of data that can be used to elucidate metabolic responses to environmental factors by microbiota. The resulting profile of total mRNA provides a complete profile of gene expression at a set time point under set conditions (Aguiar-Pulido et al., 2016). In the context of HABs this mRNA profile can provide insight into the mechanisms driving the proliferation of blooms. A typical metatranscriptomic workflow is shown in Fig. 8.

The sequencing of mRNA transcripts generally occurs via RNA-Seq. RNA-Seq always begins with the extraction of RNA. For RNA-Seq, an appropriate library preparation protocol must be selected based on study requirements. A synopsis of library preparation protocols can be found in Table 1 of Kukurba and Montgomery (2015). mRNA is converted into cDNA, processed via the selected library preparation protocol and sequenced, resulting in a readout of mRNA transcripts in the sample (Hrdlickova et al., 2016). Next, a general data processing workflow begins with the alignment of reads against a reference genome or transcriptome, then assembly of reads into discrete transcripts that can be annotated de novo or against a reference. Total transcript abundance is also determined. Transcript abundance is indicative of the net outcome of gene expression (transcription) and mRNA degradation (Kukurba and Montgomery, 2015).

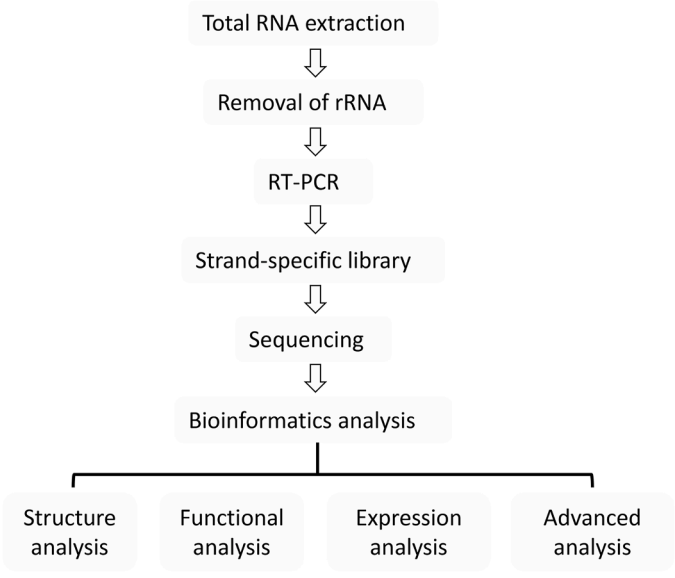

Fig. 8. Typical metatranscriptomic workflow.

Traditional understanding of how HAB species respond metabolically to a set of conditions has relied on laboratory exposure assays or field observation. These studies generally relate a set condition to an observable change in the HAB. Due to logistical limitations, these studies often can only focus on a singular or limited number of conditions. Metatranscriptomics allows in situ analysis of a multitude of variables impacting HAB metabolism and elucidation of the molecular pathways underpinning them. For instance, Yu et al. (2023) correlated environmental conditions and nutrient regime to changes in the community composition of a HAB event. The resulting metatranscriptome profile allowed for the creation of a matrix relating nutrient regimes (N, P, C, Si) to HAB species abundance. As with any biological process, no singular variable bares sole responsibility for the metabolism of a HAB. Therefore, in situ, multivariable analysis of factors impacting HAB metabolism is of great utility for the wealth of data it generates and the relative simplicity of the technique as opposed to traditional laboratory exposure assays.

In addition to allowing for multivariable analysis, metatranscriptome profiling has made significant contributions to an increased understanding of the metabolic pathways driving HAB events (Yu et al., 2020,; 2023; Zhang et al., 2019b; Zhuang et al., 2015). For example, Zhuang et al. (2015) identified genes allowing *Alexandrium catenella* to bloom under N-limited conditions. Under N-limitation, *A. catenella* was shown to have diverse genetic mechanisms for N-utilization, allowing it to utilize diverse sources of dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN). This utilization was enabled by the upregulation of genes for the creation of enzymes needed to uptake and assimilate dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN). This utilization was enabled by the upregulation of genes for the creation of enzymes need to uptake and assimilate N.

A major challenge in metatranscriptomics is to trace a gene transcript to its source species. This requires a complete database covering all species, which does not exist, the species covered in the database has increased steadily. Compromises have to be made by matching transcripts to those of the closest species in the database. For dinoflagellate blooms, a specific feature is useful such as DinoSL. This spliced leader is unique to dinoflagellate nucleus-encoded transcripts (Zhang et al., 2007), which can be used as a hook to separate dinoflagellate transcripts from transcripts of other organisms (Lin et al., 2010; Zhang et al., 2013). Recently this technique was applied to analyze the metabolic profile of dinoflagellate cysts in sediment (Deng et al., 2025). Results revealed a broadly active metabolic landscape in the dormant cysts, and that salicylic acid is important in regulating the timing of cyst germination. With the database rapidly growing, the challenge of tracing transcripts to source species is increasingly being relieved.

3.2. Metaproteomics

Metaproteomics is the study of all protein expression at a given time point (Wang et al., 2014). Two major techniques of metaproteome analysis are commonly employed for marine samples. The first is the coupling of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with mass spectroscopy (either matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF

MS) or electrospray ionization source tandem mass spectrometry (ESI-MS/MS). In this workflow, samples are loaded onto a PAGE gel for size-based separation. This PAGE gel is then selectively extracted at desired protein bands and extracted. The SDS-PAGE technique then diverges based on the type of MS used. For MALDI-TOF MS, the extracted proteins are combined with a MALDI matrix then analyzed by MALDI-TOF MS to measure protein mass spectra. These mass spectra are then compared to a database for protein identification. For ESI-MS/MS, the gel extracted proteins are further digested into smaller peptides which are analyzed by ESI-MS/MS to obtain detailed sequence information based on patterns of fragmentation. The second major technique of metaproteomic analysis is the coupling of liquid chromatography (LC) to ESI-MS/MS. In this technique, proteomic analysis is directly performed in liquid samples without the step of gel-based separation.

Environmental factors conducive to HAB formation have been determined from metaproteomic analysis. Comparisons between samples of blooms of *Heterosigma akashiwo* and *Prorocentrum shikokuense* and non-bloom samples highlighted the critical metabolic processes facilitating these blooms (Zhang et al., 2019c). While both species highly expressed light-harvesting proteins, likely as a response to their turbid environment, *H. akashiwo* showed greater expression, particularly during its dominant bloom period, conferring an advantage over *P. shikokuense* (Zhang et al., 2019c). This is consistent with the major findings of the metatranscriptomic study referred to earlier (Ji et al., 2018). In addition, another metaproteome investigation of a *P. shikokuense* bloom suggests that inorganic carbon uptake and carbon concentration were important in the maintenance of the bloom (Zhang et al., 2021).

Broadly, the ability of these species to modulate protein expression in response to variable environmental conditions highlights the ability of metaproteomics to provide a molecular explanation for HAB events.

3.3. Comparison of techniques to characterize metabolic processes and identify bloom drivers

Metagenomics, metatranscriptomics, and metaproteomics are superior in some ways and inferior in others. The genomics approach analyzes whole genomes or pathways in a species, revealing the genetic potential of the species to regulate growth and toxin production. A high-quality genome assembly provides comprehensive data for the species, including genes and regulatory elements. These provide insights into the evolution of the species that may be related to the ability to form HABs and produce toxins. However, genome sequencing and analysis is time and capital intensive. Additionally, while genes may serve as a template, their presence does not directly relate to levels of expression. Therefore, except for microbial metagenomics able to shed light on what potential impact of microbes in the community may have on HAB algae, metagenomics is not as useful as the other two omics.

In comparison, metatranscriptomics generates genome-wide gene expression profiles, providing data on what genes are active at time of sampling. This approach is much more temporally and financially efficient because non-coding DNA sequences, which account for most parts of genomes (Hou and Lin, 2009), are not sequenced. Furthermore, transcriptomic information is one step closer to the physiology of the cells and more useful for explaining what molecular functions or metabolic processes are associated with HAB development and toxin synthesis. However, for genes that are regulated at translational or post-translational levels, transcriptional data would not be as useful. In addition, genes not expressed or expressed at low levels at time of sampling would likely be missed.

The theoretically most useful of the omics, proteomics, profiles protein repertoire expressed at time of sampling, which is closest to cell physiology. However, a major limitation of proteomics is the low efficiency of the gel-based technique and strong dependence of a high-quality genome or transcriptome as database for gel-free techniques. The gel-based technique typically only allows for identification of about two thousand proteins (Abdallah et al., 2012). The number of proteins that can be identified through gel-free mass spectrometry depends on the genomic or transcriptomic database. With this protein repertoire, molecular mechanisms regulating HAB development, growth, and decline can be better understood.

4. Future prospect

4.1. The need to expedite detection and quantification of the causative species

Perhaps the most pressing, and longest standing issue pertinent to HABs lies in the need to rapidly identify and quantify the causative species. For this reason, several state, federal, and nonprofit initiatives have been established to regularly monitor for HAB events and HAB causing species. These include the Alaska Harmful Algal Bloom Network (AHABN, 2025), the Harmful Algal Event Database (HAEDAT), Phytoplankton Monitoring Network (PMN) and Soundtoxins. Many of these programs take a citizen science approach to collect phytoplankton samples and send them to a partner laboratory for analysis. The lag time between sample collection and laboratory analysis is often highlighted as a cause for concern due to the rapid changes in bloom dynamics. Another major challenge is the lack of an easily accessible high-quality database linking event characteristics, clinical symptoms, and DNA sequence to species identities. For instance, many HAEDAT entries are clinical manifestations of HAB intoxication that lack a causative organism. So called “Dock-side” techniques could serve to overcome the challenges of lag time between sample collection and result delivery as well as be useful in resource-limited areas. A dock-side eDNA method for the detection of *Asparagopsis armata,* for instance, used a simplified end-point PCR to engage stakeholders in the detection of this invasive species in Spanish ports (Ibabe et al., 2024). This technique is designed for implementation by port authority workers. Among the methods most amenable to dock-side HAB detection is LAMP. This is because of its isothermal nature, production of colorimetric results and short duration. A “suitcase lab” employing a simplified DNA extraction and LAMP reaction has allowed for dock-side detection of *Alexandrium catenella* (Fujiyoshi et al., 2020). A lateral flow dipstick (LFD) LAMP method, displaying results similar to a COVID-19 home test kit with a control and test line enabled the rapid detection of *Karenia mikimotoi* in only four steps (excluding DNA extraction) involving only simple pipetting and temperature control (Wang et al., 2020). A similar LAMP-LFD method exists for *Alexandrium catenella* (Wang et al., 2019). Major hurdles include the need to extract genomic DNA prior to the use of a LAMP method and the need to maintain isothermal temperature control. Simplifying DNA extraction and increasing the thermal range of the assay would facilitate easier use.

