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Review

# Progress and promise of omics for predicting the impacts of climate change on harmful algal blooms

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ABSTRACT

Climate change is predicted to increase the severity and prevalence of harmful algal blooms (HABs). In the past twenty years, omics techniques such as genomics, transcriptomics, proteomics and metabolomics have transformed that data landscape of many fields including the study of HABs. Advances in technology have facilitated the creation of many publicly available omics datasets that are complementary and shed new light on the mechanisms of HAB formation and toxin production. Genomics have been used to reveal differences in toxicity and nutritional requirements, while transcriptomics and proteomics have been used to explore HAB species responses to environmental stressors, and metabolomics can reveal mechanisms of allelopathy and toxicity. In this review, we explore how omics data may be leveraged to improve predictions of how climate change will impact HAB dynamics. We also highlight important gaps in our knowledge of HAB prediction, which include swimming behaviors, microbial interactions and evolution that can be addressed by future studies with omics tools. Lastly, we discuss approaches to incorporate current omics datasets into predictive numerical models that may enhance HAB prediction in a changing world. With the ever-increasing omics databases, leveraging these data for understanding climate-driven HAB dynamics will be increasingly powerful.

## 1. Introduction

Harmful algal blooms (HABs) are formed when toxic or ecosystem disruptive algal species grow to sufficient abundance. HABs can cause closures of commercial fisheries and aquaculture operations resulting in millions in economic losses (Haigh and Esenkulova, 2014; Itakura and Imai, 2014) and in some cases have even resulted in the death of humans, domestic animals and wildlife (Hallegraeff, 2010). HAB species have been identified from seven different phyla with diverse toxins, morphologies and life histories (Moestrup et al., 2009). To a large extent, omic studies are limited to species that can be cultured in the lab and are restricted by the time and effort to measure their responses to a multitude of interacting environmental variables, microbial communities and rates of evolution. The underlying mechanisms of how HAB species respond to climate change stressors must be elucidated in order to overcome these limitations. While no single analytical technique is appropriate for all HAB species, omic approaches including; genomics, transcriptomics, proteomics and metabolomics, provide a new suite of tools to aid in the understanding of HAB dynamics. Here omics are distinguished from other molecular studies by the global approach to measuring a molecule type rather than the targeted approach to quantify the abundance of specific genes (as in quantitative PCR) or toxins (as in targeted metabolite studies). The global omics approach allows for the identification of new gene, protein and metabolite targets that underpin responses to environmental change and may impact HAB species success in future ocean conditions.

### 1.1. Climate change and HABs

Anthropogenic climate change includes warming and carbonation of aquatic systems due to the release of carbon dioxide into the atmosphere from burning fossil fuels (Ciais et al., 2013). Warming and increased availability of $CO_2$ in aquatic systems can shift the balance of competition between algae (Barton et al., 2016; Dutkiewicz et al., 2015), potentially favoring harmful species. There has already been an increase in the number and shift in the seasonality of HAB events around the world which is thought to be related to climate change (Hallegraeff, 2010, 1993). Examples include a global expansion of

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detection of paralytic shellfish poisoning toxins (Anderson et al., 2012), increased abundance and northward shift of HAB species in the Northeast Atlantic (Bresnan et al., 2013; Edwards et al., 2006; Gobler et al., 2017), the first known toxic *Pseudo-nitzschia* blooms in the Gulf of Maine (Daley, 2018), *Dinophysis* blooms in the Gulf of Mexico (Campbell et al., 2010), and other pelagic HABs triggered by extreme climate events (Trainer et al., 2019). HABs in lakes and drinking water reservoirs such as *Prymnesium parvum* and the cyanobacteria *Microcystis* and *Cylindrospermopsis* are also thought to be exacerbated by climate change and represent a growing concern for public safety (Antunes et al., 2015; Paerl and Paul, 2012; Patino et al., 2014).

HAB events are expected to increase over this century with the projected increase in anthropogenic $CO_2$, warming, and changes in weather patterns. For example, model results predict a lengthened bloom season for the toxic dinoflagellate *Alexandrium* in Puget Sound (Moore et al., 2015), based on the temperature growth curves for *Alexandrium* isolates (Bill et al., 2016) and predicted warming in the region. Golden algae (*Prymnesium parvum*) blooms are also expected to increase in frequency in Texas reservoirs due to drier conditions and fewer flushing events (Roelke et al., 2012). Harmful blooms of cyanobacteria (CyanoHABs) are predicted to increase globally due to anthropogenic warming, rising $CO_2$, and longer residence times of surface waters in lakes (Michalak et al., 2013, Burford et al., 2019) because the growth kinetics of cyanobacteria can be limited by temperature and carbon at modern ambient levels (Visser et al., 2016). These predictions are increasingly informed by fundamental physiological data from lab culture studies (Table 1) that predict an increase in growth rate and/or toxicity for many HAB species under future warming and elevated $CO_2$ climate scenarios.

Predicting how HABs will respond to climate change is complicated by species or even strain level differences, interactive effects of stressors that reduce growth (*e.g.* nutrient limitation), and adaptation. Studies may disagree on the magnitude or even the direction of the growth rate response of HAB genera to climate change perturbations such as $CO_2$ (Raven and Gobler, 2019), for example *Alexandrium* species have been shown to either significantly increase growth rates (Hattenrath-Lehmann et al., 2015), significantly decrease growth rates (Hennon et al., 2017), or remain unchanged (Van de Waal et al., 2014) under elevated $CO_2$. This could be due to different species responses or methodological differences between studies, but it points to the importance of surveying a range of HAB species and strains under conditions that mimic the field as closely as possible. Other studies have revealed that stressors, such as nutrient limitation, in combination with perturbations like elevated $CO_2$, can interact to increase toxicity in some HAB species (Fu et al., 2010; Sun et al., 2011; Tatters et al., 2012a), pointing to the importance of so-called multiple stressor experiments (Boyd et al., 2015, Griffith and Gobler, 2019). Microcosm experiments have revealed both species succession and increased toxicity in complex communities with warming (Kleinteich et al., 2012) and multiple stressors (Tatters et al., 2018). Long-term culturing experiments under warming and elevated $CO_2$ have revealed that evolution can also play a role in growth rate responses and the toxicity of HABs (Flores-Moya et al., 2012). All these experiments demonstrate the importance of considering a range of environmental perturbations, stressors, community responses, and evolutionary rates in predicting HAB species responses to climate change. The overall conclusion from the multitude of climate change studies with HABs (Table 1) is that, despite the variability, many HAB species have increased growth rates or toxicity production in response to climate change perturbations, in agreement with the general trend of increasing HAB severity across systems.

