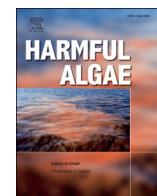




Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Revisiting chlorophyll *a* thresholds for San Francisco Bay: insights from observations of phytoplankton molecular abundance

Daniel Killam^{a,*}, Keith Bouma-Gregson^b, Martha Sutula^c, Raphe Kudela^d, James Hagy^e, Stephanie Anderson^e, David Senn^a

^a San Francisco Estuary Institute, 4911 Central Avenue, Richmond, CA 94804, United States

^b U.S. Geological Survey, California Water Science Center, 6000J Street Placer Hall, Sacramento, CA 95819, United States

^c Southern California Coastal Water Research Project, 3535 Harbor Blvd Ste. 110, Costa Mesa, CA 92626, United States

^d University of California, Santa Cruz, 1156 High St, Santa Cruz, CA 95064, United States

^e Environmental Protection Agency, 27 Tarzwell Dr, Narragansett, RI 02882, United States

ARTICLE INFO

Keywords:

Phytoplankton

Alexandrium

Saxitoxin

Heterosigma

Mytilus

Chlorophyll-*a*

Conditional probability

ABSTRACT

Harmful Algal Blooms (HABs) are a hazard for coastal environments worldwide; identifying screening thresholds of chlorophyll-*a* (chl-*a*) associated with increased risk of HABs is a management priority. Molecular surveillance of coastal phytoplankton and bivalve biotoxins could be used to link chl-*a* with HAB risk, but requires an understanding of whether the HAB risks increase uniformly as chl-*a* rises, or whether some taxa are disproportionately favored, and if these relationships vary by season. In this study, we present a novel use of molecular abundance data to investigate the scientific bases for estuarine chl-*a* thresholds protective against HABs. In San Francisco Bay (SFB), California, the relationship between molecular relative abundance (as measured by 18S metabarcoding) of nine different HAB taxa, absolute quantitative polymerase chain reaction (qPCR) abundance, and mussel toxin concentrations of a subset of the taxa were investigated for thresholds as a function of increasing chl-*a*. Our results show most HAB taxa did not increase in absolute or relative abundance during SFB's spring bloom interval, when chl-*a* levels were highest (>10 µg/L) but the assemblage was dominated by non-harmful diatoms. However, several flagellated, mixotrophic taxa did increase above their molecular baseline in fall, and the combined probability of any HAB occurring above baseline was elevated when chl-*a* reached ~4.6 µg/L in the fall. This work demonstrates the promise of molecular approaches in disentangling the seasonally complex interplay between stressors and phytoplankton/HAB community responses and has the potential to provide clearer, more cost-effective monitoring and mitigation strategies for managers.

1. Introduction

1.1. The need for threshold identification in harmful algae monitoring

Harmful Algal Blooms (HABs) are globally significant aquatic hazards impacting human and environmental health through the production of toxins, depletion of dissolved oxygen necessary for biota, and other hazards (Grattan et al., 2016; Sellner et al., 2003). Nutrient pollution and eutrophication are known to contribute to the severity and frequency of toxic HABs (Anderson et al., 2008; Heisler et al., 2008; Wurtsbaugh et al., 2019), and controlling eutrophication drivers is one of the primary long-term management strategies to reduce the risk of HAB occurrence. Management actions or strategies can be informed by

water quality goals or thresholds. “Thresholds” here are defined as disproportionately large changes in response variables relative to a small change in the environmental explanatory variable, resulting in conditions which could be of management concern. Examples of parameters used to set water quality targets include nutrient concentrations or loads, algal density, HAB abundance, or toxin concentrations (Batiuk et al., 2009; Tango and Batiuk, 2013). Establishing nutrient thresholds for each individual species can be challenging because of the complex ecological factors that shape phytoplankton community composition and HAB species-specific responses to nutrient gradients (Davidson et al., 2014). Therefore, the approach commonly taken in water quality management is to select biomass-related thresholds, such as chlorophyll *a* (chl-*a*), that are predictive of HAB abundance or toxins and use

* Corresponding author.

E-mail address: dank@sfei.org (D. Killam).

<https://doi.org/10.1016/j.hal.2026.103086>

Received 17 June 2025; Received in revised form 27 January 2026; Accepted 13 February 2026

Available online 14 February 2026

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numerical or statistical models to link these to nutrient concentrations or loads (Stevenson, 2014).

Vulnerability to toxin uptake, exposure and depuration varies among food web species. Indicator species are frequently fish and shellfish of commercial, recreational or subsistence importance. Risk evaluations have been conducted for crustaceans considering exposure to cyanotoxins (Garcia et al., 2010) and to limit toxin exposure for oysters, clam, and mussel harvests (Frolov et al., 2013; Trainer and Hardy, 2015). Mussels are abundant filter-feeders, having a multi-year life span, and therefore provide a suitable target for understanding space-time exposure to diverse toxins, accumulation of toxins relative to plankton dynamics in the system, and threshold responses in exposure risk.

Common approaches to criteria threshold derivation have included detecting differences from reference conditions (Gibson et al., 2000) or developing effects-based criteria (US EPA, 2017). Identification of change points has attracted prior research interest in terms of identifying nonlinear changes in biotic conditions which may not present ready visual evidence. Conditional probability analysis represents a possible statistical approach to relating the probability of one event to another event as a function of a continuous environmental variable measures (Sutula et al., 2017).