Autonomous and semi-autonomous instruments for the in situ detection of HABs represent a major breakthrough as they can detect HABs in near real-time and potentially sample more logistically difficult locations. To date, three such devices have been tested. Among the most widely used, Environmental sample processor (ESP) platforms have proven remarkably successful at the detection of both HAB species and HAB toxins (Doucette et al., 2009). Competitive ELISAs run onboard the ESP are sent via satellite to a user who processes the luminosity of arrays which allows for toxin detection and quantification against a standard curve. HAB species detection can also be conducted onboard the ESP using SHA adapted from Scholin et al. (1996). Custom ESP arrays can be printed for a variety of HAB species as described in NOAA Processed Report NMFS-NWFSC-PR-2025-01 (Moore et al., 2025). Surface plasmon resonance (SPR) technology has recently been added to ESP systems for the detection of quantification of particle-associated cyanobacterial microcystins (Ussler III et al., 2024). SPR targeting discreet HAB cells is thus a logical next step and would allow users to circumvent array printing. An often overlooked use of the ESP is to recover samples from difficult to access environments to aid in the

recovery and identification of HAB species. For instance, remnants of *Pseudo-nitzchia* frustules left behind on ESP arrays for toxin analysis have aided in correlating cell abundance to HAB toxin (Bowers et al., 2015). The ability of the ESP to collect samples in situ for later analysis could allow existing monitoring programs to access hard to reach locations with greater ease and potentially preserve samples for multi-omic analysis. The ability of ESPs to be configured for a wide variety of HAB species, report data in near real time and access remote locations makes it among the most promising techniques to expedite HAB testing.

The second system is the IISA-Gene system, which also shows the capacity to detect HAB species on an autonomous craft. This system has been used primarily for the detection of deep-sea microbes such as hydrothermal vent microbial assemblages using onboard PCR to detect 16S rRNA (Fukuba and Fujii, 2012). While this technology has yet to be applied to HABs, it holds promise as another autonomous detection method. The final autonomous in situ sampling device for the detection of HAB species is the autonomous microbial genosensors (AMG). This prototype instrument has conducted onboard RNA isolation for the use of RT-NASBA to detect *Karenia brevis* (Fries et al., 2007). This particular device is noteworthy for detecting mRNA instead of DNA, preventing the detection of dead, and thus non-transcriptionally active cells (Paul et al., 2007).

4.2. The need to expedite HAB multi-omics

To go beyond the identification of the particular species responsible for a HAB event, multi-omic analysis is required. Multi-omic analysis combines metatranscriptomics and metaproteomics for the analysis of HABs to provide a comprehensive picture of the molecular underpinnings of these events. The combined analysis of these provides what can be summarized as, what gene codes are expressed (the metatranscriptome) and how those codes manifest as a functional product (the metaproteome). The sum of these omic studies provides a comprehensive picture of the molecular underpinnings of HABs.

The result of multi-omic analysis could assess the potential of a bloom, what environmental triggers may prompt it, and the mechanisms allowing for the physical manifestation of bloom actions. The combined metatranscriptomic and metaproteomic facilitated discovery of metabolic pathways by which *P. shikokuense* can utilize dissolved organic carbon to bloom under otherwise dissolved inorganic carbon poor regimes is useful in this regard (Zhang et al., 2021). Shi et al. (2024) effectively used a multi-omic approach to explain the molecular mechanisms allowing for increased blooms of *Karenia brevis* under reduced salinity. Both works have direct implications for HAB prediction and management. As salinity and dissolved organic carbon regimes change, these multi-omic findings should be considered. Further multi-omic analysis of HABs is thus crucial to understanding the molecular nature of their occurrences. Impediments to multi-omics analysis of HABs include challenges in both sequencing platforms and bioinformatic pipelines. The diverse processing techniques to extract oligonucleotides and proteins also require variations in methodology. Costs associated with multi-omic analysis also form a significant barrier (Hayes et al., 2024).

Implementation of multi-omic analysis to understand the causes of HABs can be used to create so-called, “Molecular Forecasts”. For instance, the study of a 2015 *Pseudo-nitzschia australis* bloom provided several key insights with major implications to HAB prediction and management (Brunson et al., 2024). These include that changes in gene transcription can provide a one week notice prior to the detection of toxins, that adaptations to iron limitation facilitate the success of *Pseudo-nitzschia* over non-toxic diatoms, and that three members of this genus are the primary toxin producers (Brunson et al., 2024). Additionally, multi-omic analysis of cyanobacterial assemblages in wastewater treatment facilities has been presented as a promising advancement to HAB management (Romanis et al., 2021; Nagarajan et al., 2022). For instance, culturing the cyanobacterium *Pseudanabaena galeata* in wastewater demonstrated significant reductions in total bacterial content and total nitrogen (Ouhsassi et al., 2020). While a potential HAB itself, the results nonetheless could potentially be applied elsewhere using insights from multi-omic analysis. For instance, precise changes in community composition could be characterized metagenomically, potential toxin production in *P. galeata* could be monitored with metatranscriptomics and potentially functional proteins produced by *P. galeata* could be identified to explain the mechanisms by which it aids in water purification.

Lastly, multi-omic analysis may provide insight into the use of algicidal bacteria for HAB management. The algicidal ability of some bacteria has long been observed but molecular characterization has proven challenging (Coyne et al., 2022; Doucette et al., 1999; Shi et al., 2013). The co-culturing and multi-omic analysis of the bacterium, *Enterobacter hormaechei* F2 alongside the cyanobacterium, *Microcystis aeruginosa* served to characterize the algicidal process at a molecular level (Zhang et al., 2023). Extrapolating this data, the characterization of the algicidal activity of this bacterium could serve to screen candidate algicidal bacteria for HAB management. For instance, examining a library of bacterial genomes could screen for only bacteria with the prerequisite genes for algicidal activity, streamlining laboratory analysis.

4.3. The need for an integrative approach to address drivers of HABs

As discussed earlier, each HAB outbreak may be unique. The environmental trigger as well as intracellular metabolic driver varies between HAB events, even if the same species causes the outbreak in different ecosystems or separate outbreaks. Therefore, in situ analyses are imperative. In addition, to unravel the intracellular regulatory mechanisms, multi-faceted analytical techniques may be required. More importantly, the studies should be conducted on an integrative framework that includes multiple potential contributing elements. For instance, in the past several years, a framework including acquisition of energy (E) and nutrients (N), defense (D), and sexual and asexual reproduction (S), has been initiated and applied to several blooms (Wang et al., 2023,; 2023; Yu et al., 2020; Zhang et al., 2019b). Results revealed different metabolic processes significantly influencing bloom dynamics. However, this, or similar frameworks, still need to be further developed with crucial genes regulating each of the processes identified and a quantitative model constructed.

Ideally, with proper parameterization of such a model, input of expression of crucial genes regulating each of the contributing components (e.g. E, N, D, and S) will provide population growth rate. Alternatively, with net population growth rate measured, the model would allow the estimation of how much each component contributes to the bloom. Such models are challenging to construct because they require extensive omic research to identify the key metabolic processes crucial to HAB development. Both laboratory experiments and field work are needed, with the latter being particularly challenging as sample collection and on-site processing need to be quick and sample preservation requires liquid nitrogen or RNA protecting reagents (e.g. Trizol, RNALater). Furthermore, subsequent sample sequencing and bioinformatic analysis are technically demanding. These hurdles, however, are becoming less insurmountable. Analysis of a variety of HAB specific DNA/RNA preservation methods show progress towards simplifying protocols (Eckford-Soper and Daugbjerg, 2015; Harlow et al., 2006). A growing body of high throughput omic methods are presented by Dai & Shen (2022). High throughput omics have facilitated ever increasing speed of data acquisition; however, data analysis remains a major bottleneck (Vitorino, 2024). Artificial intelligence holds promise to address this Issue.

4.4. The potential of machine learning and AI to enhance molecular techniques

Recent advancements in machine learning and artificial intelligence

(AI) present several use cases for the molecular detection of HABS. While most AI applications for HABs involve spectral techniques, molecular detection can also be enhanced by AI.

Given the immense diversity of HAB species, their relatively short generation time and potential mutations, genetic programming (GP), the use of an AI model to predict genetic shifts over time, holds promise for enhancement of HAB detection and forecasting. Current GP models for HABs use ecological parameters such as chlorophyl concentration to predict changes in HAB population (Muttil and Lee, 2005; Sivapragasam et al., 2010). GP utilizes a parse tree model with genetics operators such as crossover, fitness and mutation to predict the response of a HAB to selection pressures. While the GP technique looks at a HAB population as a whole, this technique could be narrowed to examine specific genes implicated in HAB proliferation. For example, differential gene expression could be predicted with a GP model given sufficient empirical data on factors influencing the expression of the gene of interest.

Machine learning and AI show significant promise at enhancing existing molecular techniques such as CRISPR and PCR (Metsky et al., 2022; Perez-Romero et al., 2023). For example, All-inclusive Patrolling of Targets (ADAPT) has revolutionized the use of CRISPR based detection technologies. By screening diagnostic target-pairs ADAPT facilitated a lower limit of detection than non-AI assisted CRISPR techniques while maintaining similar specificity and sensitivity (Metsky et al., 2022). Selection of primers for RT-qPCR for emerging variants of SARS-CoV-2 was also assisted by an AI primer design tool (Perez-Romero et al., 2023).

A majority of HAB detection techniques require the interpretation of spectral data to determine a result. This process can be laborious and potentially introduce human error. Advancements in spectral interpretation via machine learning/AI have been shown to rapidly and accurately interpret spectral data in a variety of formats. Many of these advancements have occurred in the medical field. For instance, AI interpretation of FISH fluorescence to determine gene amplification status has proven a rapid and reliable technique in oncology (Xue et al., 2023). Subjectivity of LAMP results has been reduced with AI image interpretation (Rohaim et al., 2020). Result interpretation of field deployed ELISAs has been enhanced by AI to remove effects of ambient lighting (Duan et al., 2023). These advancements could likewise be applied to HAB based detection techniques.

5. Conclusion

At present, a great diversity of techniques exists to detect HAB-causing organisms with 12 such techniques being covered in this review. These techniques can be broadly categorized as being either antibody or nucleic acid-based with advantages and disadvantages to each. Multi-omic techniques including metatranscriptomics and metaproteomics have been shown to enhance the understanding of the molecular underpinning of HAB events and provide novel insights. Future prospects highlight needs in HAB research field as well as offering insights into how these needs may be addressed. Two major advancements addressing the most pressing need, the rapid detection of HAB species, include the use of Dock-side techniques and autonomous/semi-autonomous HAB detection instruments. Multi-omic analysis shows promise in enhancing the ability to predict and respond to HAB events. Integrating detection, multi-omics and in situ techniques will be an area of further research. AI has also been shown to be a powerful tool for the enhancement of current techniques as well as the interpretation of results by end users. Overall, this review highlights the diversity of techniques to detect HABs and understand their causes, providing a resource of technical options for use in HAB research and monitoring, and shedding light on areas further research and technical development are warranted.