## 2. Omics of HAB species

A number of studies have combined physiological measurements with targeted gene expression studies to reveal potential mechanisms of HAB acclimation to climate change perturbations. For example, responses to elevated $CO_2$ include shifts from high to low affinity bicarbonate transporters in *Microcystis aeruginosa* (Sandrini et al., 2015), changes in toxin biochemistry in *Alexandrium tamarense* (Van de Waal et al., 2014) and decreased expression of putative carbon concentrating mechanism genes in *Prorocentrum minimum* and *Alexandrium monilatum* (Hennon et al., 2017) that underpin the physiological response to rising $CO_2$. Understanding the genetically-determined mechanisms of HAB responses to climate change perturbations will be crucial for predicting the responses of species whose full responses cannot be characterized in the lab due to difficulty obtaining cultures or due to constraints on research time and resources. The promise of omic-based techniques is that the underlying responses to environmental perturbations are thought to be predictable based on gene complement and the resulting cell metabolic networks. Non-targeted molecular studies for HAB species have only recently become possible due to the rapid expansion of genomic, transcriptomic, proteomic and metabolomics techniques. Here we review representative studies for each omics technique and provide a selection of the data resources available for HAB genera. Since the year 2000, and particularly in the last decade 2008–2018, there has been a rapid increase in the approximate number of omics data sets for the major HAB phyla (Fig. 1, Supplemental Table 1). Sequencing technology has advanced to drastically reduce the cost per base over the past ten years (Wetterstrand, 2018), allowing a large increase in the number of genomes (Fig. 1A) and transcriptomes (Fig. 1B).

### 2.1. Genomics

Genome sequencing of HAB species has been dominated by cyanobacteria, due to their smaller genome sizes. The Joint Genome Institute sponsored a project to increase the coverage of cyanobacteria diversity as part of the Genomic Encyclopedia of Bacteria and Archaea (CyanoGEBA) leading to a large increase in available sequences (Shih et al., 2013). The findings from genomes of the toxic cyanobacteria *Microcystis aeruginosa* have been extensively reviewed (Harke et al., 2016b), there are also a growing number of genomes for additional CyanoHABs such as *Cylindrospermopsis* (Sinha et al., 2014; Stucken et al., 2010) and *Nodularia* (Popin et al., 2016; Voß et al., 2013). Many draft cyanobacterial genomes from other toxic genera are also publicly available (Supplemental Table 1). These genomes are instrumental for the identification and distribution of toxin-related genes (Pearson et al., 2016), comparative studies (Humbert et al., 2013), and of course to facilitate the analysis of other omic data that relies on having a sequence database for analysis.

Relative to the CyanoHABs, there are few whole genomes for the eukaryotic HABs. Genomic reference data, even if incomplete, can serve as an important database for downstream omic analyses, and highlight aspects of HAB biology such as genome organization and toxin production. Genome surveys for selected HAB genera like the dinoflagellates *Alexandrium*, *Prorocentrum* and *Heterocapsa* (Jaeckisch et al., 2011; McEwan et al., 2008; Ponmani et al., 2016), and the haptophyte *Chrysochromulina* (John et al., 2010) produced libraries of genomic sequences that served as references for understanding eukaryotic HAB toxins and genome structures. The initial work on dinoflagellate genomes revealed massive genome sizes (up to 245 Gb) with many recombinations, repeats, and a high percentage of non-coding sequence (Lin, 2011; Wisecaver and Hackett, 2011), making closing a full genome for dinoflagellates currently impractical (see Box 1). Other eukaryotes have proved more tractable, if still quite complex, a draft genome for the toxic diatom *Pseudo-nitzschia multiseries* is publicly available, yet unpublished (Supplemental Table 1). The *Pseudo-nitzschia* genome is also relatively large and repetitive. However, using the genome sequence in concert with gene expression data, the genes for synthesis of the domoic acid toxin were recently elucidated (Brunson et al., 2018), providing new avenues for studying toxicity in the

**Table 1**
Overview of examples of harmful algal bloom species responses to climate change stressors. Responses of HAB species to climate change conditions including warming and elevated $CO_2$ (abbreviated as $CO_2$) in concert with other factors such as nutrient limitation, and various light levels and salinities. The species responses in terms of growth and toxicity are characterized as increasing (+), decreasing (-), not measured (nm) or not applicable (NA) in response to climate change conditions of warming and/or elevated $CO_2$. Other responses are briefly described along with the citations for each study.

| Species | Climate change conditions | growth | toxicity | other responses | citations |
|---|---|---|---|---|---|
| *Pseudo-nitzschia spp.* | warming | + | + | lower cell yield | (Lewis et al., 1993) |
| | $CO_2$ | + | – | altered $CO_2$ uptake kinetics | (Lundholm et al., 2004; Trimborn et al., 2008) |
| | warming, $CO_2$ | + | nm | warming and $CO_2$ promoted growth | (Zhu et al., 2017) |
| | $CO_2$ and nutrient limitation | + | + | nutrient limitation and elevated $CO_2$ synergistically increase DA production | (Sun et al., 2011; Tatters et al., 2012a,b) |
| *mixed community* | $CO_2$, warming, nutrient limitation | nm | + | shifted community composition | (Tatters et al., 2018) |
| *Chattonella spp.* | warming, salinity and nutrients | + | NA | | (Zhang et al., 2006) |
| | warming, salinity, light | + | NA | | (Yamaguchi et al., 2010) |
| *Heterosigma akashiwo* | $CO_2$ | + | NA | increased down swimming | (Hennon et al., 2019; Kim et al., 2013; Xu et al., 2010) |
| | warming, $CO_2$ | + | NA | | (Fu et al., 2008) |
| | warming, salinity and nutrients | + | NA | | (Zhang et al., 2006) |
| *Phaeocystis spp.* | $CO_2$ | ns | NA | shift in elemental composition and increased colony formation | (Wang et al., 2010) |
| | $CO_2$ and light | – | NA | high light and $CO_2$ reduced growth | (Hoogstraten et al., 2012) |
| | warming, $CO_2$ | – | NA | | (Zhu et al., 2017) |
| *Prymnesium parvum* | $CO_2$ | +, - | – | reduction in toxin potency | (Lysgaard et al., 2018; Prosser et al., 2012; Ulitzur and Shilo, 1964; Valenti et al., 2010) |
| | warming | +,- | – | unimodal temperature max in growth | (Baker et al., 2007; Larsen and Bryant, 1998) |
| | salinity and nutrient limitation | – | + | | (Padilla, 1970) |
| *Chrysochromulina polylepis* | $CO_2$ | + | NA | increased CCM gene expression | (Hennon et al., 2017) |
| *Alexandrium spp.* | $CO_2$ | +,- | +,- | decreased CCM gene expression | (Hattenrath-Lehmann et al., 2015; Hennon et al., 2017; Van de Waal et al., 2014) |
| | warming, salinity | + | nm | | (Bill et al., 2016) |
| | $CO_2$ and warming | + | + | slower growing strains had most growth stimulation | (Kremp et al., 2012; Tatters et al., 2013) |
| | $CO_2$, warming, | + | +, - | strains evolved to grow faster under future warming and $CO_2$ conditions, but toxicity varied | (Flores-Moya et al., 2012) |
| *Dinophysis acuminata* | warming | + | + | | (Kamiyama et al., 2010) |
| *Gambierdiscus spp.* | warming, salinity, light | +,- | nm | unimodal growth rate responses suggesting a range of locally adapted genotypes | (Kibler et al., 2012; Xu et al., 2016) |
| *Heterocapsa circularisquama* | warming, salinity | + | nm | | (Yamaguchi et al., 1997) |
| *Karenia brevis* | warming, salinity, light | +,- | nm | | (Magana and Villareal, 2006) |
| *Karlodinium veneficum* | $CO_2$ and nutrient limitation | + | + | $CO_2$ and nutrient limitation interactions increased toxicity | (Fu et al., 2010) |
| *Prorocentrum minimum* | $CO_2$ | – | nm | decreased CCM gene expression | (Hennon et al., 2017) |
| | warming, $CO_2$ | ns | nm | | (Fu et al., 2008) |
| *Microcystis aeruginosa* | $CO_2$ | + | + | shift from high to low afinity bicarbonate uptake | (Sandrini et al., 2015) |
| | $CO_2$ and nutrient limitation | nm | ns | light limitation stimulated toxicity | (Van De Waal et al., 2009) |
| *Cylindrospermopsis raciborskii* | warming | + | nm | wide range of temperature tolerance among strains | (Chonudomkul et al., 2004) |
| *mixed community* | warming | nm | + | shift in community composition with warming | (Kleinteich et al., 2012) |