Past efforts to establish chl-*a* based numeric thresholds of HAB risk have often relied on the reasonable hypothesis of a “proportionate increase” or “all boats rise” paradigm, in which increasing algal biomass leads to proportional increases in the abundance of all phytoplankton species, including harmful ones (Sutula et al., 2017). An advantage of the proportionate increase paradigm is that it does not necessarily require any information about how the prevalence of a particular HAB species changes along a eutrophication gradient. The PI paradigm applies to HAB species that dominate a significant proportion of total phytoplankton biomass, such as diatom-dominated phytoplankton communities in coastal ecosystems (e.g., well documented correlations between chl-*a* and *Pseudo-nitzschia* abundance and toxin concentrations in the southern California Current System; Sandoval-Belmar et al., 2023). However, it is unlikely that all taxa covary equally with chl-*a*, particularly in different seasons when other factors such as light availability, salinity and temperature are at play. An alternative paradigm that could be used to set thresholds is a “disproportionate increase” paradigm, i.e., HAB species disproportionately increase in abundance along gradients of increasing eutrophication (Glibert, 2020). Two causal mechanisms for this disproportionate increase have been documented in experimental studies. First, different HAB taxa respond in opposite ways to seasonal changes in salinity, temperature, nutrients and other environmental variables (Camargo and Alonso, 2006; Kudela et al., 2008; Nejad et al., 2018; Smayda and Trainer, 2010). Second, flagellated HAB-forming species have been shown to be selected for by turbulence and light limitation in the water column, since they can migrate to maximize their access to light (Shikata et al., 2015; Smayda, 2010; Zheng et al., 2023). Generally in Northern Hemisphere temperate estuaries, diatoms proliferate in spring months when higher flows and salinity stratification provide them with plentiful access to a nutrient and light-rich surface layer, while dinoflagellates and raphidophytes dominate in fall, when mixotrophy and swimming ability provides them with an advantage in nutrient acquisition (Burkholder et al., 2008; Smayda and Trainer, 2010). In a case when winners and losers are expected to be present among phytoplankton, the hypothesis to test becomes whether individual harmful algae taxa proliferate in line with their general group: does *Pseudo-nitzschia* increase alongside other diatoms in spring, and does *Dinophysis* increase along with other dinoflagellates in fall? Do abundance patterns of HAB-forming taxa follow the broader covariation of dinoflagellate, raphidophyte and diatoms in an estuarine system? Confirming whether phytoplankton disproportionately increase relative to each other in field studies has been difficult due to both natural variability and potential limitations of methods used for detecting, classifying, and quantifying relevant taxa (e.g., microscopy is sometimes unable to differentiate between closely related taxa,

or quantify phytoplankton community across the entire size spectrum; Zingone et al., 2015).

1.2. Metabarcoding as a useful but noisy monitoring tool: accounting for error

Metabarcoding and quantitative polymerase chain reaction (qPCR) methods can be used to cost-effectively and consistently document phytoplankton community structure in extended time series that are highly complementary to traditional microscopy approaches. The ability to measure counts per volume is a major advantage of qPCR analysis, but if approaches demand the quantification of increasing numbers of distinct taxa, metabarcoding can be more scalable due to its high throughput nature (Esenkulova et al., 2020). However, interpreting metabarcoding results requires accounting for the varying sensitivities and biases of different molecular assays (Ayala et al., 2023). Because of the amplification needed for metabarcoding analyses, the data are expressed in terms of relative rather than absolute abundance. Due to this amplification, metabarcoding can be considered a more qualitative monitoring analysis, more appropriate for surveillance approaches rather than exact quantification (Santoferrara, 2019). It has been proposed to be more useful for presence/absence based detection or approaches accounting for the stochastic factors influencing molecular detection (Gold et al., 2023). Because of the difficulty of disentangling confounding impacts from amplification, care must be taken to account for these probabilistic factors. Despite these shortcomings, metabarcoding approaches have advantages in observing taxa that are difficult to monitor reliably over time microscopically, such as dinoflagellates, where small sizes and general similarity of forms can lead to misidentifications via microscopy (Culverhouse et al., 2003; Kim et al., 2024). The ability to improve identifications of different harmful algae through time as taxonomic databases improve is another major advantage for molecular approaches. Alongside microscopy, the depth and breadth of identification from metabarcoding is particularly useful for differentiating fine-scale seasonal differences in relative abundances (Hammond et al., 2022).

Dichotomization (also called discretization) of a continuous dependent variable into a binary one based on a chosen cut-off point has been used in studies where a parameter is difficult to compare to a continuous predictor variable (Murtaugh, 1996), or due to contamination by additive errors which introduce noise, as is common in molecular data (Shentu and Xie, 2010). The choice of cut-off point used to evaluate the dependent variable is important, as setting too high or low can reduce statistical power, and ideally the specific cut-off will be informed by corroborating data (Murtaugh, 1996). Such an approach is useful to inform a binary output based on uncertain or error-prone data (Ge et al., 2010; Liu et al., 2002). The binary dependent variable can then be compared to a continuous explanatory variable via approaches like Conditional Probability Analysis (CPA), which quantifies the probability of one event occurring as a function of another event (Hollister et al., 2008; Paul and McDonald, 2005; Sutula et al., 2017). For datasets like metabarcoding where some stochasticity is introduced by the amplification process, a probabilistic approach like CPA helps to quantify those uncertainties.

1.3. Study area

SFB is the largest estuary in California, consisting of six sub-embayments (Jassby et al., 1997). Phytoplankton production has historically been dominated by a spring bloom event, when chl-*a* values over the channel regularly exceed 10 µg/L, levels which are unusual in the remainder of the year (Jassby et al., 2002). SFB is among the most nutrient-enriched estuaries globally (Cloern et al., 2020), but has been historically resistant to typical symptoms of eutrophication such as high phytoplankton biomass and resulting low dissolved oxygen (Cloern et al., 2020; Novick and Senn, 2014). This resistance has previously been

attributed to the attenuation of light by high turbidity, intensive tidal flushing to remove biomass and replenish dissolved oxygen, and top-down control by large populations of invasive grazing bivalves, but some research suggests those controls may be degrading in recent decades (Cloern et al., 2020, 2007). To that end, chl-*a* concentrations in SFB have been increasing during the spring blooms, during the summertime baseline, and with the emergence of a fall bloom (Cloern and Jassby, 2012; Sutula and Senn, 2015). There is also growing evidence that multiple harmful phytoplankton taxa are commonly detected in SFB (Sutula et al., 2017; Peacock et al., 2018), leading to heightened concerns over the potential risk of HAB events (Sutula et al., 2017).