CRediT authorship contribution statement

Jackson Sanders: Writing – review & editing, Writing – original draft. **Senjie Lin:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdallah, C., Dumas-Gaudot, E., Renaut, J., Sergeant, K., 2012. Gel-based and gel-free quantitative proteomics approaches at a glance. Int. J. Plant Genom. 2012 (1), 494572. https://doi.org/10.1155/2012/494572.

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., Narasimhan, G., 2016. Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis: supplementary issue: bioinformatics methods and applications for big Metagenomics data. Evol. Bioinform. Online 12 (1), 5–16. https://doi.org/10.4137/EBO.S36436.

Alaska Harmful Algal Bloom Network (AHABN), 2025. Alaska Shellfish and HAB Data. https://ahab.portal.aoos.org/Top.

Ahn, S., Kulis, D.M., Erdner, D.L., Anderson, D.M., Walt, D.R., 2006. Fiber-optic microarray for simultaneous detection of multiple harmful algal bloom species. Appl. Environ. Microbiol. 72 (9), 5742–5749. https://doi.org/10.1128/AEM.00332-06.

Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., 2012. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Ann. Rev. Mar. Sci. 4, 143–176. https://doi.org/10.1146/annurev-marine-120308-081121.

Anderson, D.M., Fensin, E., Gobler, C.J., Hoeglund, A.E., Hubbard, K.A., Kulis, D.M., Landsberg, J.H., Lefebvre, K.A., Provoost, P., Richlen, M.L., Smith, J.L., Solow, A.R., Trainer, V.L., 2021. Marine harmful algal blooms (HABs) in the United States: history, current status and future trends. Harmful Algae 102, 101975. https://doi.org/10.1016/j.hal.2021.101975.

Andree, K.B., Fernández-Tejedor, M., Elandaloussi, L.M., Quijano-Scheggia, S., Sampedro, N., Garcés, E., Camp, J., Diogène, J., 2011. Quantitative PCR coupled with melt curve analysis for detection of selected *Pseudo-nitzschia* spp. (Bacillariophyceae) From the Northwestern Mediterranean Sea. Appl. Environ. Microbiol. 77 (5), 1651–1659. https://doi.org/10.1128/AEM.01978-10.

Antonella, P., Luca, G., 2013. The quantitative real-time PCR applications in the monitoring of marine harmful algal bloom (HAB) species. Environ. Sci. Pollut. Res. Int. 20 (10), 6851–6862. https://doi.org/10.1007/s11356-012-1377-z.

Backer, L.C., McGillicuddy, D.J.J., 2006. Harmful algal blooms: at the interface between coastal oceanography and human health. Oceanography 19 (2), 94–106. https://doi.org/10.5670/oceanog.2006.72.

Bari, M.L., Yeasmin, S., 2014. Water quality assessment modern microbiological techniques. In: Batt, C.A., Tortorello, M.L. (Eds.), Encyclop. Food Microbiol., 2 Academic Press, pp. 755–765. https://doi.org/10.1016/B978-0-12-384730-0.00353-0.

Bartlett, J.M.S., Stirling, D., 2003. A short history of the polymerase chain reaction. In: Bartlett, J.M.S., Stirling, D. (Eds.), PCR Protocols. Humana Press, pp. 3–6. https://doi.org/10.1385/1-59259-384-4:3.

Bartolo, A., Zammit, G., Peters, A., Küpper, F., 2020. The current state of DNA barcoding of macroalgae in the Mediterranean Sea: presently lacking but urgently required. Bot. Mar. 63 (3), 253–272. https://doi.org/10.1515/bot-2019-0041.

Bauman, J.G.J., Wiegant, J., Borst, P., van Duijn, P., 1980. A new method for fluorescence microscopical localization of specific DNA sequences by in situ hybridization of fluorochrome-labelled RNA. Exp. Cell Res. 128 (2), 485–490. https://doi.org/10.1016/0014-4827(80)90087-7.

Berdalet, E., Tester, P.A., Chinain, M., Fraga, S., Lemée, R., Litaker, W., Penna, A., Usup, G., Vila, M., Zingone, A., 2017. Harmful algal blooms in benthic systems: recent progress and future research. Oceanography 30 (1), 36–45. https://doi.org/10.5670/oceanog.2017.108.

Bowers, H.A., Marin III, R., Birch, J.M., Scholin, C.A., Doucette, G.J., 2015. Recovery and identification of *Pseudo-nitzschia* (Bacillariophyceae) frustules from natural samples acquired using the environmental sample processor. J. Phycol. 52 (1), 135–140. https://doi.org/10.1111/jpy.12369.

Bowers, H.A., Marin III, R., Birch, J.M., Scholin, C.A., 2017. Sandwich hybridization probes for the detection of *Pseudo-nitzschia* (Bacillariophyceae) species: an update to existing probes and a description of new probes. Harmful Algae 70, 37–51. https://doi.org/10.1016/j.hal.2017.10.005.

Brunson, J.K., McKinnie, S.M., Chekan, J.R., McCrow, J.P., Miles, Z.D., Bertrand, E.M., Bielinski, V.A., Luhavaya, H., Oborník, M., Smith, G.J., Hutchins, D.A., Allen, A.E., Moore, B.S., 2018. Biosynthesis of the neurotoxin domoic acid in a bloom-forming diatom. Science 361 (6409), 1356–1358. https://doi.org/10.1126/science.aau0382.

Brunson, J.K., Thukral, M., Ryan, J.P., Anderson, C.R., Kolody, B.C., James, C.C., Chavez, F.P., Leaw, C.P., Rabines, A.J., Venepally, P., Fussy, Z., Zheng, H., Kudela, R. M., Smith, G.J., Moore, B.S., Allen, A.E., 2024. Molecular forecasting of domoic acid during a pervasive toxic diatom bloom. Proc. Natl. Acad. Sci. U.S.A. 121 (40), e2319177121. https://doi.org/10.1073/pnas.2319177121.

Burki, F., Sandin, M.M., Jamy, M., 2021. Diversity and ecology of protists revealed by metabarcoding. Curr. Biol. 31 (19), 1267–1280. https://doi.org/10.1016/j.cub.2021.07.066.

Carrera, M., Garet, E., Barreiro, A., Garcés, E., Pérez, D., Guisande, C., González-Fernández, Á., 2010. Generation of monoclonal antibodies for the specific immunodetection of the toxic dinoflagellate *Alexandrium minutum* Halim from Spanish waters. Harmful Algae 9 (3), 272–280. https://doi.org/10.1016/j.hal.2009.11.004.

Casabianca, S., Cornetti, L., Capellacci, S., Vernesi, C., Penna, A., 2017. Genome complexity of harmful microalgae. Harmful Algae 63, 7–12. https://doi.org/10.1016/j.hal.2017.01.003.

Casero, M.C., Velázquez, D., Medina-Cobo, M., Quesada, A., Cirés, S., 2019. Unmasking the identity of toxigenic cyanobacteria driving a multi-toxin bloom by high-throughput sequencing of cyanotoxins genes and 16S rRNA metabarcoding. Sci. Total Environ. 665, 367–378. https://doi.org/10.1016/j.scitotenv.2019.02.083.

Casper, E.T., Patterson, S.S., Bhanushali, P., Farmer, A., Smith, M., Fries, D.P., Paul, J.H., 2007. A handheld NASBA analyzer for the field detection and quantification of *Karenia brevis*. Harmful Algae 6 (1), 112–118. https://doi.org/10.1016/j.hal.2006.11.001.

Chen, G., Cai, P., Zhang, C., Wang, Y., Zhang, S., Guo, C., Lu, D.D., 2015. Hyperbranched rolling circle amplification as a novel method for rapid and sensitive detection of *Amphidinium carterae*. Harmful Algae 47, 66–74. https://doi.org/10.1016/j.hal.2015.05.012.

Chen, G., Ma, C., Zhang, C., Zhou, J., Wang, Y., Wang, G., Zhang, B., Xu, Z., Lu, D.D., 2013. A rapid and sensitive method for field detection of *Prorocentrum donghaiense* using reverse transcription-coupled loop-mediated isothermal amplification. Harmful Algae 29, 31–39. https://doi.org/10.1016/j.hal.2013.08.001.

Chen, X., Zhou, Q., Duan, W., Zhou, C., Duan, L., Zhang, H., Sun, A., Yan, X., Chen, J., 2016. Development and evaluation of a DNA microarray assay for the simultaneous detection of nine harmful algal species in ship ballast and seaport waters. Chin. J. Oceanol. Limnol. 34 (1), 86–101. https://doi.org/10.1007/s00343-015-4265-9.

Clancy, E., Burke, M., Arabkari, V., Barry, T., Kelly, H., Dwyer, R.M., Kerin, M.J., Smith, T.J., 2017. Amplification-free detection of microRNAs via a rapid microarray-based sandwich assay. Anal. Bioanal. Chem. 409 (14), 3497–3505. https://doi.org/10.1007/s00216-017-0298-6.

Cohen, A.B., Novkov-Bloom, A., Wesselborg, C., Yagudaeva, M., Aranguiz, E., Taylor, G. T., 2021. Applying fluorescence in situ hybridization to aquatic systems with cyanobacteria blooms: autofluorescence suppression and high-throughput image analysis. Limnol. Oceanogr. Methods 19 (7), 457–475. https://doi.org/10.1002/lom3.10437.

Cooper, E.D., Bentlage, B., Gibbons, T.R., Bachvaroff, T.R., Delwiche, C.F., 2014. Metatranscriptome profiling of a harmful algal bloom. Harmful Algae 37, 75–83. https://doi.org/10.1016/j.hal.2014.04.016.

Coyne, K.J., Wang, Y., Johnson, G., 2022. Algicidal bacteria: a review of current knowledge and applications to control harmful algal blooms. Front. Microbiol. 13. https://doi.org/10.3389/fmicb.2022.871177.

Daher, R.K., Stewart, G., Boissinot, M., Bergeron, M.G., 2016. Recombinase polymerase amplification for diagnostic applications. Clin. Chem. 62 (7), 947–958. https://doi.org/10.1373/clinchem.2015.245829.

Dai, X., Shen, L., 2022. Advances and trends in Omics technology development. Front. Med. 9, 911861. https://doi.org/10.3389/fmed.2022.911861.

Dai, Y., Yang, S., Zhao, D., Hu, C., Xu, W., Anderson, D.M., Li, Y., Song, X., Boyce, D.G., Gibson, L., Zheng, C., Feng, L., 2023. Coastal phytoplankton blooms expand and intensify in the 21st century. Nature 615 (7951), 280–284. https://doi.org/10.1038/s41586-023-05760-y.