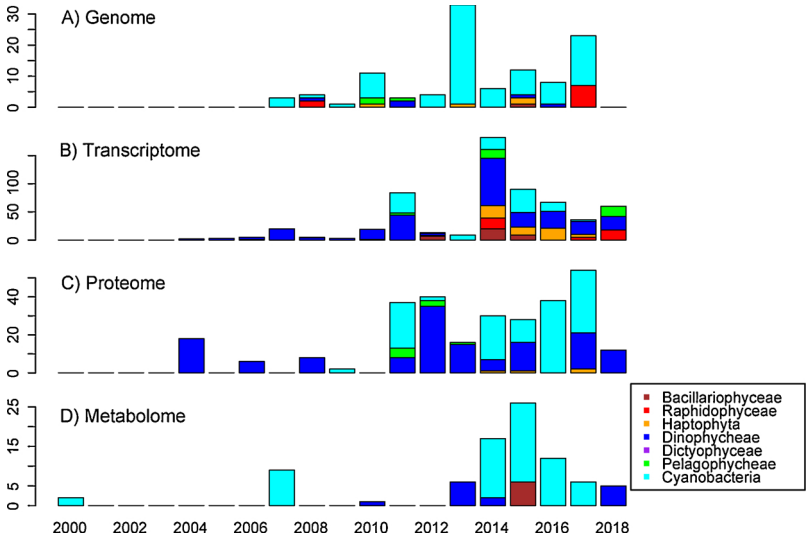


**Fig. 1.** Survey of published and publicly available omics datasets for harmful algal bloom genera.
Approximate number of published and publicly available data sets for HAB genera in the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al., 2009) by year for genomes (A), transcriptomes (B), proteomes (C), and metabolomes (D). Data sets were identified in part by searching Google Scholar for each genus name in combination with each of the keywords 'genome', 'transcriptome', 'proteome', and 'metabolome'. Colors indicate the phyla of each species according to the legend. See Supplemental Table 1 for citations or accession information.

environment and how toxicity might be modulated in the future ocean.

The pelagophyte, *Aureococcus anophagefferens* was the first HAB species with a whole genome sequence (Gobler et al., 2011). The availability of the genome sequence not only enabled many subsequent omic papers (Frischkorn et al., 2014; Gobler et al., 2013; Wurch et al., 2011b), but the genome content also provided a mechanistic view of how this species is able to reach bloom densities in the anthropogenically modified estuaries where it occurs (Gobler and Sunda, 2012). Plastid genomes are also available for select HAB genera such as *Karlodinium* (Gabrielsen et al., 2011), *Heterosigma* (Cattolico et al., 2008; Seoane et al., 2017), *Aureoumbra and Aureococcus* (Ong et al., 2010). Plastid genomes are smaller and more tractable to sequence than nuclear genomes, providing a glimpse into the complex evolutionary history of groups such as the dinoflagellates (Gabrielsen et al., 2011, see also Box 1). Additional genome sequences for HAB species would facilitate future analyses, but due to the large size of eukaryotic HAB genomes, particularly for the dinoflagellates (Box 1), recent community sequencing efforts have largely focused on the set of expressed genes (Fig. 1B) rather than full genomes.

### 2.2. Transcriptomics

Transcriptional profiling has been the most utilized omics method for HABs over the past five years (Fig. 1B). This is in part due to the expansion of reference transcriptomes. The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) (Keeling et al., 2014) leveraged next-generation sequencing and de-novo assembly approaches, releasing hundreds of RNA sequences and assembled transcriptomes for eukaryotes including over 150 belonging to HAB genera in 2014. This database has been a valuable resource in exploring the diversity of genes within HAB species (Di Dato et al., 2015; Liu et al., 2016; Murray et al., 2012) as well as the effects of nutrient-limitation (Frischkorn et al., 2014; Haley et al., 2017; Harke et al., 2017) and elevated $CO_2$ (Hennon et al., 2019, 2017) on gene expression.

Initially, surveys of expressed sequence tags (ESTs) were generated with clone libraries for select HAB groups such as dinoflagellates (Bachvaroff et al., 2004; Hackett et al., 2005; Lidie et al., 2005), making it possible to study gene content, develop microarrays, and study expression dynamics. For example, microarray approaches have been

**Box 1**
Dinoflagellate genomes: a mystery wrapped in an enigma

Dinoflagellates produce a wide variety of toxins and exhibit flexible metabolisms (*e.g.*: mixotrophy) and complex behaviors (*e.g.*: kleptoplasty). Most HABs are caused by dinoflagellates, yet to date no nuclear genome has been sequenced for a free-living species of this group. This is because dinoflagellates have large genome sizes (3–245 Gb) with many atypical features (Wisecaver and Hackett, 2011; Lin, 2011), that defy the current evolutionary theory that free-living cells bear a substantial fitness cost for carrying excess genes (*e.g.*: Black Queen Hypothesis, Morris et al., 2012). In contrast, the plastid genomes of dinoflagellates are highly reduced, composed of minicircles (Howe et al., 2008), apparently the result of horizontal transfer of most genes to the nuclear genome where some are stored in tandem repeats (Zhang and Lin, 2003). Additionally, nuclear genes are highly multi-copy with some arranged in tandem repeats (Bachvaroff and Place, 2008). Extensive replication of nuclear gene families and horizontally transferred genes were also observed in the smaller genomes of symbiotic dinoflagellates (Aranda et al., 2016). Therefore, sequencing a free-living dinoflagellate genome would likely contain a large percentage of repetitive, recombined, and non-coding 'junk' sequence that would be computationally challenging to assemble from short reads, requiring longer read sequencing. These difficulties have so far been side-stepped by deeply sequencing and assembling the expressed genes (transcriptomes, Section 2.2). However, transcriptome reads differ substantially from genomic sequences due to mRNA editing in organelles (Mungpakdee et al., 2014) and spliced-leader sequences in nuclear genes (Zhang et al., 2007) and do not reveal total gene copy numbers or organization. New methods such as nanopore technology are facilitating rapid and inexpensive long-read sequencing (Venkatesan and Bashir, 2011) that could aid in scaffold assembly for dinoflagellate genomes. As sequencing technology and assembly tools improve, the first free-living dinoflagellate nuclear genome may soon be within reach. However, it seems likely that the genetic secrets of dinoflagellates will remain difficult to decode without an understanding of the role of 'junk' DNA, tandem repeats and mRNA splicing/editing in fitness. These difficult questions may require an expansion of evolutionary theory to accommodate single-celled organisms like dinoflagellaes with large genomes, in addition to advances in technology. Understanding the basis of dinoflagellate success and toxicity through genomics in the modern ocean will aid in predicting the future impacts of dinoflagellate HABs in a warmer, acidified ocean.