Previous work identified that average monthly chl-*a* thresholds in SFB (i.e. 13 $\mu\text{g/L}$) were associated with an increase in the probability of higher harmful taxa abundances and toxin concentrations, an analysis based on the “proportionate increase” paradigm (Sutula et al., 2017). However, the dataset used in this prior study had limitations in being able to test either hypothesis, including low data density and uncertainty in taxonomic identification via microscopy, particularly for small sized or rare phytoplankton. The reduced data density also reduced its usefulness for assessing the relative risk of particular HAB taxa between

different seasons. This uncertainty led to major investments in monitoring for shellfish toxin, metabarcoding and limited qPCR monitoring, which has generated a dataset that can now be queried to disentangle seasonal and spatial relationships among HABs and chl-*a*, and to investigate the scientific basis for HAB related thresholds.

Continued and expanded monitoring has increased our knowledge on the seasonality of HAB species found in SFB, with emerging information on their drivers (Kudela et al., 2023). Prior studies have established that the dinoflagellate *Alexandrium*, which generates the neurotoxin saxitoxin associated with Paralytic Shellfish Poisoning, is consistently present in SFB (Sutula et al., 2017; Peacock et al., 2018), and in 2018 the occurrence of saxitoxin was noted to exceed the human health warning threshold (Egel, 2018; *Marine Biotoxin Monitoring Report*, 2018). Other HAB taxa are known to be present in SFB in low to moderate relative abundances (Kudela et al., 2023), including *Heterosigma akashiwo*, a raphidophyte with ichthyotoxic properties which caused an unprecedented bloom event in August 2022, leading to mass fish deaths and hypoxic conditions throughout SFB (Kudela et al., 2024). *Dinophysis* and other similar harmful unarmored, mixotrophic dinoflagellates have also been identified as present within SFB, and its toxin has previously