Dearth, N.M., Jones, W.J., Espinosa, J.I., Mortensen, R.A., Pinckney, J.L., Greenfield, D. I., 2022. A new sandwich hybridization assay method to identify and quantify *Microcystis* spp. Limnol. Oceanogr. Methods 20 (4), 210–221. https://doi.org/10.1002/lom3.10481.

Deng, Y., Yue, C., Yang, H., Li, F., Hu, X., Shang, L., Chai, Z., Lin, S., Tang, Y., 2025. Broad active metabolic pathways, autophagy, and antagonistic hormones regulate dinoflagellate cyst dormancy in marine sediments. Sci. Adv. 11 (6), eads7789. https://doi.org/10.1126/sciadv.ads7789.

Diercks, S., Metfies, K., Medlin, L.K., 2008. Molecular probe sets for the detection of toxic algae for use in sandwich hybridization formats. J. Plankton. Res. 30 (4), 439–448. https://doi.org/10.1093/plankt/fbn009.

Doucette, G.J., McGovern, E.R., Babinchak, J.A., 1999. Algicidal bacteria active against *Gymnodinium breve* (Dinophyceae). I. Bacterial isolation and characterization of killing activity. J. Phycol. 35 (6), 1447–1454. https://doi.org/10.1046/j.1529-8817.1999.3561447.x.

Doucette, G.J., Mikulski, C.M., Jones, K.L., King, K.L., Greenfield, D.I., Marin, R., Jensen, S., Roman, B., Elliott, C.T., Scholin, C.A., 2009. Remote, subsurface detection of the algal toxin domoic acid onboard the Environmental Sample Processor: assay development and field trials. Harmful Algae 8 (6), 880–888. https://doi.org/10.1016/j.hal.2009.04.006.

Duan, S., Cai, T., Zhu, J., Yang, X., Lim, E.G., Huang, K., Hoettges, K., Zhang, Q., Fu, H., Guo, Q., Liu, X., Yang, Z., Song, P., 2023. Deep learning-assisted ultra-accurate smartphone testing of paper-based colorimetric ELISA assays. Anal. Chim. Acta 1248, 340868. https://doi.org/10.1016/j.aca.2023.340868.

Duan, W., Guo, L., Sun, D., Zhu, S., Chen, X., Zhu, W., Xu, T., Chen, C., 2011. Morphological and molecular characterization of free-floating and attached green macroalgae *Ulva* spp. In the Yellow Sea of China. J. Appl. Phycol. 24 (1), 97–108. https://doi.org/10.1007/s10811-011-9654-7.

Dunn, A.R., Hassell, J.A., 1977. A novel method to map transcripts: evidence for homology between an adenovirus mRNA and discrete multiple regions of the viral genome. Cell 12 (1), 23–36. https://doi.org/10.1016/0092-8674(77)90182-9.

Durán-Vinet, B., Araya-Castro, K., Chao, T.C., Wood, S.A., Gallardo, V., Godoy, K., Abanto, M., 2021. Potential applications of CRISPR/Cas for next-generation biomonitoring of harmful algae blooms: a review. Harmful Algae 103, 102027. https://doi.org/10.1016/j.hal.2021.102027.

Eckford-Soper, L.K., Daugbjerg, N., 2015. Examination of six commonly used laboratory fixatives in HAB monitoring programs for their use in quantitative PCR based on Taqman probe technology. Harmful Algae 42, 52–59. https://doi.org/10.1016/j.hal.2014.12.007.

Eckford-Soper, L.K., Davidson, K., Bresnan, E., 2013. Identification and quantification of toxic and nontoxic strains of the harmful dinoflagellate *Alexandrium tamarense* using fluorescence in situ hybridization and flow cytometry. Limnol. Oceanogr. Methods 11 (10), 540–548. https://doi.org/10.4319/lom.2013.11.540.

Feuillie, C., Merheb, M.M., Gillet, B., Montagnac, G., Daniel, I., Hänni, C., 2011. A novel SERRS sandwich-hybridization assay to detect specific DNA target. PLoS One 6 (5), e17847. https://doi.org/10.1371/journal.pone.0017847.

Fries, D., Paul, J., Smith, M., Farmer, A., Casper, E., Wilson, J., 2007. The autonomous microbial genosensor, an in situ sensor for marine microbe detection. Microsc. Microanal. 13, 514–515. https://doi.org/10.1017/S1431927607078816.

Fu, M., Chen, G., Zhang, C., Wang, Y., Sun, R., Zhou, J., 2019. Rapid and sensitive detection method for *Karlodinium veneficum* by recombinase polymerase amplification coupled with lateral flow dipstick. Harmful Algae 84, 1–9. https://doi.org/10.1016/j.hal.2019.01.011.

Fujiyoshi, S., Yarimizu, K., Miyashita, Y., Rilling, J., Acuña, J.J., Ueki, S., Gajardo, G., Espinoza-González, O., Guzmán, L., Jorquera, M.A., Nagai, S., Maruyama, F., 2020. Suitcase Lab: new, portable, and deployable equipment for rapid detection of specific harmful algae in Chilean coastal waters. Environ. Sci. Pollut. Res. Int. 28 (11), 14144–14155. https://doi.org/10.1007/s11356-020-11567-5.

Fukuba, T., Fujii, T., 2012. Microfabricated flow-through device for in situ gene analysis. In: Tiquia-Arashiro, S.M. (Ed.), Molecular Biological Technologies For Ocean Sensing. Humana Press, pp. 59–72. https://doi.org/10.1007/978-1-61779-915-0_3.

Galluzzi, L., Bertozzini, E., Penna, A., Perini, F., Garcés, E., Magnani, M., 2009. Analysis of rRNA gene content in the Mediterranean dinoflagellate *Alexandrium catenella* and *Alexandrium taylori*: implications for the quantitative real-time PCR-based monitoring methods. J. Appl. Phycol. 22 (1), 1–9. https://doi.org/10.1007/s10811-009-9411-3.

Galluzzi, L., Penna, A., Bertozzini, E., Vila, M., Garcés, E., 2004. Development of a real-time PCR assay for rapid detection and quantification of *Alexandrium minutum* (a dinoflagellate). Appl. Environ. Microbiol. 70 (2), 1199–1206. https://doi.org/10.1128/AEM.70.2.1199-1206.2004.

Gan, N., Huang, Q., Zheng, L., Song, L., 2010. Quantitative assessment of toxic and nontoxic *microcystis* colonies in natural environments using fluorescence in situ hybridization and flow cytometry. Sci. China Life Sci. 53 (8), 973–980. https://doi.org/10.1007/s11427-010-4038-9.

Gao, S., Chen, X., Yi, Q., Wang, G., Pan, G., Lin, A., Peng, G., 2010. A strategy for the proliferation of *Ulva prolifera*, main causative species of green tides, with formation of sporangia by fragmentation. PLoS One 5 (1), e8571. https://doi.org/10.1371/journal.pone.0008571.

Garafutdinov, R.R., Sakhabutdinova, A.R., Gilvanov, A.R., Chemeris, A.V., 2021. Rolling circle amplification as a universal method for the analysis of a wide range of biological targets. Russ. J. Bioorg. Chem. 47 (6), 1172–1189. https://doi.org/10.1134/S1068162021060078.

Garcés, E., Camp, J., 2012. Habitat changes in the Mediterranean Sea and the consequences for harmful Algal blooms formation. In: Stambler, N. (Ed.), Life in the Mediterranean Sea: a Look at Habitat Changes. Nova Science Publishers, pp. 519–541.

Gas, F., Pinto, L., Baus, B., Gaufres, L., Crassous, M., Compere, C., Quéméneur, E., 2009. Monoclonal antibody against the surface of *Alexandrium minutum* used in a whole-cell ELISA. Harmful Algae 8 (3), 538–545. https://doi.org/10.1016/j.hal.2008.08.027.

GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms Programme), 2001. In: Gilbert, P.M., Pitchr, G. (Eds.), Global Ecology and Oceanography of Harmful Algal Blooms Science Plan. Scientific Committee on Oceanic Research & Intergovernmental Oceanographic Commission UNESCO, pp. 1–87.

Gobler, C.J., 2020. Climate change and harmful algal blooms: insights and perspective. Harmful Algae 91, 101731. https://doi.org/10.1016/j.hal.2019.101731.

Gobler, C.J., Doherty, O.M., Hattenrath-Lehmann, T., Griffith, A.W., Kang, Y., Litaker, R. W., 2017. Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. Proc. Natl. Acad. Sci. U.S.A. 114 (19), 4975–4980. https://doi.org/10.1073/pnas.1619575114.

Goldman, J.M., Zhang, L.A., Manna, A., Armitage, B.A., Ly, D.H., Schneider, J.W., 2013. High affinity γpna sandwich hybridization assay for rapid detection of short nucleic acid targets with single mismatch discrimination. Biomacromolecules 14 (7), 2253–2261. https://doi.org/10.1021/bm400388a.