used to measure transcriptional changes in response to environmental perturbations in HABs such as the dinoflagellates *Karenia* (Morey et al., 2011; Van Dolah et al., 2007) and *Alexandrium* (Wohlrab et al., 2010; Yang et al., 2011), and the CyanoHAB *Microcystis* (Makower et al., 2015; Sandrini et al., 2015; Straub et al., 2011). Yet microarray approaches lack the sensitivity of RNA sequencing and rely on having an assembled transcriptome or genome as a reference to create the microarray chip. As sequencing technology and costs started to decline, tag based (MPSS, SAGE) sequencing approaches were applied to genera like *Alexandrium* (Erdner and Anderson, 2006; Moustafa et al., 2010) and *Aureococcus* (Wurch et al., 2011b) to identify how transcriptional patterns were modified as a function of limitation or changes in physiology. However, these approaches still largely relied on *a priori* knowledge of gene sequences for interpretation.

With further advances in sequencing and assembly approaches, and the reduction in sequencing costs, it is now possible to sequence short-reads from transcripts, and assess gene content and expression patterns at unprecedented resolution, moving from 10,000 sequence tags, to 60 M or more short-reads (Mardis, 2008). These next-generation sequencing methods have been used to explore the gene expression responses of the CyanoHAB *Microcystis* (Harke and Gobler, 2015, 2013) to nutrient limitation and profile diel responses during a toxic CyanoHAB event (Penn et al., 2014). Similar approaches were used to generate the MMETSP database, and such approaches have been used to track the gene expression of a growing set of HAB groups like diatoms, pelagophytes, raphidophytes, and prymnesiophytes among others: *Pseudo-nitschia* (Amin et al., 2015), *Aureococcus* (Frischkorn et al., 2014), *Heterosigma* (Haley et al., 2017) and *Chrysochromulina* (Hovde et al., 2015), as just a few examples. Transcriptome approaches have also been critical to studies of dinoflagellates, for example identifying the genes related to saxitoxin production in the genus *Alexandrium* (Stüken et al., 2011) and narrowing the gene candidates for production of ciguatoxins in *Gambierdiscus polynesiensis* (Pawlowiez et al., 2014). The widespread use of transcriptomics have led to insights such as spliced-leader sequences (Zhang et al., 2007) and limited transcriptional regulation in dinoflagellates (Lin, 2011; Morey and Van Dolah, 2013; Moustafa et al., 2010). Due to the importance of dinoflagellates, with nearly 100 recognized HAB species (Moestrup et al., 2009), and their limited transcriptional regulation, alternative methods for assessing the mechanisms of cell response to environmental stimuli have been explored. Such methods include suppression subtraction hybridization transcriptomics (Toulza et al., 2010; Zhang et al., 2014) to restrict analysis to genes that have substantial changes in expression.

Moving from cultured isolates to field studies of the community gene transcription, termed metatranscriptomics, is increasingly tractable and becoming a powerful tool to evaluate gene compliment and understand species responses in a natural community (Caron et al., 2017). Metatranscriptome studies have focused on assemblies and expression profiling of key groups in a mixed community, including diatom (Marchetti et al., 2012), pelagophyte (Wurch et al., 2019), raphidophyte (Ji et al., 2018), and dinoflagellate populations (Cooper et al., 2014; Gong et al., 2017; Lin et al., 2010). For example, metatranscriptome sequences used to assemble the expressed gene compliment of *Prorocentrum minimum*, showed that the gene catalog was similar to culture-derived assemblies but that the relative expression patterns differed, particularly for toxin biosynthesis (Cooper et al., 2014). Ultimately, the interpretation of next-generation sequencing techniques in the field is strengthened by the ever-expanding database of reference sequences like those produced in the MMETSP (Keeling et al., 2014). Transcriptomics have been criticized for measuring gene expression as a proxy for metabolism when the true engines of metabolism, proteins, are not necessarily correlated with changes in coding gene expression (Waldbauer et al., 2012). Regardless, transcriptomic approaches have, and continue to provide key information about the biology of HAB species and how they respond to a changing environment.

### 2.3. Proteomics

Proteomics is another important technique (e.g.: Chan et al., 2004; Zhang et al., 2018) that allows measurement of protein abundance as a proxy for enzymatic activity (Fig. 1C). Proteomics has lower resolution than transcriptomic approaches, where only a fraction of the transcripts can be related to the observed proteome (*e.g.*: Dyhrman et al., 2012; Wurch et al., 2011a). Proteomics also relies on either a draft genome or transcriptome assembly to assign peptide fragments to full protein sequences, so the technique is best utilized for a species that has been previously sequenced or are highly similar to a previously sequenced species. Early proteomics studies of HAB species were qualitative and unable to annotate proteins due to the lack of sequence data (Chan et al., 2006, 2004). With the increasing availability of sequence data peptides can now often be assigned *in silico* to translated proteome sequences (Zhang et al., 2018), allowing the authors to interpret changes in peptide abundances as a proxy for changes in abundance of proteins, some of which have functional annotations. Recent proteomics studies have leveraged the availability of sequence databases to identify how the proteomes of HAB genera like *Mycrocystis* (Chen et al., 2017; Yue et al., 2015), *Prorocentrum*, *Alexandrium* (Wang et al., 2012, 2013), *Aureoumbra* (Sun et al., 2012) and *Aureococcus* (Wurch et al., 2011a) are modulated by changes in physiology and in response to changes in the environment. Utilizing multiple omics techniques in the study of HABs can increase the power to determine the underlying mechanism of cell response and reveal the limitations and strengths of each technique. For example, Steffen et al. (2014) utilized transcriptomics, proteomics and metabolomics to explore the response of *Microcystis* to nutrient-limitation, and found that transcription was sensitive to changes in nutrients, yet metabolites were maintained at relatively constant levels by cell homeostasis. Either technique measured alone, may have led to different conclusions, but when omics techniques were combined it became clear that the cell was capable of balancing metabolic fluxes in response to the environmental stimulus. Although there are still challenges in identifying proteins in a species-specific manner from a mixed community, proteomics, especially when applied with other omic approaches offers a path forward for understanding how HAB species will respond to climate-driven changes in their environment.