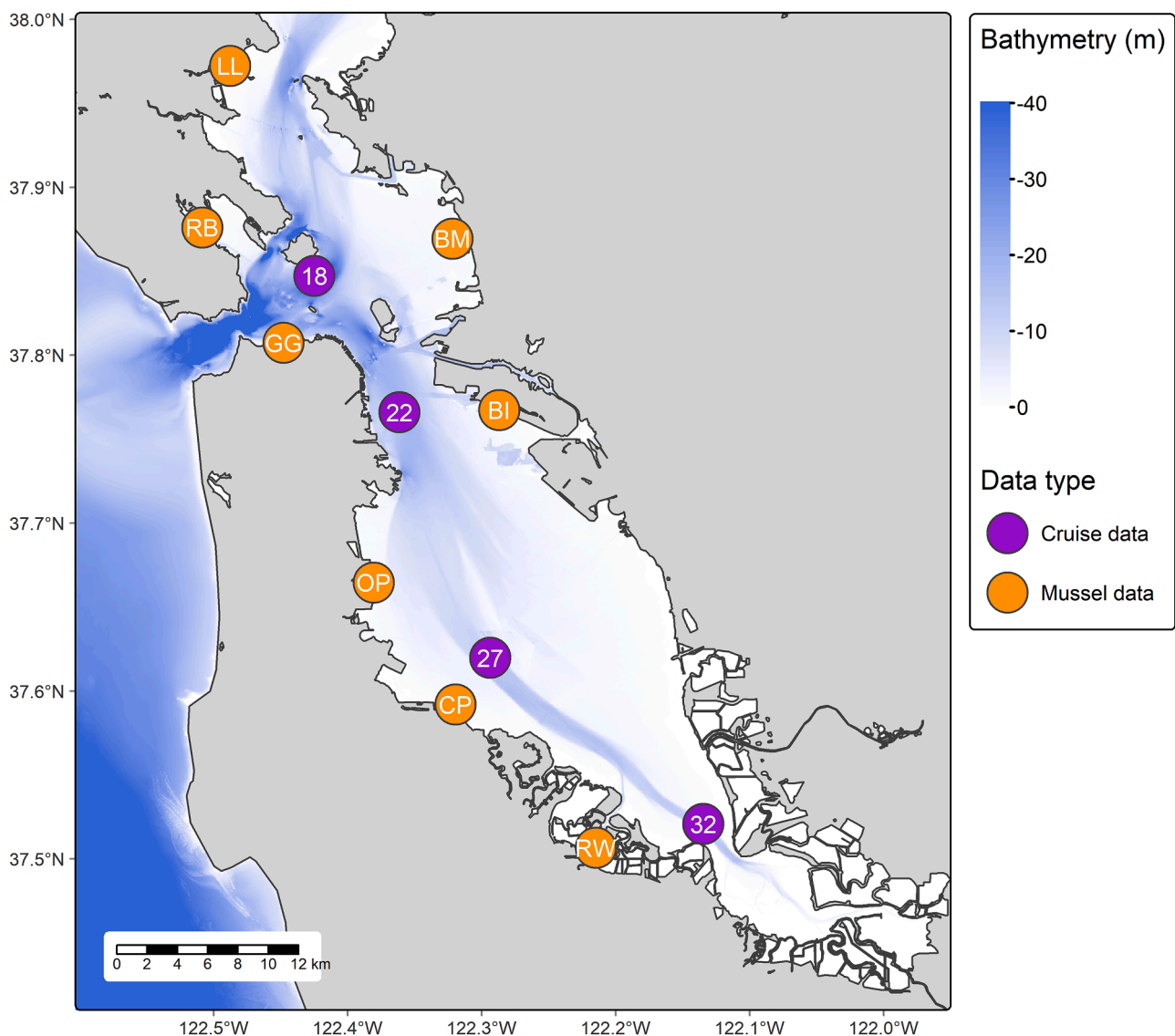


Fig. 1. Map of San Francisco Bay, showing the distribution of research vessel (R/V) Peterson cruise stations along the channel in purple, and the mussel toxin sampling sites are around the Bay in orange. Letters indicate site names. Bathymetry data from National Centers for Environmental Information and California Natural Resources Agency (National Centers for Environmental Information, 2018).

been determined to bio-accumulate in excess of public health standards in local mussels (Peacock et al., 2018). *Heterosigma* (Jeong, 2011; Nygaard and Tobiesen, 1993), *Dinophysis* and a selection of other similar HAB-forming taxa are united by a mixotrophic life habit, flagellar motility, and other adaptations which may enhance their ability to seek optimal conditions in estuarine settings like the tidally dynamic SFB and to seek alternative energy sources when photosynthesis is limited by light attenuation from suspended sediments (Burkholder et al., 2008). TheseF HAB taxa may be particularly favored in SFB during the dry summer and fall, when there is opportunity for longer residence times and stratification is at its annual lowest point (Cloern, 1991).

We compiled a 5-year time series (2015 through 2020) of HAB molecular data, shellfish toxins, and chl-*a* data at stations throughout SFB (Fig. 1, Table 1), and used that data set to investigate several research directions. The goals of this study were to investigate the following: (1) document the status, trends and seasonal covariance of chl-*a* with molecular measures of HAB abundance (qPCR) or relative abundance (metabarcoding) and shellfish toxins, (2) investigate the validity of the disproportionate increase paradigm, and (3) for HAB taxa with viable relationships, identify thresholds in either qPCR-measured harmful genus abundance or chl-*a* that signal changes in the risk in the magnitude of the HAB event. We also investigated whether any of these changes in probability were nonlinear in nature with any jumps in probability at diagnostic chl-*a* levels, which would merit selecting abundance thresholds applicable across taxa. These relationships and thresholds represent an application of molecular data with promise for similar coastal and estuarine settings around the globe.

2. Methods

2.1. Compilation of data: ship-based sampling: chl-*a*, metabarcoding and qPCR sequencing

After compiling the 5 year time series of shellfish toxins, HAB molecular data, and chl-*a* data at stations throughout SFB (Fig. 1, Table 1), we used that data set to: (1) document the temporal and spatial correspondence of bulk phytoplankton biomass (as measured by chl-*a*), HAB species abundances, and shellfish toxin concentration, (2) document the relationships between shellfish toxins, HAB absolute or relative abundance and chl-*a*, in order to test the “disproportionate increase” hypothesis in SFB, and (3) develop risk-based models and identify thresholds for HAB abundance and/or chl-*a* (Fig. 2). Ship-based data used in this study consisted of chl-*a* samples, 18S metabarcoding relative abundances, and qPCR absolute counts per mL, collected between 2014 and 2020. Molecular (18S and qPCR) and chl-*a* samples were collected on cruises on the Research Vessel (R/V) *Polaris* from 2014–2015 and R/V *Peterson* (after 2016) traveling along the SFB channel, which occurred on a monthly basis from 2014–2020, with the majority of samples occurring at stations 33, 32, 27, 22, and 18, and a small minority of samples occurring at other stations (Fig. 1, Table 1). Samples were collected via a submerged fixed intake 1–2 m below the bow of the ship. 500 mL of water was passed through a 47 mm, 0.7-micron glass fiber filter (Whatman GF/F, Cytiva Life Sciences, Marlborough, MA, USA), with a small minority of samples (~5 % of samples) during high turbidity conditions being collected from 250 mL of filtered water.

Table 1

Information on the duration, number of samples and sampling platform for the different data types used in this report.

Data type	Years measured	# samples in this study (n)	Shore/ship based
18S Metabarcoding	2014–2020	280	Ship
<i>Alexandrium</i> quantitative polymerase chain reaction	2015–2020	270	Ship
Chlorophyll <i>a</i>	2014–2020	280	Ship
Mussel saxitoxin	2015–2020	849	Shore

Filters were stored at -80°C and later cut in half with sterile scalpels (to create an archive). DNA extraction was conducted on one filter half using the Qiagen PowerSoil DNA extraction kit (Qiagen, Venlo, Netherlands). The extracted DNA was amplified using polymerase chain reaction and sequenced for 18S rRNA genes using a MiSeq sequencer (Illumina, San Diego, CA, USA) (Otten et al., 2021). Primers spanning the V5 and V7 regions (Euk1132F, Euk1423R) of the 18S rRNA gene were used (Hugerth et al., 2014). Sequencing reads (counts of appearance of a sequence) were demultiplexed and alignment and taxonomic identification was conducted in QIIME2 (Bolyen et al., 2019). Primer sequences were removed with cutadapt in R (Martin, 2011). A curated SILVA database based on version 132 was used for 18S sequences. HAB taxa mentioned in this report were validated via BLAST against the National Center for Biotechnology Information database. More information on the workflow used to process molecular data can be found in Otten et al. (2021).