Gong, P., Antrim, A.K., Bickman, S.R., Cooley, E.G., Chung, S.H., 2022. Sandwich hybridization assay for in situ real-time cyanobacterial detection and monitoring: a review. Biosensors 12 (8), 640. https://doi.org/10.3390/bios12080640.
Gray, M., Wawrik, B., Paul, J., Casper, E., 2003. Molecular detection and quantitation of the red tide dinoflagellate *Karenia brevis* in the marine environment. Appl. Environ. Microbiol. 69 (9), 5726–5730. https://doi.org/10.1128/AEM.69.9.5726-5730.2003.
Greenfield, D.I., Marin III, R., Doucette, G.J., Mikulski, C., Jones, K., Jensen, S., Roman, B., Alvarado, N., Feldman, J., Scholin, C., 2008. Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007. Limnol. Oceanogr. Methods 6 (12), 667–679. https://doi.org/10.4319/lom.2008.6.667.
Greenfield, D.I., Marin III, R., Jensen, S., Massion, E., Roman, B., Feldman, J., Scholin, C. A., 2006. Application of environmental sample processor (ESP) methodology for quantifying *Pseudo-nitzschia* australis using ribosomal RNA-targeted probes in sandwich and fluorescent in situ hybridization formats. Limnol. Oceanogr. Methods 4 (11), 426–435. https://doi.org/10.4319/lom.2006.4.426.
Groben, R., Medlin, L., 2005. In situ hybridization of phytoplankton using fluorescently labeled rRNA probes. Methods Enzymol. 395, 299–310. https://doi.org/10.1016/S0076-6879(05)95018-0.
Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32 (2), 79–99. https://doi.org/10.2216/i0031-8884-32-2-79.1.
Hallegraeff, G.M., Anderson, D.M., Belin, C., Bottein, M.D., Bresnan, E., Chinain, M., Enevoldsen, H., Iwataki, M., Karlson, B., McKenzie, C.H., Sunesen, I., Pitcher, G.C., Provoost, P., Richardson, A., Schweibold, L., Tester, P.A., Trainer, V.L., Yñiguez, A. T., Zingone, A., 2021. Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. Commun. Earth Environ. 2 (1), 117. https://doi.org/10.1038/s43247-021-00178-8.
Harlow, L.D., Koutoulis, A., Hallegraeff, G.M., 2006. A novel, simplified technique for preservation and rapid isolation of total RNA from the toxic dinoflagellate *Alexandrium catenella* (Dinophyceae). Phycologia 45 (3), 311–318. https://doi.org/10.2216/05-29.1.
Harmsen, M.M., De Haard, H.J., 2007. Properties, production, and applications of camelid single-domain antibody fragments. Appl. Microbiol. Biotechnol. 77 (1), 13–22. https://doi.org/10.1007/s00253-007-1142-2.
Hattenrath-Lehmann, T.K., Yu, Z., Wallace, R.B., Tang, Y.Z., Gobler, C.J., 2016. Mapping the distribution of cysts from the toxic dinoflagellate *Cochlodinium polykrikoides* in bloom-prone estuaries by a novel fluorescence in situ hybridization assay. Appl. Environ. Microbiol. 82 (4), 1114–1125. https://doi.org/10.1128/AEM.03457-15.
Hayes, C.N., Nakahara, H., Ono, A., Tsuge, M., Oka, S., 2024. From omics to multi-omics: a review of advantages and tradeoffs. Genes 15 (12), 1551. https://doi.org/10.3390/genes15121551.
Haywood, A.J., Scholin, C.A., Marin III, R., Steidinger, K.A., Heil, C., Ray, J., 2007. Molecular detection of the brevetoxin-producing dinoflagellate *Karenia brevis* and closely related species using rRNA-targeted probes and a semiautomated sandwich hybridization assay. J. Phycol. 43 (6), 1271–1286. https://doi.org/10.1111/j.1529-8817.2007.00407.x.
Heller, M.J., 2002. DNA microarray technology: devices, systems, and applications. Annu Rev. Biomed. Eng. 4, 129–153. https://doi.org/10.1146/annurev.bioeng.4.020702.153438.
Hebert, P.D., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. Biol. Sci. 270 (1512), 313–321. https://doi.org/10.1098/rspb.2002.2218.
Hou, Y., Lin, S., 2009. Distinct gene number-genome size relationships for eukaryotes and non-eukaryotes: gene content estimation for dinoflagellate genomes. PLoS One 4 (9), e6978. https://doi.org/10.1371/journal.pone.0006978.
Hrdlickova, R., Toloue, M., Tian, B., 2016. RNA-seq methods for transcriptome analysis. WIREs RNA 8 (1), e1364. https://doi.org/10.1002/wrna.1364.
Huang, H., Gao, W., Zhu, P., Zhou, C., Qiao, L., Dang, C., Pang, J., Yan, X., 2020. Molecular method for rapid detection of the red tide dinoflagellate *Karenia mikimotoi* in the coastal region of Xiangshan Bay, China. J. Microbiol. Methods 168, 105801. https://doi.org/10.1016/j.mimet.2019.105801.
Huang, H., Xu, Q., Gibson, K., Chen, Y., Chen, N., 2021. Molecular characterization of harmful algal blooms in the Bohai Sea using metabarcoding analysis. Harmful Algae 106, 102066. https://doi.org/10.1016/j.hal.2021.102066.
Huang, H., Zhu, P., Zhou, C., He, S., Yan, X., 2017. The development of loop-mediated isothermal amplification combined with lateral flow dipstick for detection *of Karlodinium veneficum*. Harmful Algae 62, 20–29. https://doi.org/10.1016/j.hal.2016.11.022.
Huber, D., Voith von Voithenberg, L., Kaigala, G.V., 2018. Fluorescence in situ hybridization (FISH): history, limitations and what to expect from micro-scale FISH? Micro Nano Eng. 1, 15–24. https://doi.org/10.1016/j.mne.2018.10.006.
Ibabe, A., Menéndez-Teleña, D., Soto-López, V., Ardura, A., Arias, A., Bartolomé, M., Borrell, Y.J., Fernandez, S., Machado-Schiaffino, G., Mateo, J.L., Dopico, E., Garcia-Vazquez, E., 2024. New dockside eDNA based protocol to detect the seaweed *Asparagopsis armata* evaluated by stakeholders. Mar. Pollut. Bull. 201, 116259. https://doi.org/10.1016/j.marpolbul.2024.116259.
IOCCG, 2021. Observation of harmful algal blooms with ocean colour radiometry. In: Bernard, S., Kudela, R., Robertson Lain, L., Pitcher, G. (Eds.), Reports of the International Ocean Colour Coordinating Group Report, Reports of the International Ocean Colour Coordinating Group Report, 20. International Ocean Colour Coordinating Group, pp. 1–165. https://doi.org/10.25607/OBP-1042.
Jacobs-Palmer, E., Gallego, R., Cribari, K., Keller, A.G., Kelly, R.P., 2021. Environmental DNA metabarcoding for simultaneous monitoring and ecological assessment of many harmful algae. Front. Ecol. Evol. 9, 612107. https://doi.org/10.3389/fevo.2021.612107.
Jiang, W., Cossey, S., Rosenberg, J.N., Oyler, G.A., Olson, B.J.S.C., Weeks, D.P., 2014. A rapid live-cell ELISA for characterizing antibodies against cell surface antigens of *Chlamydomonas reinhardtii* and its use in isolating algae from natural environments with related cell wall components. BMC Plant Biol. 14 (1), 244. https://doi.org/10.1186/s12870-014-0244-0.
Ji, N., Lin, L., Li, L., Yu, L., Zhang, Y., Luo, H., Li, M., Shi, X., Wang, D.Z., Lin, S., 2018. Metatranscriptome analysis reveals environmental and diel regulation of a *Heterosigma akashiwo* (*Raphidophyceae*) bloom. Environ. Microbiol. 20 (3), 1078–1094. https://doi.org/10.1111/1462-2920.14045.
Jie, J., Hu, S., Liu, W., Wei, Q., Huang, Y., Yuan, X., Ren, L., Tan, M., Yu, Y., 2020. Portable and battery-powered PCR device for DNA amplification and fluorescence detection. Sensors 20 (9), 2627. https://doi.org/10.3390/s20092627.
Joffroy, B., Uca, Y.O., Prešern, D., Doye, J.P.K., Schmidt, T.L., 2018. Rolling circle amplification shows a sinusoidal template length-dependent amplification bias. Nucleic Acids Res. 46 (2), 538–545. https://doi.org/10.1093/nar/gkx1238.
Kawaguchi, Y.W., Tsuchikane, Y., Tanaka, K., Taji, T., Suzuki, Y., Toyoda, A., Ito, M., Watano, Y., Nishiyama, T., Sekimoto, H., Tsuchimatsu, T., 2023. Extensive copy number variation explains genome size variation in the unicellular zygnematophycean alga, *Closterium peracerosum–Strigosum–littorale* complex. Genome Biol. Evol. 15 (8), evad115. https://doi.org/10.1093/gbe/evad115.
Khehra, N., Padda, I.S., Swift, C.J., 2023. Polymerase chain reaction (PCR). *StatPearls* (Internet). StatPearls Publishing, Treasure Island (FL), 2025 Jan–. PMID: 36943981.
Kim, C., Kim, C., Sako, Y., 2005. Development of molecular identification method for genus *Alexandrium* (Dinophyceae) using whole-cell FISH. Mar. Biotechnol. 7 (3), 215–222. https://doi.org/10.1007/s10126-004-0424-2.
Kukurba, K.R., Montgomery, S.B., 2015. RNA sequencing and analysis. Cold. Spring Harb. Protoc. 951–969. https://doi.org/10.1101/pdb.top084970.
Lahaye, R., van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G., Savolainen, V., 2008. DNA barcoding the floras of biodiversity hotspots. Proc. Natl. Acad. Sci. U.S.A. 105 (8), 2923–2928. https://doi.org/10.1073/pnas.0709936105.
Lapointe, B.E., Bedford, B.J., 2007. Drift rhodophyte blooms emerge in Lee County, Florida, USA: evidence of escalating coastal eutrophication. Harmful Algae 6 (3), 421–437. https://doi.org/10.1016/j.hal.2006.12.005.
Lee, P., Wong, Y., Othman, S., Chee, H., 2021. Room-temperature stable loop-mediated isothermal amplification (LAMP) reagents to detect leptospiral DNA. Asian Biomed. 15 (4), 183–189. https://doi.org/10.2478/abm-2021-0023.
Lin, S., 2008. Use of molecular markers for early warning detection of harmful algal blooms. Int. J. Environ. Pollut. 33 (4), 381–400. https://doi.org/10.1504/IJEP.2008.020569.
Lin, S., Carpenter, E.J., 1996. An empirical protocol for whole-cell immunofluorescence of marine phytoplankton. J. Phycol. 32 (6), 1083–1094. https://doi.org/10.1111/j.0022-3646.1996.01083.x.
Lin, S., Feinstein, T.N., Zhang, H., Carpenter, E.J., 2003. Development of an immunofluorescence technique for detecting *Pfiesteria piscicida*. Harmful Algae 2 (3), 223–231. https://doi.org/10.1016/S1568-9883(03)00043-X.
Lin, S., Wang, L., Zheng, L., Dong, Y., Liu, S., Ding, S., Ye, N., Cao, W., Zhuang, Z., 2014. Current status and future prospect of DNA barcoding in marine biology. Acta Oceanol. Sin. 36 (12), 1–17. https://doi.org/10.3969/j.issn.0253-4193.2014.12.001 (in Chinese with English abstract and illustrations).
Lin, S., Zhang, H., Dubois, A., 2006. Low abundance distribution of *Pfiesteria piscicida* in Pacific and Western Atlantic as detected by mtDNA-18S rDNA real-time polymerase chain reaction. J. Plankton Res. 28 (7), 667–681. https://doi.org/10.1093/plankt/fbi150.
Lin, S., Zhang, H., Zhuang, Y., Tran, B., Gill, J., 2010. Spliced leader–based metatranscriptomic analyses lead to recognition of hidden genomic features in dinoflagellates. Proc. Natl. Acad. Sci. U.S.A. 107 (46), 20033–20038. https://doi.org/10.1073/pnas.1007246107.
Liu, F.G., Chen, G.F., Zhang, C.Y., Wang, Y.Y., Zhou, J., 2019. Exponential rolling circle amplification coupled with lateral flow dipstick strips as a rapid and sensitive method for the field detection of *Karlodinium veneficum*. J. Appl. Phycol. 31 (4), 2423–2436. https://doi.org/10.1007/s10811-019-01762-4.
Liu, F., Zhang, C., Yang, Y., Yang, Y., Wang, Y., Chen, G., 2020. Detection of *Prorocentrum minimum* by hyperbranched rolling circle amplification coupled with lateral flow dipstick. Environ. Sci. Pollut. Res. Int. 27 (36), 44995–45007. https://doi.org/10.1007/s11356-020-10391-1.
Lobato, I.M., O'Sullivan, C.K., 2018. Recombinase polymerase amplification: basics, applications and recent advances. Trends Analyt. Chem. 98, 19–35. https://doi.org/10.1016/j.trac.2017.10.015.
Loukas, C., Mowlem, M.C., Tsaloglou, M., Green, N.G., 2018. A novel portable filtration system for sampling and concentration of microorganisms: demonstration on marine microalgae with subsequent quantification using IC-NASBA. Harmful Algae 75, 94–104. https://doi.org/10.1016/j.hal.2018.03.006.
Lundholm, N., Bernard, C., Churro, C., Escalera, L., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø., Murray, S., Salas, R., Tillmann, U. & Zingone, A. (Eds) (2009). IOC-UNESCO taxonomic reference list of harmful micro algae. doi:10.14284/362.
Mary, L., Quere, J., Latimier, M., Rovillon, G., Hégaret, H., Réveillon, D., Le Gac, M., 2022. Genetic association of toxin production in the dinoflagellate *Alexandrium minutum*. Microb. Genom. 8 (11), 000879. https://doi.org/10.1099/mgen.0.000879.
Mazzega, E., Beran, A., Cabrini, M., de Marco, A., 2019. In vitro isolation of nanobodies for selective *Alexandrium minutum* recognition: a model for convenient development of dedicated immuno-reagents to study and diagnostic toxic unicellular algae. Harmful Algae 82, 44–51. https://doi.org/10.1016/j.hal.2019.01.002.
McGirr, S., Clarke, D., Kilcoyne, J., Salas, R., Koehler, H., Silke, J., Touzet, N., 2021. Insights into the discrepancy between *Azadinium* spp. And azaspiracid toxins near