### 2.4. Metabolomics

Metabolomics are growing in popularity as an omics technique (Fig. 1D), with uses in characterizing commercially-important or toxic metabolites (Briand et al., 2016) among others. The metabolites measured are highly dependent on the study methodology and analytical techniques, so the metabolome is not as directly comparable between studies as it would be with sequencing methods. Metabolomics cannot reflect fluxes of metabolites, but measures of standing stocks can reveal important environmental signals governing interactions between bacteria and HAB species (Amin et al., 2015) or allelopathic interactions with competing algae (Poulin et al., 2018; Song et al., 2017), as just a few examples. A combination of metabolomics and proteomics was used to determine the impact of *Karenia brevis* allelopathy on two competing species of diatoms; the more naïve competing species *Thalassiosira pseudonana*, had a drastically reduced growth rate in co-culture with *K. brevis* and exhibited major shifts in cell metabolome and proteomes indicating a decrease in photosynthesis and an increase in oxidative stress (Poulson-Ellestad et al., 2014). Metabolomics can also be used to evaluate cellular responses to changing environments, evaluating osmotic stress, and nutrient limitation scenarios. For example, targeted metabolomics aided in the interpretation of proteomic and transcriptome data (Wurch et al., 2011a, 2011b), showing that *Aureococcus* strips phosphate from intracellular nucleotides under phosphate limitation (Kujawinski et al., 2017). This ability may help maintain phosphate for key functions in an environment of fluctuating

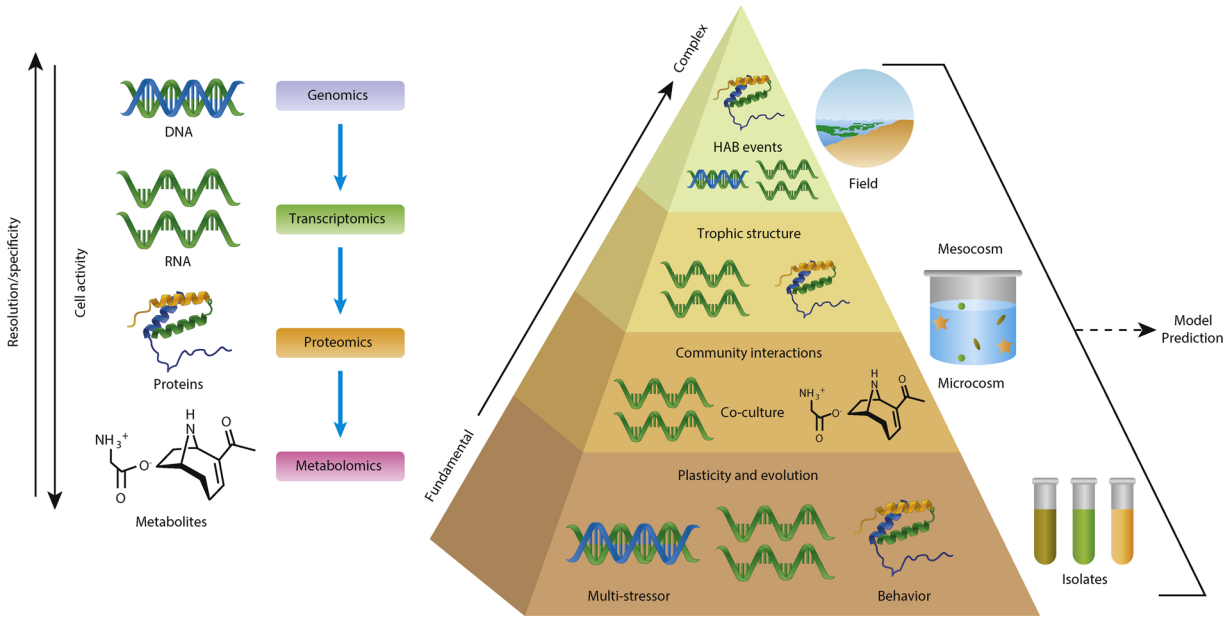


**Fig. 2.** Hierarchy of complexity in climate change experiments on HAB species.
Conceptual diagram of experimental and field approaches for measuring impacts of climate change and the role of omics in disentangling HAB species responses.

phosphorus supply. It is likely that the trend of increasing numbers of omics studies will continue with great potential for expansion of metabolomics data sets from both the lab and field, necessitating the creation of new databases and analysis tools (Longnecker et al., 2015). These trends suggest that standardized techniques for both proteomics and metabolomics should be developed and public databases should be created to store data similar to sequencing databases to facilitate meta-analysis of omics data.

## 3. Integrating climate omics studies for HAB species

Experimental designs for using omics techniques to predict the impacts of climate change can be organized in a hierarchy of increasing complexity, with fundamental studies of single species forming the basis of interpretation for more complex communities, ranging from co-cultures to mesocosms and field studies (Fig. 2). Similarly, omics techniques range in specificity and sensitivity from highly specific and sensitive techniques like genomics and transcriptomics to lower resolution techniques such as proteomics and metabolomics which are more proximate to cell metabolic activity (Fig. 2). Integrating multiple omic methods and studies at many levels of community complexity will best facilitate predictive modeling.

### *3.1. Integrating omic studies of isolates to interpret responses to environmental change*

Omic approaches are increasingly being integrated into the fundamental studies with HAB species isolates that are crucial for interpretation of higher complexity systems in the context of a changing environment. For example, genome sequencing of *Microcystis aeruginosa* (Kaneko et al., 2007) and *Planktothrix agardhii* (Christiansen et al., 2014; Tooming-Klunderud et al., 2013), made it possible for meta-transcriptome sequence analysis to uncover the nutrient-driven niche separation of CyanoHABs in Lake Erie (Harke et al., 2016a). If nutrient changes select for certain strains of HAB species then this could have large scale ramifications for predicting the dynamics of these species with increasing eutrophication or climate-driven changes in nutrient supply (Riebesell et al., 2009). Studies of CyanoHAB metagenomes and metatranscriptomes in Lake Erie also revealed extensive horizontal gene transfer among *Microcystis* sp. (Meyer et al., 2017), and thus provide insight on how resilient these populations might be to environmental change (Polz et al., 2013). Gobler et al. (2011) sequenced the first partial genome of a eukaryotic HAB species, *Aureococcus anophagefferens*, and with this resource was able to interpret metaproteome data from a brown tide HAB event in Long Island Sound. Subsequent work investigating the transcriptome and proteome responses of *A. anophagefferens* to nutrient and light limitation aided in understanding how these drivers might influence the bloom dynamics of this species both now and with potential changes in light and resources in the future (Frischkorn et al., 2014; Wurch et al., 2011a). More recent work has also focused on omic responses to increases in $CO_2$ for selected HAB taxa (Hennon et al., 2017; Sandrini et al., 2015). Carbon concentrating mechanisms have been shown to respond in a species-specific manner, lacking commonality in the $CO_2$-responsive gene families or even direction of expression change in response to elevated $CO_2$ within the same phyla (Hennon et al., 2017). These results indicate that more studies are required to characterize the response of HAB species to rising $CO_2$, as genetic mechanisms are not conserved at the phylum level. Such fundamental experiments provide valuable context for the subsequent analysis of complex communities, and provide insight into how HAB species may respond to climate-driven changes in the environment.