As an additional validation step, microscopy-based monitoring data on phytoplankton community composition from the same water quality cruise stations was downloaded (Nejad et al., 2018), and matches were identified based on collection at the same station and date as the molecular samples present in our dataset. *Pseudo-nitzschia*, *Heterosigma* and *Dinophysis* were identified as genera sharing the most shared analysis dates between the datasets. Microscopy-based percent relative abundance was calculated by dividing counts by the total counts present in the sample. Values were cross-plotted to investigate the correspondence between microscopy-based relative abundance and metabarcoding-based relative abundance (Fig. S1A).

Two genera, *Alexandrium* and *Pseudo-nitzschia*, were also analyzed with quantitative Polymerase Chain Reaction (qPCR), a technique more directly translatable to cell density (Coyne et al., 2022), using a LUNA Universal Probe or SYBR Master Mix on an iQ5 Multicolor Real Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) (Otten et al., 2021). qPCR for *Alexandrium* was conducted with primers Alex_5.8S_F/R (Galluzzi et al., 2005), while detection of *Pseudo-nitzschia* used PnAll F/R (Hubbard et al., 2008). Samples from the same day and station were cross-plotted to compare variations in metabarcoding percent abundance of each of the two taxa to the cells/mL estimates from qPCR (Fig. S1B,C). Because the counts/mL output of qPCR is not directly translatable to the metabarcoding measures of relative percent abundance, we instead used a logistic regression approach to relate the probability of metabarcoding-based detection to the qPCR-measured abundances.

Metabarcoding data have an advantage of being able to resolve identifications to species level in some cases, but the nine taxa investigated in our study vary in terms of their respective coverage in taxonomic databases and in the diversity of sequences present at the 18S locus. This means that some genera are more difficult to identify to species, particularly among the dinoflagellates (Hoppenrath, 2017; Stern et al., 2010). Because a major focus of this study is about assessing relative risk of different taxa proliferating above their baseline, we have conducted all taxonomic identification to the genus level for greatest comparability between groups. Though this does not account for species-specific differences in toxin production (Anderson et al., 2012), most HAB monitoring programs assess HABs at the genus level using microscopy, so this method provides a comparable assessment of relative risk. To provide a conservative aspect to our surveillance approach, we included taxa such as *Gyrodinium* in our analysis due to the presence of HAB-forming species in each respective genus, despite currently not being able to resolve identifications in our dataset to conclusively demonstrate that particular species' presence.

2.2. Mussel toxin sampling

Samples of *Mytilus* spp. mussels (a multi-species assemblage of *M. californianus* and members of the *M. edulis* complex) were collected biweekly for phycotoxin analysis from ten floating docks bordering SFB

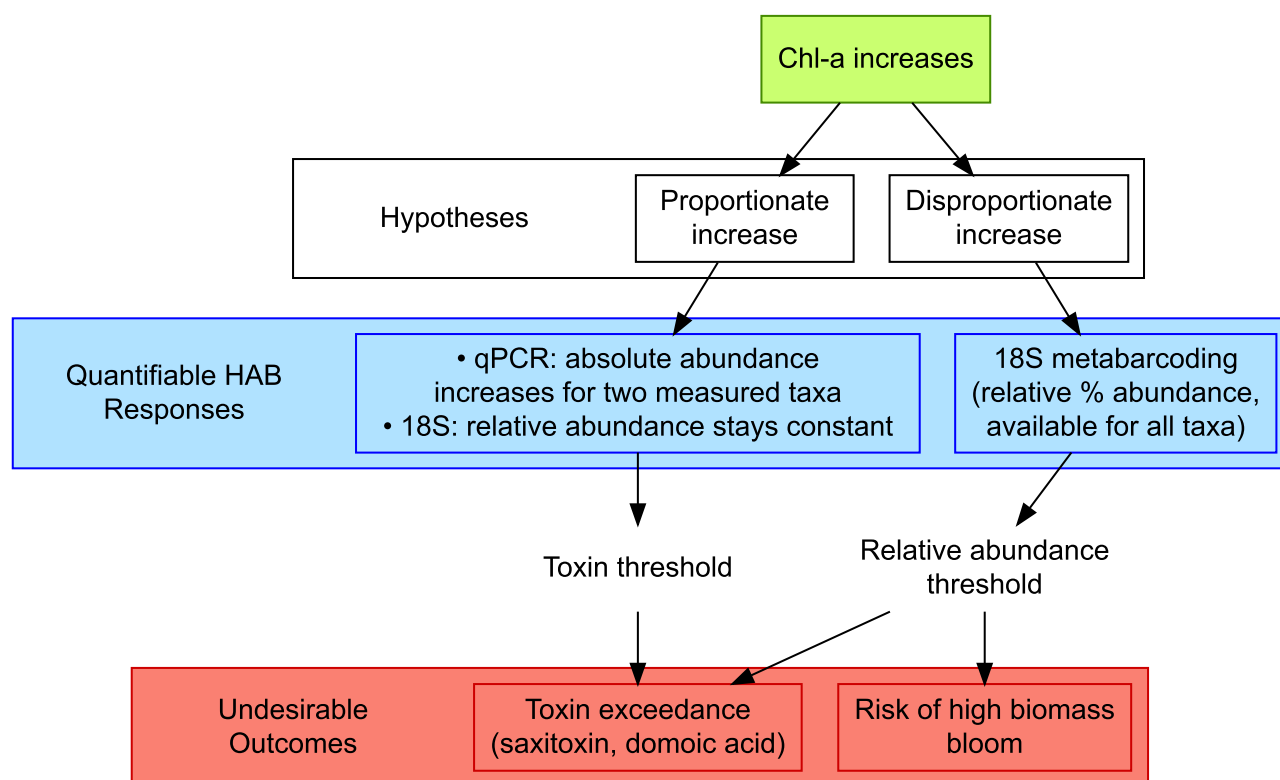


Fig. 2. Schematic illustration of connections between the different data types utilized in this study, including comparing chlorophyll *a* (chl-*a*) concentration to metabarcoding or quantitative polymerase chain reaction (qPCR) data, and how each of those data types can be compared to a risk-related analysis of undesirable outcomes.