strategically important aquaculture operations in the west and southwest of Ireland. Estuar. Coast. Shelf Sci. 262, 107622. https://doi.org/10.1016/j.ecss.2021.107622.
Medlin, L.K., Orozco, J., 2017. Molecular techniques for the detection of organisms in aquatic environments, with emphasis on harmful algal bloom species. Sensors 17 (5), 1184. https://doi.org/10.3390/s17051184.
Metsky, H.C., Welch, N.L., Pillai, P.P., Haradhvala, N.J., Rumker, L., Mantena, S., Zhang, Y.B., Yang, D.K., Ackerman, C.M., Weller, J., Blainey, P.C., Myhrvold, C., Mitzenmacher, M., Sabeti, P.C., 2022. Designing sensitive viral diagnostics with machine learning. Nat. Biotechnol. 40 (7), 1123–1131. https://doi.org/10.1038/s41587-022-01213-5.
Meyer, T., 2010. Competitive ELISA. In: Kontermann, R., Dübel, S. (Eds.), Antibody Engineering. Springer, Berlin Heidelberg, pp. 739–742. https://doi.org/10.1007/978-3-642-01144-3_47.
Miller, P.E., Scholin, C.A., 1996. Identification of cultured *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes. J. Phycol. 32 (4), 646–655. https://doi.org/10.1111/j.0022-3646.1996.00646.x.
Miranda, L.N., 2009. Gene expression in the toxic dinoflagellate, Alexandrium fundyense: emphasis on cell cycle- and growth-regulatory genes. Ph.D.Dissertation, University of Connecticut. https://digitalcommons.lib.uconn.edu/dissertations/AAI3367447.
Moore, S.K., Adams, N.G., Danyluk, L., Mikulski, C., Doucette, G., Mickett, J.B., Michel-Hart, N., Roman, B., Ussler, B., Birch, J.M., Engstrom, I., Palladino, D., Gossiaux, D., 2025. Standard Operating Procedures for Environmental Sample Processor Deployments. U.S. Department of Commerce, NOAA, pp. 1–181. https://doi.org/10.25923/svhf-yp57.
Murray, S.A., Wiese, M., Stüken, A., Brett, S., Kellmann, R., Hallegraeff, G., Neilan, B.A., 2011. sxtA-based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters. Appl. Environ. Microbiol. 77 (19), 7050–7057. https://doi.org/10.1128/AEM.05308-11.
Muttil, N., Lee, J.H.W., 2005. Genetic programming for analysis and real-time prediction of coastal algal blooms. Ecol. Modell. 189 (3), 363–376. https://doi.org/10.1016/j.ecolmodel.2005.03.018.
Nagai, S., 2013. Species-specific detection of six *Alexandrium* species from single vegetative cells by a loop-mediated isothermal amplification method. DNA Testing 5, 33–46.
Nagai, S., Yamamoto, K., Hata, N., Itakura, S., 2012. Study of DNA extraction methods for use in loop-mediated isothermal amplification detection of single resting cysts in the toxic dinoflagellates *Alexandrium tamarense* and *A. catenella*. Mar. Genom. 7, 51–56. https://doi.org/10.1016/j.margen.2012.03.002.
Nagarajan, D., Lee, D., Varjani, S., Lam, S.S., Allakhverdiev, S.I., Chang, J., 2022. Microalgae-based wastewater treatment – Microalgae-bacteria consortia, multi-omics approaches and algal stress response. Sci. Total Environ. 845, 157110. https://doi.org/10.1016/j.scitotenv.2022.157110.
Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28 (12), e63. https://doi.org/10.1093/nar/28.12.e63.
Ørberg, S.B., Krause-Jensen, D., Geraldi, N.R., Ortega, A., Díaz-Rúa, R., Duarte, C.M., 2021. Fingerprinting Arctic and North Atlantic macroalgae with eDNA – application and perspectives. Environ. DNA 4 (2), 385–401. https://doi.org/10.1002/edn3.262.
Ouhsassi, M., Khay, E.O., Bouyahya, A., El Ouahrani, A., Harsal, A.E., Abrini, J., 2020. Evaluation of self-purifying power of cyanobacteria *Pseudanabaena galeata*: case of dairy factory effluents. Appl. Water Sci. 10 (7), 181. https://doi.org/10.1007/s13201-020-01270-8.
Paerl, H.W., Fulton, R.S., Moisander, P.H., Dyble, J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci. World J. 1 (2), 76–113. https://doi.org/10.1100/tsw.2001.16.
Paul, J., Scholin, C., van den Engh, G., Perry, M.J., 2007. In situ instrumentation. Oceanography 20 (2), 70–78. https://doi.org/10.5670/oceanog.2007.50.
Penna, A., Battocchi, C., Garcés, E., Anglès, S., Cucchiari, E., Totti, C., Kremp, A., Satta, C., Grazia Giacobbe, M., Bravo, I., Bastianini, M., 2010. Detection of microalgal resting cysts in European coastal sediments using a PCR-based assay. Deep. Sea Res. 2. Top. Stud. Oceanogr. 57 (3), 288–300. https://doi.org/10.1016/j.dsr2.2009.09.010.
Penna, A., Magnani, M., 1999. Identification of *Alexandrium* (Dinophyceae) species using PCR and rDNA-targeted probes. J. Phycol. 35 (3), 615–621. https://doi.org/10.1046/j.1529-8817.1999.3530615.x.
Penna, A., Magnani, M., 2008. A PCR immunoassay method for the detection of *Alexandrium* (Dinophyceae) species. J. Phycol. 36 (6), 1183–1186. https://doi.org/10.1046/j.1529-8817.2000.99149.x.
Perez-Romero, C., Mendoza-Maldonado, L., Tonda, A., Coz, E., Tabeling, P., Vanhomwegen, J., MacSharry, J., Szafran, J., Bobadilla-Morales, L., Corona-Rivera, A., Claassen, E., Garssen, J., Kraneveld, A.D., Lopez-Rincon, A., 2023. An innovative AI-based primer design tool for precise and accurate detection of SARS-CoV-2 variants of concern. Sci. Rep. 13 (1), 15782. https://doi.org/10.1038/s41598-023-42348-y.
Peters, R. P. H., van Agtmael, M.A., Simoons-Smit, A.M., Danner, S.A., Vandenbroucke-Grauls, C.M.J.E., Savelkoul, P.H.M., 2006. Rapid identification of pathogens in blood cultures with a modified fluorescence in situ hybridization assay. J. Clin. Microbiol. 44 (11), 4186–4188. https://doi.org/10.1128/jcm.01085-06.
Pickar-Oliver, A., Gersbach, C.A., 2019. The next generation of CRISPR–Cas technologies and applications. Nat. Rev. Mol. Cell Biol. 20 (8), 490–507. https://doi.org/10.1038/s41580-019-0131-5.
Pitz, K.J., Richlen, M.L., Fachon, E., Smith, T.B., Parsons, M.L., Anderson, D.M., 2021. Development of fluorescence in situ hybridization (FISH) probes to detect and enumerate *Gambierdiscus* species. Harmful Algae 101, 101914. https://doi.org/10.1016/j.hal.2020.101914.
Pohanka, M., 2009. Monoclonal and polyclonal antibodies production - preparation of potent biorecognition element. J. Appl. Biomed. 7 (3), 115–121. https://doi.org/10.32725/jab.2009.012.
Qin, Y., Chen, G., Zhang, C., Wang, Y., Zhou, J., 2019. Development of loop-mediated isothermal amplification combined with a chromatographic lateral-flow dipstick for rapid detection of *Chattonella marina*. Harmful Algae 89, 101666. https://doi.org/10.1016/j.hal.2019.101666.
Qin, Y., Zhang, C., Liu, F., Chen, Q., Yang, Y., Wang, Y., Chen, G., 2020. Establishment of double probes rolling circle amplification combined with lateral flow dipstick for rapid detection of *Chattonella marina*. Harmful Algae 97, 101857. https://doi.org/10.1016/j.hal.2020.101857.
Reen, D.J., 1994. Enzyme-linked immunosorbent assay (ELISA). In: Walker, J.M. (Ed.), Basic Protein and Peptide Protocols. Humana Press, pp. 461–466. https://doi.org/10.1385/0-89603-268-X:461.
Robledo, D., Vázquez-Delfín, E., Freile-Pelegrín, Y., Vásquez-Elizondo, R.M., Qui-Minet, Z., Salazar-Garibay, A., 2021. Challenges and opportunities in relation to *Sargassum* events along the Caribbean Sea. Front. Mar. Sci. 8, 699664. https://doi.org/10.3389/fmars.2021.699664.
Rodríguez-Martínez, R.E., Medina-Valmaseda, A.E., Blanchon, P., Monroy-Velázquez, L. V., Almazán-Becerril, A., Delgado-Pech, B., Vásquez-Yeomans, L., Francisco, V., García-Rivas, M.C., 2019. Faunal mortality associated with massive beaching and decomposition of pelagic *Sargassum*. Mar. Pollut. Bull. 146, 201–205. https://doi.org/10.1016/j.marpolbul.2019.06.015.
Rohaim, M.A., Clayton, E., Sahin, I., Vilela, J., Khalifa, M.E., Al-Natour, M., Bayoumi, M., Poirier, A.C., Branavan, M., Tharmakulasingam, M., Chaudhry, N.S., Sodi, R., Brown, A., Burkhart, P., Hacking, W., Botham, J., Boyce, J., Wilkinson, H., Williams, C., Munir, M., 2020. Artificial intelligence-assisted loop mediated isothermal amplification (AI-LAMP) for rapid detection of SARS-CoV-2. Viruses 12 (9), 972. https://doi.org/10.3390/v12090972.
Romanis, C.S., Pearson, L.A., Neilan, B.A., 2021. Cyanobacterial blooms in wastewater treatment facilities: significance and emerging monitoring strategies. J. Microbiol. Methods 180, 106123. https://doi.org/10.1016/j.mimet.2020.106123.
Ruvindy, R., Barua, A., Bolch, C.J.S., Sarowar, C., Savela, H., Murray, S.A., 2023. Genomic copy number variability at the genus, species and population levels impacts in situ ecological analyses of dinoflagellates and harmful algal blooms. ISMe Commun. 3 (1), 70. https://doi.org/10.1038/s43705-023-00274-0.
Sahoo, P.R., Sethy, K., Mohapatra, S., Panda, D., 2016. Loop mediated isothermal amplification: an innovative gene amplification technique for animal diseases. Vet. World 9 (5), 465–469. https://doi.org/10.14202/vetworld.2016.465-469.
Saunders, G.W., McDevit, D.C., 2012. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. Methods Mol. Biol. 858, 207–222. https://doi.org/10.1007/978-1-61779-591-6_10.
Scholin, C.A., Buck, K.R., Britschgi, T., Cangelosi, G., Chavez, F.P., 1996. Identification of *Pseudo-nitzschia* australis (Bacillariophyceae) using rRNA-targeted probes in whole cell and sandwich hybridization formats. Phycologia 35 (3), 190–197. https://doi.org/10.2216/i0031-8884-35-3-190.1.
Scholin, C., Miller, P., Buck, K., Chavez, F., Harris, P., Haydock, P., Howard, J., Cangelosi, G., 1997. Detection and quantification of *Pseudo-nitzschia* australis in cultured and natural populations using LSU rRNA-targeted probes. Limnol. Oceanogr. 42 (5), 1265–1272. https://doi.org/10.4319/lo.1997.42.5_part_2.1265.
Schönhuber, W., Boris, Z., Stella, E., Rosmarie, R., Michael, H., Wolfgang, L., Rudolf, A., 1999. In situ identification of cyanobacteria with horseradish peroxidase-labeled, rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 65 (3), 1259–1267. https://doi.org/10.1128/AEM.65.3.1259-1267.1999.
Shi, R., Huang, H., Qi, Z., Hu, W., Tian, Z., Dai, M., 2013. Algicidal activity against *Skeletonema costatum* by marine bacteria isolated from a high frequency harmful algal blooms area in southern Chinese coast. World J. Microbiol. Biotechnol. 29 (1), 153–162. https://doi.org/10.1007/s11274-012-1168-1.
Shi, X., Zou, Y., Zhang, Y., Ding, G., Xiao, Y., Lin, S., Chen, J., 2024. Salinity decline promotes growth and harmful blooms of a toxic alga by diverting carbon flow. Glob. Change Biol. 30 (6), 17348. https://doi.org/10.1111/gcb.17348.
Shumway, S.E., Burkholder, J.M., Morton, S.L., 2018. Harmful Algal Blooms: a Compendium Desk Reference. Wiley-Blackwell, pp. 561–638. ISBN: 978-1-118-99465-8.
Sidabutar, T., Cappenberg, H., Srimariana, E.S., Muawanah, A., Wouthuyzen, S., 2021. Harmful algal blooms and their impact on fish mortalities in Lampung Bay: an overview. IOP Conf. Ser.: Earth Environ. Sci. 944, 1–13. https://doi.org/10.1088/1755-1315/944/1/012027.
Sivapragasam, C., Muttil, N., Muthukumar, S., Arun, V.M., 2010. Prediction of algal blooms using genetic programming. Mar. Pollut. Bull. 60 (10), 1849–1855. https://doi.org/10.1016/j.marpolbul.2010.05.020.
Smith, K.F., Biessy, L., Argyle, P.A., Trnski, T., Halafihi, T., Rhodes, L.L., 2017. Molecular identification of *Gambierdiscus* and *Fukuyoa* (Dinophyceae) from environmental samples. Mar. Drugs 15 (8). https://doi.org/10.3390/md15080243.
Stauffer, B.A., Schaffner, R.A., Catherine, W., Caron, D.A., 2008. Immunofluorescence flow cytometry technique for enumeration of the brown-tide alga, *Aureococcus anophagefferens*. Appl. Environ. Microbiol. 74 (22), 6931–6940. https://doi.org/10.1128/AEM.00996-08.
Stüken, A., Orr, R.J.S., Kellmann, R., Murray, S.A., Neilan, B.A., Jakobsen, K.S., 2011. Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates. PLoS One 6 (5), e20096. https://doi.org/10.1371/journal.pone.0020096.
Toebe, K., 2013. Whole cell hybridisation for monitoring harmful marine microalgae. Environ. Sci. Pollut. Res. 20 (10), 6816–6823. https://doi.org/10.1007/s11356-012-1416-9.
Toldrà, A., Alcaraz, C., Andree, K.B., Fernández-Tejedor, M., Diogène, J., Katakis, I., O'Sullivan, C.K., Campàs, M., 2019. Colorimetric DNA-based assay for the specific