Other critical types of studies include so-called multi-stressor experiments. Multi-stressor experiments have shown that phytoplankton physiology can be altered in ways that are difficult to predict due to significant interactions between predicted future environmental conditions such as warmer temperatures, nutrient limitation and elevated $CO_2$ (Boyd et al., 2015). Physiology experiments measuring the domoic acid (DA) toxicity of the diatom *Pseudo-nitzschia* have found an interaction between $CO_2$ and nutrient limitation (Sun et al., 2011; Tatters et al., 2012a) that explains the finding of increased DA in a natural community of phytoplankton with nutrient limitation and elevated $CO_2$ (Tatters et al., 2018). Now that the genetic basis of DA production has been elucidated (Brunson et al., 2018), omics techniques may be used to assess the probability of DA toxicity events in the field. Omics approaches show useful promise in understanding the mechanistic drivers of multi-stressor experiments in cyanobacteria like *Trichodesmium* (Walworth et al., 2016), and expanding these types of approaches for both eukaryotic species and the CyanoHABs is warranted.

HAB species behavior is another fundamental variable that can

contribute to bloom formation, via changes in swimming rate, directionality, or buoyancy. There are only a few studies focused on how HAB species behavior may change with climate-driven variables like increased $CO_2$. For example, Kim et al. (2013), showed enhanced downswimming behavior of ichthyotoxic raphidophyte *Heterosigma akashiwo* with elevated $CO_2$, which could have important impacts on bloom formation and forecasts of future bloom dynamics for this species. The expression of motility genes were found to be significantly correlated with $CO_2$ in a transcriptomics experiment with *H. akashiwo* (Hennon et al., 2019), and these genes could serve as biomarkers of behavior to aid interpretation of metatranscriptomes collected from *H. akashiwo* blooms (Ji et al., 2018). As another example, changes in *Microcystis* cell composition associated with elevated $CO_2$ have been hypothesized to increase buoyancy and thereby enhance harmful bloom formation potential (Sandrini et al., 2015). In brief, omic approaches can identify how behaviors, like swimming and buoyancy, are modulated in culture and help to develop the tools to screen field populations and to define the parameters needed to develop more highly resolved HAB predictions.

Changes in relative fitness have been measured in phytoplankton species maintained for hundreds of generations under future climate scenarios (Collins and Bell, 2004; Hutchins et al., 2015; Lohbeck et al., 2012) and will likely be an important parameter to constrain for predicting the relative fitness of HAB species. Flores-Moya et al. (2012) found growth rates increased after 150–200 generations for two strains of *Alexandrium minutum* grown under elevated temperature and $CO_2$, yet the toxicity became more variable within evolved lines indicating that while the cells adapted to maximize growth, the toxicity was subject to random drift. The $N_2$-fixing cyanobacterium, *Trichodesmium,* has been shown to increase rates of $N_2$-fixation after hundreds of generations of elevated $CO_2$ exposure, possibly as a result of epigenetic modifications (Hutchins et al., 2015), although these results have recently been questioned as a potential artifact of pH changes in the media (Hong et al., 2017). Understanding the impacts of $CO_2$ on the physiology and genetic response of cyanobacteria such as *Trichodesmium* could have important implications for $N_2$-fixing CyanoHABs. Transcriptome and epigenome sequencing could help disentangle how epigenetic modifications impact gene expression and acclimation to environmental variables in cyanobacteria (Walworth et al., 2017). *Alexandrium* cysts can be preserved for hundreds of years in sediments revealing correlations between cyst abundance and ocean warming (Feifel et al., 2012), these cysts could potentially be revived for physiological studies (Ribeiro et al., 2013) and sequenced providing a natural time series of adaptation to climate change. Physiological plasticity has also been shown to influence the trajectory of evolution in phytoplankton (Schaum and Collins, 2014), which has yet to be comprehensively explored in HAB species. Both omics and physiology experiments will be important to constrain the rates and range of adaptation and plasticity of HAB species to climate change.

### 3.2. Leveraging omic approaches to study interactions and mixed communities

Moving from fundamental studies of single strains towards more complex systems (Fig. 2), there is a growing recognition of the importance of microbial interactions in shaping the behavior and fitness of phytoplankton species. Allelopathy, in particular, has been a focus of research in HAB species as an evolutionary driver of toxin production and bloom formation (Graneli and Salomon, 2010; Prince et al., 2010; Song et al., 2017). Microbial interactions can change the competitive outcomes of evolution experiments for dinoflagellates (Tatters et al., 2012b), with some species having much more success in mixed cultures than would be predicted based upon growth rate alone. Mutualism may also play an important role in HAB species success, Amin et al. (2015) found that the growth rate of toxic diatom *Psuedo-nitzschia* was significantly enhanced in co-culture with a heterotrophic bacterium. HAB species have complex microbiomes with many possible interactions (Amin et al., 2012; Hattenrath-Lehmann and Gobler, 2017). Hennon et al. (2018) found that beneficial microbial interaction between a cosmopolitan cyanobacterium and heterotrophic bacterium could be significantly and rapidly altered by projected future $CO_2$, indicating that interactions between HAB species and their microbiomes may also be subject to disruption by climate change. Both metabolomics and transcriptomics were instrumental tools in these studies. Transcriptomics can provide species-specific information from a mixed community while metabolomics can provide clues about the metabolites that are exchanged between species and these and related omics approaches are likely to continue to be utilized in future investigations of microbial interactions focused on HAB genera.

HAB traits such as toxicity, metabolic potential, and even response to $CO_2$, frequently do not match the phylogenetic history of the group, because key functional genes can be lost or acquired through horizontal gene transfer (Humbert et al., 2013; Koid et al., 2014; Tooming-Klunderud et al., 2013). Recent work with transcriptomics and proteomics has focused on identifying signatures of trophic status (Liu et al., 2016; Shim et al., 2011), which are fundamental to understanding the tropic structure of complex communities. This is particularly important for organisms such as dinoflagellates; their degree of mixotrophy is plastic depending on prey availability or light levels (Liu et al., 2016). Signatures of trophic strategy could aid in understanding the complex dynamics of natural communities (Hu et al., 2018) and how HAB species persist in shifting environments.

With the growing availability of assembled genomes and transcriptomes, it is possible to quantify the copy number of key functional genes that have the potential to respond to elevated $CO_2$ such as the carbon concentration and photorespiration genes across multiple HAB phyla (Hennon et al., 2017, Fig. 3). With more research this method termed 'functional gene fingerprinting', could serve as a proxy for traits, reducing the experimental space to a few major 'functional groups' of algae, which share the same functional responses to rising $CO_2$ (Fig. 3). This tool could focus the experimental effort on species with unique genetic signatures and reduce the effort spent on species that have similar functional gene patterns as compared with well-studied organisms. Such functional gene signatures could be applied to a variety of gene types including heat shock factors to predict responses to changes in temperature or genes that could indicate the potential for mixotrophy or motility. Functional gene fingerprinting methods could also be applied to uncultured organisms in the field to aid in trait-based categorization of natural populations. Such an approach is attractive because it leverages the vast omics databases to improve ecosystem prediction and avoids the phylogenetic approaches that fail to identify important trait differences and improves upon the subjective classifications of many current trait-based and functional group approaches.

The ultimate goal of these experimental approaches is to leverage omics tools to understand how to predict how climate change will either promote or disfavor HABs (Fig. 2). This requires a fundamental understanding of both HAB species and their relative competitive fitness in the microbial community. Isolate studies with genomics, transcriptomics and proteomics are a powerful approach for understanding species responses to climate change variables, yet given the incredible diversity of aquatic microbes we cannot hope to perform every possible experiment. In this context, omics approaches can help with field observations of mixed communities. Omics techniques can help to resolve species-specific responses and to uncover fundamental connections between genes and organism responses to climate change stressors. Such mechanistic knowledge could reduce the number of experiments needed to characterize how key groups of phytoplankton such as HABs respond to future conditions.