from 2015–2020, though the majority of samples were collected at sites RB, GG, BM, BI, OP, CP and RW (Fig. 1, Table 1). At each site, five mussels were collected, stored frozen at -20°C , thawed, shucked, and homogenized into a representative pooled sample for each station and date. Samples were analyzed for saxitoxin concentration at the University of California at Santa Cruz. Saxitoxin was measured via enzyme-linked immunosorbent assay (ELISA), both following the approaches of Peacock et al. (2018). The method detection limits are 3 ng/g for 1 g of tissue for saxitoxin. Tissue toxin concentrations were compared against toxin thresholds derived from regulatory guidelines and literature sources for saxitoxin (Table 2). Regulatory shellfish advisory thresholds were infrequently exceeded; instead, ecological thresholds relevant for chronic sublethal effects and the potential for trophic bioaccumulation were the primary focal point of this analysis.

2.3. Analytical approach

2.3.1. Overview

Different HAB-forming taxa have different baseline abundances (the relative abundance at the lowest chl-*a* level), methods of detection, and thresholds of risk available for their abundance and associated hazards,

Table 2

Summary of shellfish toxin screen thresholds employed in this study.

Threshold name	Concentration
Human consumption advisory level	800 ng/g (California Department of Public Health threshold) (Egel, 2018)
Bioaccumulation or reporting limit	100 ng/g = toxin dietary accumulation above background levels (this study, Fig. 4)
Sublethal threshold	300 ng/g = Sublethal toxicity exhibiting neurotoxin symptoms, including slowed valve closure and elevated stress indicator metabolites (Beaulercq et al., 2023; Lavaud et al., 2021)

which resulted in a variable ability to test one or both hypotheses (Tables 2, 3). For *Alexandrium* shellfish toxin, qPCR abundance, and metabarcoding data were available, which allowed us to investigate the basis for disproportionate increases. If the disproportionate increase hypothesis was supported, we would expect to observe a rise in the qPCR-based absolute abundance of *Alexandrium* and *Pseudo-nitzschia* as a function of chl-*a*. For all other HAB taxa listed in Table 3, only metabarcoding data were available, which limited the testing to the disproportionate increases and generation of chl-*a* thresholds when relationships were documented.

We assessed metabarcoding-determined relative abundance data for a broad assemblage of HAB taxa, including *Alexandrium* and *Pseudo-*

Table 3

Table of percent abundances required for different taxa to reach their genus-specific 87th percentile of relative abundance. Some taxa are inherently rare in the dataset, like *Pseudo-nitzschia*, while others are quite common, such as *Gyrodinium*. These abundances are in terms of percent of total reads, and not necessarily directly related to absolute cell-based abundance. This is due to the differential response of different sequences to amplification as well as the ability of some taxa, particularly the dinoflagellates, to produce replicate genomes.

Taxon	Percent abundance
<i>Alexandrium</i>	1.2
<i>Dinophysis</i>	0.083
<i>Gonyaulax</i>	0.85
<i>Gyrodinium</i>	4.4
<i>Heterocapsa</i>	2.5
<i>Heterosigma</i>	0.14
<i>Noctiluca</i>	0.65
<i>Prymnesium</i>	0.22
<i>Pseudo-nitzschia</i>	0.040

nitzschia, and seven additional taxa (*Dinophysis*, *Heterosigma*, *Heterocapsa*, *Gonyaulax*, *Noctiluca*, *Gyrodinium*, and *Prymnesium*), that have been identified as taxa of concern in other coastal and estuarine systems and are included in international HAB databases (Intergovernmental Oceanographic Commission of UNESCO, 2021; Lundholm et al., 2009). We then developed risk-based models that described how the probability of exceeding relative abundance thresholds increased with chl-*a*. Since relative abundance thresholds for HAB taxa are not generally available, they were instead set by investigating the relative abundance at which a known harmful alga coincided with elevated toxin levels in grazers, with that abundance threshold being applied to investigate the other HABs. The disproportionate increase paradigm can be tested by comparing the probability of different taxa increasing or decreasing in relation to abundance-based thresholds as a function of chl-*a*, as described below.

2.3.2. Statistical analysis

All metabarcoding results are presented in terms of percent of total reads (count of reads for that taxon from that sample, divided by the total reads for the filter). Mussel samples were matched to qPCR and metabarcoding relative abundances from ship-based samples, according to geographic proximity and close temporal matches. Geographic matches were identified based on sub-embayment, with various matches of time intervals and geographic proximity being tested, before settling on matched mussel stations being assigned to a zone of influence of approximately 10 km surrounding a ship station. The temporal matches were identified based on mussel samples occurring within 14 days after a ship-collected sample. Chl-*a* and metabarcoding samples were synoptically collected from a ship. Observations were seasonally binned based on the climatological trends in temperature, salinity and photosynthetically active radiation typically observed in SF Bay (Fig. S2), as follows: (1) Fall = August through October, (2) Winter = November through January, (3) Spring = February through April, and (4) Summer = May through July.