detection and quantification of *Ostreopsis* cf. *ovata* and *Ostreopsis* cf. *siamensis* in the marine environment. Harmful Algae 84, 27–35. https://doi.org/10.1016/j.hal.2019.02.003.

Toldrà, A., Jauset-Rubio, M., Andree, K.B., Fernández-Tejedor, M., Diogène, J., Katakis, I., O'Sullivan, C.K., Campàs, M., 2018. Detection and quantification of the toxic marine microalgae *Karlodinium veneficum* and *Karlodinium armiger* using recombinase polymerase amplification and enzyme-linked oligonucleotide assay. Anal. Chim. Acta 1039, 140–148. https://doi.org/10.1016/j.aca.2018.07.057.

Toldrà, A., O'Sullivan, C.K., Diogène, J., Campàs, M., 2020. Detecting harmful algal blooms with nucleic acid amplification-based biotechnological tools. Sci. Total Environ. 749, 141605. https://doi.org/10.1016/j.scitotenv.2020.141605.

Tönnies, H., 2010. Molecular cytogenetics in Molecular diagnostics. In: Patrinos, G.P., Ansorge, W.J. (Eds.), Molecular Diagnostics, 2nd Edition. Academic Press, pp. 133–153. https://doi.org/10.1016/B978-0-12-374537-8.00010-9.

Trinh, T.N.D., Lee, N.Y., 2018. A rapid and eco-friendly isothermal amplification microdevice for multiplex detection of foodborne pathogens. Lab Chip 18, 2369–2377. https://doi.org/10.1039/c8lc00424b.

Ulrich, R.M., Casper, E.T., Campbell, L., Richardson, B., Heil, C.A., Paul, J.H., 2010. Detection and quantification of *Karenia mikimotoi* using real-time nucleic acid sequence-based amplification with internal control RNA (IC-NASBA). Harmful Algae 9 (1), 116–122. https://doi.org/10.1016/j.hal.2009.08.010.

Ussler III, W., Doucette, G.J., Preston, C.M., Weinstock, C., Allaf, N., Roman, B., Jensen, S., Yamahara, K., Lingerfelt, L.A., Mikulski, C.M., Hobson, B.W., Kieft, B., Raanan, B., Zhang, Y., Errera, R.M., Ruberg, S.A., Den Uyl, P.A., Goodwin, K.D., Soelberg, S.D., Scholin, C.A., 2024. Underway measurement of cyanobacterial microcystins using a surface plasmon resonance sensor on an autonomous underwater vehicle. Limnol. Oceanogr. Methods 22 (9), 681–699. https://doi.org/10.1002/lom3.10627.

Vitorino, R., 2024. Transforming clinical research: the power of high-throughput omics integration. Proteomes 12 (3), 25. https://doi.org/10.3390/proteomes12030025.

Wang, B., Zhang, C., Liu, F., Li, R., Wang, Y., Chen, G., 2021. Development of a recombinase polymerase amplification combined with lateral flow dipstick assay for rapid and sensitive detection of *Heterosigma akashiwo*. J. Appl. Phycol. 33 (5), 3165–3178. https://doi.org/10.1007/s10811-021-02538-5.

Wang, D., Huang, X., Chan, L.L., Hong, H., 2007. Development of an immunofluorescence technique for detecting *Prorocentrum donghaiense* Lu. J. Appl. Phycol. 19 (4), 325–332. https://doi.org/10.1007/s10811-006-9139-2.

Wang, D., Xie, Z., Zhang, S., 2014. Marine metaproteomics: current status and future directions. J. Proteom. 97, 27–35. https://doi.org/10.1016/j.jprot.2013.08.024.

Wang, L., Chen, G., Zhang, C., Wang, Y., Sun, R., 2019. Application of loop-mediated isothermal amplification combined with lateral flow dipstick to rapid and sensitive detection of *Alexandrium catenella*. Environ. Sci. Pollut. Res. 27 (4), 4246–4257. https://doi.org/10.1007/s11356-019-06889-y.

Wang, L., Chen, X., Pan, F., Yao, G., Chen, J., 2023. Development of a rapid detection method for *Karenia mikimotoi* by using CRISPR-Cas12a. Front. Microbiol. 14, 1205765. https://doi.org/10.3389/fmicb.2023.1205765.

Wang, L., Li, L., Alam, M.J., Geng, Y., Li, Z., Yamasaki, S., Shi, L., 2008. Loop-mediated isothermal amplification method for rapid detection of the toxic dinoflagellate *Alexandrium*, which causes algal blooms and poisoning of shellfish. FEMS Microbiol. Lett. 282 (1), 15–21. https://doi.org/10.1111/j.1574-6968.2008.01074.x.

Wang, L., Wu, Y., Pan, S., Pan, F., Chen, J., 2025. Rapid and sensitive detection of *Karlodinium veneficum* using RAA and CRISPR-Cas12a technologies. Harmful Algae 146, 102864. https://doi.org/10.1016/j.hal.2025.102864.

Wang, L., Zhang, C., Chen, G., Wang, Y., Fu, M., 2020. Development of a rapid screening test for *Karenia mikimotoi* by using loop-mediated isothermal amplification and lateral flow dipstick. J. Appl. Phycol. 32 (5), 3143–3155. https://doi.org/10.1007/s10811-020-02203-3.