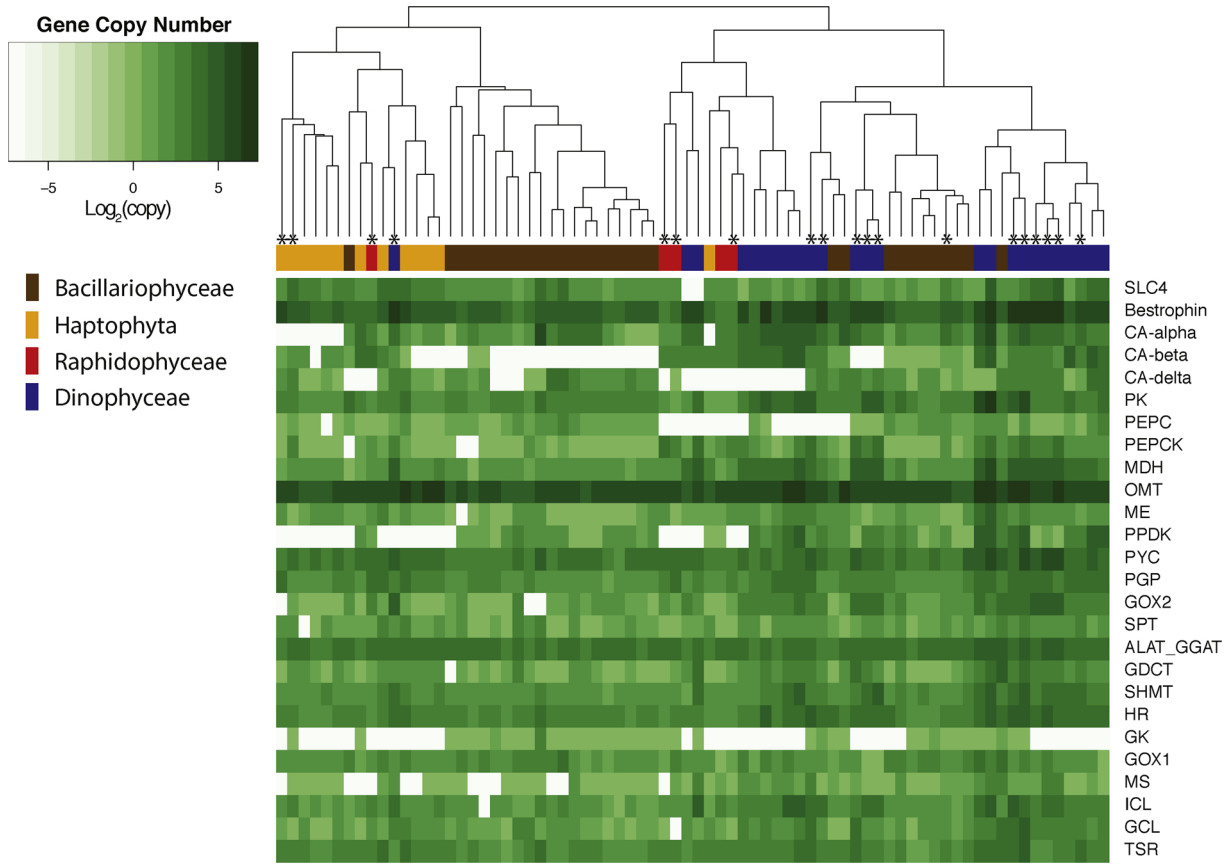


**Fig. 3.** $CO_2$-responsive functional gene 'fingerprint' of four HAB phyla.
Hierarchical clustering by carbon concentration and photorespiration gene abundance reveals functional groups of phytoplankton that share similar expressed gene complements for responding to elevated $CO_2$. Color bar indicates gene copy number in assembled consensus transcriptomes, Bacillariophyceae in brown, Haptophyta in orange, Raphidophyceae in red and Dinophyceae in blue, asterisks indicate species with potential for HAB formation (data from Hennon et al., 2017). Abbreviations for putative carbon concentration mechanism genes: SLC4, SLC-4 bicarbonate transporter; Bestrophin, bestrophin-like gated ion channel; CA, carbonic anhydrase; PK, pyruvate kinase; PEPC, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; MDH, malate dehydrogenase; OMT, oxoglutarate/malate transporter; ME, malic enzyme; PPDK, pyruvate orthophosphate dikinase; PYC, pyruvate carboxylase. Abbreviations for putative photorespiration genes: PGP, 2-phosphoglycolate phosphatase; GOX, glycolate oxidase; SPT, serine-pyruvate aminotransferase; ALAT_GGAT, alanine aminotransferase; GDCT, glycine decarboxylase; SHMT, serine hydroxymethyl transferase; HR, hydroxypyruvate reductase; GK, glycerate kinase; MS, malate synthase; ICL, isocitrate lyase; GCL, glyoxylate carboligase; TSR, tartronate semialdehyde reductase.

## 4. Potential approaches for incorporating omics into predictive models for HABs

One of the major challenges in the context of studying HABs is incorporating the wealth of omic data into ecosystem-level predictions. Arguably, the next frontier in HAB climate prediction is to incorporate information about cell mechanisms, evolution and interactions, such as those highlighted above, into a global or regional framework. We propose that several existing modeling frameworks can be augmented by omics information from various experimental methods (Fig. 4). For example, cell mechanism models can be created from transcriptome or proteome data for a few well-studied model phytoplankton using systems biology (Ashworth et al., 2013; Hennon et al., 2015) or flux-balance analysis techniques (Levering et al., 2017). Cell models could be created for well-studied HAB species based on the omics and physiology data from climate projection multi-stressor experiments (Table 1, Fig. 4).

These cell mechanistic models could be incorporated into regional HAB species models (Fig. 4) similar to the model created to forecast *Alexandrium* abundance in the Gulf of Maine (McGillicuddy et al., 2011). Regional models take into account fine-scale seasonal forcings, physical transport and inter-annual variability. For example, behavioral data such as changes in swimming for motile organisms with increasing $CO_2$ (Kim et al., 2013) could be incorporated into these regional models as it would likely have a large impact on HAB formation. Similarly, organismal plasticity has been shown to impact nitrogen to phosphorus ratios of macromolecules with rising temperature (Toseland et al., 2013) and elemental ratios and metabolic rates under elevated $CO_2$ (Hennon et al., 2014), leading to potential differences in nutrient requirements in a warming and acidifying environment that could be taken into account in regional HAB models. Longer-term prediction will require regional models to consider evolution rates such as improvements in growth rate measured for *Alexandrium minutum* under increased temperature and $CO_2$ (Flores-Moya et al., 2012). Microbial interactions such as allelopathy can favor toxic species in a competitive ecosystem (Tatters et al., 2012b) or promote HAB growth via mutualism (Amin et al., 2015). Physiological traits can also be rapidly transferred between species due to horizontal gene transfer, for example in *Microcystis* sp. (Meyer et al., 2017). Integrating physiology and omics data could improve the resolution of regional models for future climate forcings on HAB events (Fig. 4).