Chl-*a*, molecular and mussel toxin data were visualized as parallel time series to initially diagnose whether the observed values align with prior literature observations of phytoplankton dynamics. Additionally, the relative seasonal occurrence of toxins in mussels were analyzed. Because the toxin concentration data were not normally distributed as determined by Shapiro-Wilks tests, a Kruskal-Wallis comparison of medians was used as a non-parametric alternative to Analysis of Variance. qPCR and toxin data were compared to investigate positive trends in co-occurrence of higher *Alexandrium* density and the concentration of the corresponding toxin in nearby mussels. Quantile Generalized Additive Models (GAMs) were included for the 75th and 90th percentiles of toxin concentrations (Fasiolo et al., 2021) to identify positive relationships at distinct quantiles, and to further investigate threshold effects via conditional probability analyses (see below). All data analysis was conducted with R version 4.2.2 (R Core Team, 2025), with code available in the Supplemental Materials. Metabarcoding taxonomic assignment data files are available at the Environmental Data Initiative (Killam et al., 2025). Metabarcoding reads are deposited in the National Center for Biotechnology Information (NCBI) Sequencing Read Archive (SRA; Sayers et al., 2025) under bioproject ###. R code is available at Zenodo (Killam, 2026).

2.3.3. HAB absolute and relative abundance thresholds

To identify levels of chl-*a* associated with an increased risk of HABs, it was first necessary to identify intermediary absolute or relative abundance thresholds for harmful taxa, or disproportionately large changes in a response variable relative to a small change in the environmental explanatory variable.

For harmful taxa in which shellfish toxin data were available, the relation between the probability that toxin thresholds would be exceeded in nearby mussels as a function of increasing metabarcoding relative abundance and qPCR absolute abundance was assessed using CPA

(Hollister et al., 2008; Paul and McDonald, 2005; Sutula et al., 2017). CPA relates the probability of one event, such as a threshold being exceeded, as a function of another continuous variable. Paired metabarcoding-toxin observations were ordered by increasing percent abundance. Toxin threshold exceedance was then set as a binary variable (1 if the threshold was exceeded and 0 if not). Following the approach of Hollister et al. (2008), the estimates were then bootstrapped, resampling the dataset 1000 times with replacement (Frick et al., 2023), and the same CPA was conducted. Of the 1000 simulations, the 97.5th percentile and 2.5th percentiles were used to construct a 95 % confidence interval around the data. qPCR and metabarcoding relative abundance thresholds were identified at the point of the upper bound of 95 % confidence interval of the CPA indicating 50 % probability of the chosen mussel toxin threshold.

For harmful taxa in which toxin data were not available, CPA was performed to observe the occurrence of nine HAB taxa of concern above background relative percent abundance levels. For these analyses, the percent abundance threshold of a particular taxon could not be set at a static level across taxa, as the HAB taxa all have different baseline percent abundances in the dataset. Some taxa like *Heterocapsa* are quite common, while others like *Dinophysis* are often rare. We instead identified increases in molecular relative abundance above baseline as a function of chlorophyll concentration. Therefore, the change in the baseline relative abundance threshold was used as a metric to investigate whether those changes co-occurred with increasing chl-*a*. *Alexandrium* reaching 1.2 % of reads represented the relative abundance level at which 100 ng/g toxin was >50 % likely to be detected in nearby mussels, as indicated by the upper confidence interval of the CPA crossing 50 % probability. That abundance corresponded to the 87th percentile of all *Alexandrium* abundances in the dataset, which was used as a threshold by which to assess if other taxa were above or below their respective baseline abundances (Table 3).

2.3.4. Chl-*a* relative abundance thresholds

The taxon-specific relative abundance thresholds were then gathered and used to create a series of CPAs for each taxon among the nine HABs as a function of chl-*a*, subset by season. Some taxa rose above their respective abundance baseline as a function of chl-*a* concentration, while others stayed flat or declined. To standardize comparisons between taxa, probabilities were converted to represent a “risk multiple” above baseline by dividing calculated probabilities by the baseline probabilities (the probability at the lowest chl-*a* level). The probability baselines for each taxon are viewable in Table 4. Among the analyzed taxa, a subset were identified which were of particular interest in terms of their disproportionate increase in abundance as a function of chl-*a*, and the nonlinearity of that increase. Such nonlinear increases in molecular abundance could be suggestive of the early stages of bloom-like proliferation in terms of cellular division in the water column (Metfies et al., 2020; Needham and Fuhrman, 2016).

Table 4

Baseline probabilities of 87th percentile threshold being exceeded. These represent “global” probabilities of the event occurring across the chlorophyll distribution. For each Conditional Probability Analysis plot, the increasing or decreasing probabilities are divided by this baseline to obtain a probability multiple.

Taxon	Winter	Spring	Summer	Fall
<i>Alexandrium</i>	17.9	17.4	8.6	4.7
<i>Dinophysis</i>	16.1	7.2	7.1	21.9
<i>Gonyaulax</i>	3.6	10.1	21.4	15.6
<i>Gyrodinium</i>	8.9	10.1	5.7	25.0
<i>Heterocapsa</i>	7.1	13.0	17.1	12.5
<i>Heterosigma</i>	5.4	15.9	5.7	25.0
<i>Noctiluca</i>	8.9	11.6	20.0	10.9
<i>Prymnesium</i>	10.7	5.8	18.6	17.2
<i>Pseudo-nitzschia</i>	21.4	18.8	10.0	3.1

All HAB taxa were subsequently combined together and a grouped CPA was conducted to investigate the probability of any HAB taxon rising as a function of chl-*a* concentration. The package ‘EnvCpt’ was used to identify statistically significant change-points in the data (Killick et al., 2025), with the ideal change-point model and resulting change-points being selected from among twelve possible models by selecting the one with the minimum Akaike Information Criterion/Bayesian Information Criterion ratio. To assist in visualization of nonlinear increases probability in relation to chl-*a*, an approximate instantaneous slope along the combined CPAs was visualized using a GAM (Wood, 2023), using the REML method and 30 knots. The derivatives along the fitted GAM were then identified using the finite differences method, via the “derivatives” function in the gratia package (Simpson and Singmann, 2024), to identify areas of nonlinear increase or decrease in probability of occurrence. A derivative value of 2 would indicate that the probability was doubling per additional $\mu\text{g/L}$ of chl-*a*.