Wong, Y., Othman, S., Lau, Y., Radu, S., Chee, H., 2018. Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. J. Appl. Microbiol. 124 (3), 626–643. https://doi.org/10.1111/jam.13647.

Wood, S.A., Kelly, L., Bouma-Gregson, K., Humbert, J.F., Laughinghouse, H.D., Lazorchak, J., McAllister, T., McQueen, A., Pokrzywinski, K., Puddick, J., Quiblier, C., Reitz, L.A., Ryan, K., Vadeboncoeur, Y., Zastepa, A., Davis, T.W., 2020. Toxic benthic freshwater cyanobacterial proliferations: challenges and solutions for enhancing knowledge and improving monitoring and mitigation. Freshw. Biol. 65 (10), 1824–1842. https://doi.org/10.1111/fwb.13532.

Wood, S.A., Smith, K.F., Banks, J.C., Tremblay, L.A., Rhodes, L., Mountfort, D., Cary, S. C., Pochon, X., 2013. Molecular genetic tools for environmental monitoring of New Zealand's aquatic habitats, past, present and the future. N. Z. J. Mar. Freshw. Res. 47 (1), 90–119. https://doi.org/10.1080/00288330.2012.745885.

Xiao, J., Fan, S., Wang, Z., Fu, M., Song, H., Wang, X., Yuan, C., Pang, M., Miao, X., Zhang, X., 2020. Decadal characteristics of the floating *Ulva* and *Sargassum* in the Subei Shoal, Yellow Sea. Hai Yang Xue Bao 39 (10), 1–10. https://doi.org/10.1007/s13131-020-1655-4.

Xin, Z., Yu, Z., Wang, T., Hui, X., Gou, W., Sun, J., Qi, H., Li, R., 2005. Identification and quantification of the toxic dinoflagellate *Gymnodinium* sp. with competitive enzyme-linked immunosorbent assay (cELISA). Harmful Algae 4 (2), 297–307. https://doi.org/10.1016/j.hal.2004.05.004.

Xu, H., Wu, X., Liu, Q., Yang, C., Shen, M., Wang, Y., Liu, S., Zhao, S., Xiao, T., Sun, M., Ding, Z., Bao, J., Chen, M., Gao, M., 2023. A universal strategy for enhancing the circulating miRNAs' detection performance of rolling circle amplification by using a dual-terminal stem-loop padlock. ACS Nano 18 (1), 436–450. https://doi.org/10.1021/acsnano.3c07721.

Xue, T., Chang, H., Ren, M., Wang, H., Yang, Y., Wang, B., Lv, L., Tang, L., Fu, C., Fang, Q., He, C., Zhu, X., Zhou, X., Bai, Q., 2023. Deep learning to automatically evaluate HER2 gene amplification status from fluorescence in situ hybridization images. Sci. Rep. 13 (1), 9746. https://doi.org/10.1038/s41598-023-36811-z.

Yarimizu, K., Sildever, S., Hamamoto, Y., Tazawa, S., Oikawa, H., Yamaguchi, H., Basti, L., Mardones, J.I., Paredes-Mella, J., Nagai, S., 2021. Development of an absolute quantification method for ribosomal RNA gene copy numbers per eukaryotic single cell by digital PCR. Harmful Algae 103, 102008. https://doi.org/10.1016/j.hal.2021.102008.

Yu, L., Li, T., Li, H., Ma, M., Li, L., Lin, S., 2023. In situ molecular ecological analyses illuminate distinct factors regulating formation and demise of a harmful dinoflagellate bloom. Microbiol. Spectr. 11 (3), 5157. https://doi.org/10.1128/spectrum.05157-22.

Yu, L., Zhang, Y., Li, M., Wang, C., Lin, X., Li, L., Shi, X., Guo, C., Lin, S., 2020. Comparative metatranscriptomic profiling and microRNA sequencing to reveal active metabolic pathways associated with a dinoflagellate bloom. Sci. Total Environ. 699, 134323. https://doi.org/10.1016/j.scitotenv.2019.134323.

Zammatteo, N., Alexandre, I., Ernest, I., Le, L., Brancart, F., Remacle, J., 1997. Comparison between microwell and bead supports for the detection of human cytomegalovirus amplicons by sandwich hybridization. Anal. Biochem. 253 (2), 180–189. https://doi.org/10.1006/abio.1997.2352.

Zeller, P., Ploux, O., Méjean, A., 2016. A simple protocol for attenuating the auto-fluorescence of cyanobacteria for optimized fluorescence in situ hybridization (FISH) imaging. J. Microbiol. Methods 122, 16–19. https://doi.org/10.1016/j.mimet.2016.01.006.

Zhang, B., Yang, Y., He, W., Liu, W., 2023. Algicidal process and mechanisms of *Enterobacter hormaechei* F2 revealed by an integrated transcriptomic and metabolomic approach. Genomics 115 (2), 110586. https://doi.org/10.1016/j.ygeno.2023.110586.

Zhang, C., Chen, G., Wang, Y., Zhou, J., Li, C., 2019a. Establishment and application of hyperbranched rolling circle amplification coupled with lateral flow dipstick for the sensitive detection of *Karenia mikimotoi*. Harmful Algae 84, 151–160. https://doi.org/10.1016/j.hal.2019.03.015.

Zhang, C., Sun, R., Wang, Y., Chen, G., Guo, C., Zhou, J., 2018. Comparative detection of *Karenia mikimotoi* by exponential rolling circle amplification (E-RCA) and double-ligation E-RCA. J. Appl. Phycol. 31 (1), 505–518. https://doi.org/10.1007/s10811-018-1584-1.

Zhang, C., Wang, Y., Wang, Y., Liu, F., Chen, G., 2024. Establishment of a multiplex polymerase chain reaction assay for the detection of marine harmful algae. J. Appl. Phycol. 36 (1), 243–258. https://doi.org/10.1007/s10811-023-03112-x.

Zhang, F., Ma, L., Xu, Z., Zheng, J., Shi, Y., Lu, Y., Miao, Y., 2009. Sensitive and rapid detection of *Karenia mikimotoi* (Dinophyceae) by loop-mediated isothermal amplification. Harmful Algae 8 (6), 839–842. https://doi.org/10.1016/j.hal.2009.03.004.

Zhang, H., Lin, S., 2005. Development of a cob-18S rRNA gene real-time PCR assay for quantifying *Pfiesteria shumwayae* in the natural environment. Appl. Environ. Microbiol. 71 (11), 7053–7063. https://doi.org/10.1128/AEM.71.11.7053-7063.2005.

Zhang, H., Hou, Y., Miranda, L., Campbell, D.A., Sturm, N.R., Gaasterland, T., Lin, S., 2007. Spliced leader RNA trans-splicing in dinoflagellates. Proc. Natl. Acad. Sci. U.S. A. 104 (11), 4618–4623. https://doi.org/10.1073/pnas.0700258104.

Zhang, H., Xu, H., Zhang, S., Zhou, Y., He, Y., Amin, S.A., Chen, J., Yan, K., Lin, L., Liu, S., Wang, D., 2021. Metaproteomics reveals the molecular mechanism underlying bloom maintenance of a marine dinoflagellate under low ambient CO_2 and inorganic nutrients. Sci. Total Environ. 768, 144515. https://doi.org/10.1016/j.scitotenv.2020.144515.

Zhang, H., Zhuang, Y., Gill, J., Lin, S., 2013. Proof that dinoflagellate spliced leader (DinoSL) is a useful hook for fishing dinoflagellate transcripts from mixed microbial samples: *Symbiodinium kawagutii* as a case study. Protist 164 (4), 510–527. https://doi.org/10.1016/j.protis.2013.04.002.

Zhang, H., Lin, S., 2002. Detection and quantification of *Pfiesteria piscicida* by using the mitochondrial cytochrome b gene. Appl. Environ. Microbiol. 68 (2), 989–994. https://doi.org/10.1128/AEM.68.2.989-994.2002.

Zhang, H., He, Y., Wu, P.S., Xie, Z., Li, D., Lin, L., Chen, F., Wang, D.-Z., 2019c. Functional differences in the blooming phytoplankton *Heterosigma akashiwo* and *prorocentrum donghaiense* revealed by comparative metaproteomics. Appl. Environ. Microbiol. 85 (19), 1425. https://doi.org/10.1128/AEM.01425-19.

Zhang, Y., Lin, X., Shi, X., Lin, L., Luo, H., Li, L., Lin, S., 2019b. Metatranscriptomic signatures associated with phytoplankton regime shift from diatom dominance to a dinoflagellate bloom. Front. Microbiol. 10, 590. https://doi.org/10.3389/fmicb.2019.00590.

Zhen, Y., Mi, T., Yu, Z., 2009. Detection of several harmful algal species by sandwich hybridization integrated with a nuclease protection assay. Harmful Algae 8 (5), 651–657. https://doi.org/10.1016/j.hal.2008.12.001.

Zhi, W., Ge, Z., He, Z., Zhang, H., 2014. Methods for understanding microbial community structures and functions in microbial fuel cells: a review. Bioresour. Technol. 171, 461–468. https://doi.org/10.1016/j.biortech.2014.08.096.

Zhou, L., Ji, N., Sprecher, B.N., Lin, S., 2025. Estimating carbon biomass using DNA: Phytoplankton as a case study. DNA 5 (1). https://doi.org/10.3390/dna5010013.

Zhou, L., Peng, R., Zhang, R., Li, J., 2018. The applications of CRISPR/Cas system in molecular detection. J. Cell Mol. Med. 22 (12), 5807–5815. https://doi.org/10.1111/jcmm.13925.

Zhu, J.P., Du, S., Li, X., 2012a. Detection of microcystin-producing *microcystis* in Guanqiao Lake using a sandwich hybridization assay. Can. J. Microbiol. 58 (4), 442–447. https://doi.org/10.1139/w2012-008.

Zhu, J.P., Li, X., Du, S., 2012b. Identification and enumeration of *microcystis* using a sandwich hybridization assay. J. Microbiol. 50 (2), 186–190. https://doi.org/10.1007/s12275-012-1418-9.

Zhuang, Y., Zhang, H., Hannick, L., Lin, S., 2015. Metatranscriptome profiling reveals versatile N-nutrient utilization, CO_2 limitation, oxidative stress, and active toxin production in an *Alexandrium fundyense* bloom. Harmful Algae 42, 60–70. https://doi.org/10.1016/j.hal.2014.12.006.

Zu, Y., Miao, W., Luo, Y., Xu, C., Pan, Q., Chen, S., Li, J., 2022. Screening of nanobody against microcystis from a human phage display nanobody library. J. Oceanol. Limnol. 40 (5), 1696–1705. https://doi.org/10.1007/s00343-022-1361-5.