Functional gene fingerprinting (*e.g.*: Fig. 3) could be used to assign or extrapolate traits to HAB species that are uncultured or lack detailed physiological data. Trait-based approaches can simplify complex community dynamics into tractable functional groups that are defined by common traits (Litchman and Klausmeier, 2008). The contribution of omics data sets would be to create data-driven functional groups that recognize distinct physiological types independent of phytoplankton

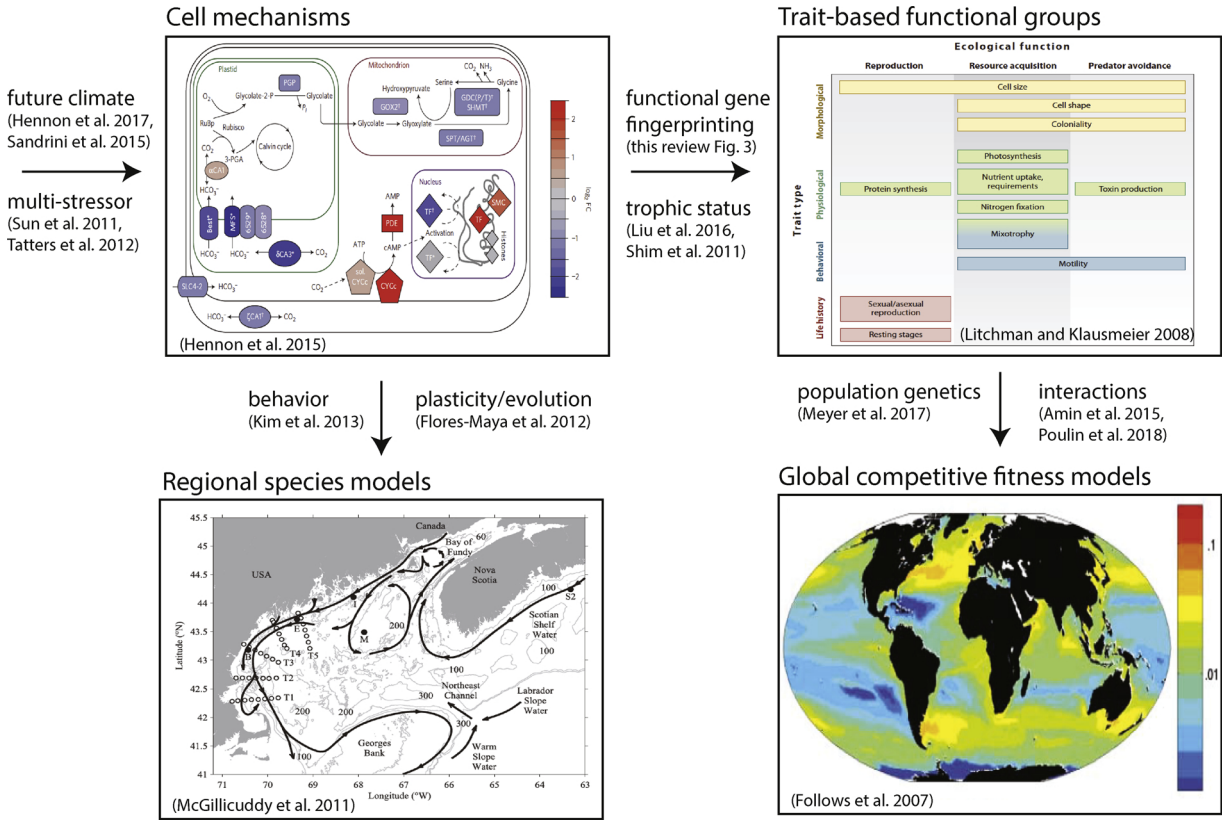


**Fig. 4.** Integrating climate-related omics data into HAB models.
Potential routes for incorporating omics data into existing modeling frameworks. Arrows indicate how omics data from climate change and other single isolate experiments (text) could be integrated into existing modeling frameworks (images) to improve modeling products related to HABs in a future ocean. Citations provide examples of datasets that could be leveraged for modeling and are not intended to be comprehensive.

phylogeny (Fig. 4). For example, such a functional characterization could be based on the distribution of genes involved in $CO_2$-responses (Fig. 3). Newly-defined functional groups could then be incorporated into existing global ecological models such as the Darwin Project (Follows et al., 2007) to predict the impacts of climate change on the global distributions of HAB species.

Global-scale modeling will likely prove particularly important for marine HABs, as observed changes in ocean temperature have already caused large scale poleward shifts and changes in phytoplankton community structure (Barton et al., 2016) and predicted changes in phytoplankton communities with elevated $CO_2$ are highly dependent on interspecies competition (Dutkiewicz et al., 2015). To get the predictions correct it is important to not only consider the impact of climate change on HAB species, but also on other key functional groups such as coccolithophores (Schlüter et al., 2014) and diazotrophs (Hutchins et al., 2015) to determine the changes in competitive fitness landscape for HABs. Interactions of major functional groups such as cyanobacteria with heterotrophic bacteria (Braakman et al., 2017; Hennon et al., 2018) may also greatly influence the fitness landscape for HABs. Incorporating information from omics studies about species trophic status such as capacity for mixotrophy or parasitism (Worden et al., 2015) could also prove important in predicting the phytoplankton community structure in these global competitive fitness models (Fig. 4). The role of omics would be to reduce the complexity of these many biological interactions and trophic behaviors into a few select functional responses defined by functional gene fingerprinting to make them tractable for computationally expensive global modeling.

Both global and regional models augmented by omics will provide new testable hypotheses focused on how environmental change impacts community structure. These hypotheses could be tested by measuring omics in field studies of natural communities (Alexander et al., 2015a) and HAB events (Gobler et al., 2011; Meyer et al., 2017) in an iterative process to improve model performance. Recent work by Coles et al. (2017) pioneered this approach by using field data to parameterize how biogeochemistry shapes metagenomes and metatranscriptomes in the tropical Atlantic. Many metatranscriptome datasets already exist from important ocean regions (Alexander et al., 2015b; Dupont et al., 2015; Marchetti et al., 2012) and modeling frameworks are now poised to incorporate these omics datasets (Stec et al., 2017). Predicting the impacts of climate change on HAB frequency and severity remains an important goal for managing wildlife, food and water security. By leveraging omics datasets with cutting-edge numerical modeling techniques, we may be able to advance both seasonal and longer term prediction capabilities.

## 5. Conclusions

Harmful algal blooms are a growing global concern that studies suggest are likely to be exacerbated by climate change in many regions. Many genome, transcriptome, proteome and metabolome datasets are now available for HAB species due to advances in technology. These omics datasets are complementary, for example genomes and transcriptomes allow peptides to be identified in proteomics. Therefore, the power of omics data sets increases non-linearly, creating many opportunities for synthesis and meta-analysis. Systems biology and functional gene fingerprinting may enhance our ability to understand how changes in gene expression or complement impact functional traits in HAB species. Promising avenues exist for integration of omics studies into numerical modeling, which will be essential in improving prediction of HAB events in the face of climate change.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hal.2019.03.005.

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