3. Results

3.1. Temporal occurrences of saxitoxin and *Alexandrium*

Saxitoxin was frequently detected in mussel tissue, and its concentration showed temporal association with elevated presence of *Alexandrium* in the metabarcoding data. Saxitoxin exceeded the California Department of Public Health (CDPH) threshold of 800 ng/g in March 2018 (Fig. 3B), at which time CDPH issued a saxitoxin warning. This event also was a peak of *Alexandrium* in the relative abundance data (~10 percent abundance; Fig. 3A). Saxitoxin concentrations occurred in excess of 300 ng/g several times in the 2017–2018 period and more

regularly exceeded the 100 ng/g threshold during that interval. *Alexandrium* was noted across the Central SFB and South Bay down from Station 18 near the Bay entrance (the Golden Gate Bridge) to Station 32 (at the entrance to the Lower South Bay).

Comparing the seasonal median toxin concentrations, the majority of observations of saxitoxin above 100 ng/g occurred in spring (Fig. S3), but most of those spring observations coincided with chl-*a* concentrations around 5 $\mu\text{g/L}$ (Fig. S4). A Kruskal-Wallis test indicates that saxitoxin median concentrations were significantly different across seasons (chi-squared = 31.323, df = 3, p-value < 0.0001).

3.2. Exceedance of toxin thresholds in mussels as a function of absolute and relative abundance

Saxitoxin concentrations in mussels show a positive relationship with qPCR counts/mL for *Alexandrium* (Fig. 4A). There is a positive inflection in the probability of mussels recording saxitoxin above 100 ng/g when *Alexandrium* reaches 1000 qPCR counts/mL (Fig. 4A). *Alexandrium* declines in absolute abundance at higher chl-*a* concentrations consistent with the spring phytoplankton bloom (Fig. 4B,D).

Comparing the metabarcoding relative abundances to the probability of exceeding different concentrations of their toxins in mussels, *Alexandrium* shows a positive relationship with the occurrence of saxitoxin above a given concentration (Fig. 5). The probability of saxitoxin exceeding 100 ng/g begins at around 20 % probability as a baseline. The probability that the concentration in mussels exceeded 100 ng/g passed 50 % when *Alexandrium* reached 1.2 % of 18S reads. Mussels reached a 50 % probability of exceeding 300 ng/g when *Alexandrium* reached 2.4 % of reads (Fig. 5).

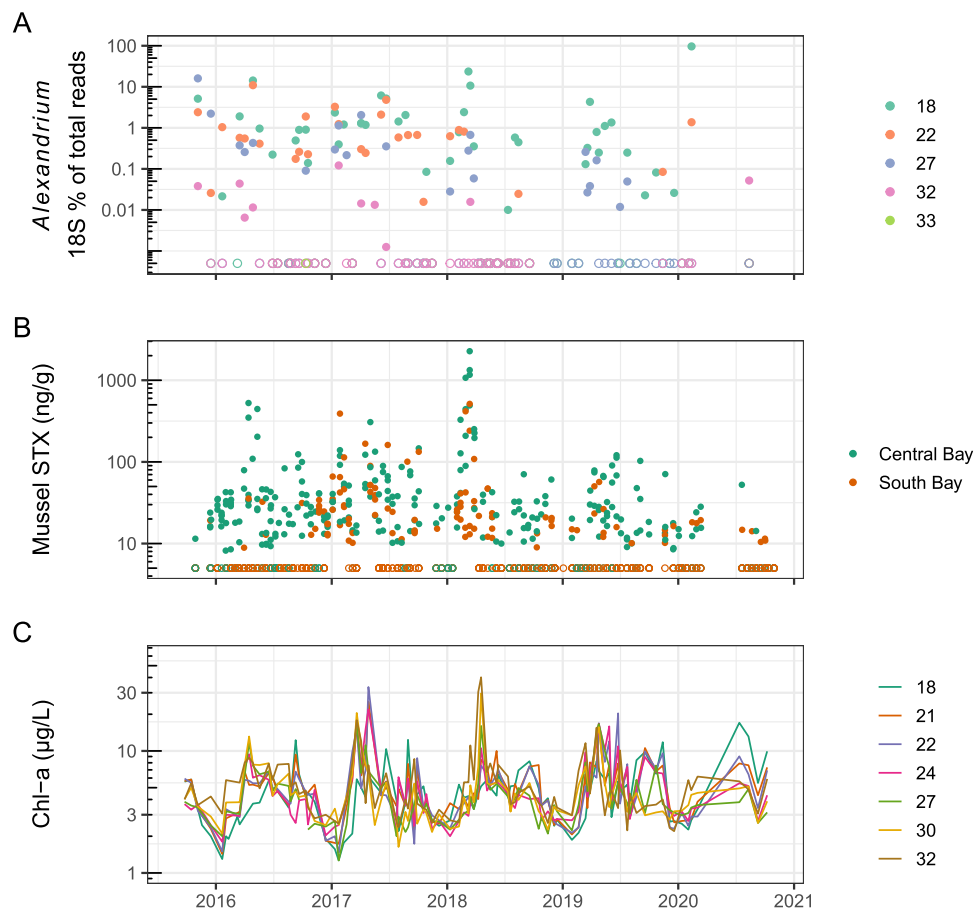


Fig. 3. Temporal correspondence of chlorophyll *a* (chl-*a*) with mussel toxins and corresponding harmful algal bloom (HAB) species relative abundance. Numbered points (in plot A) are channel cruise stations, while points in plot B are colored by regions of SFB.

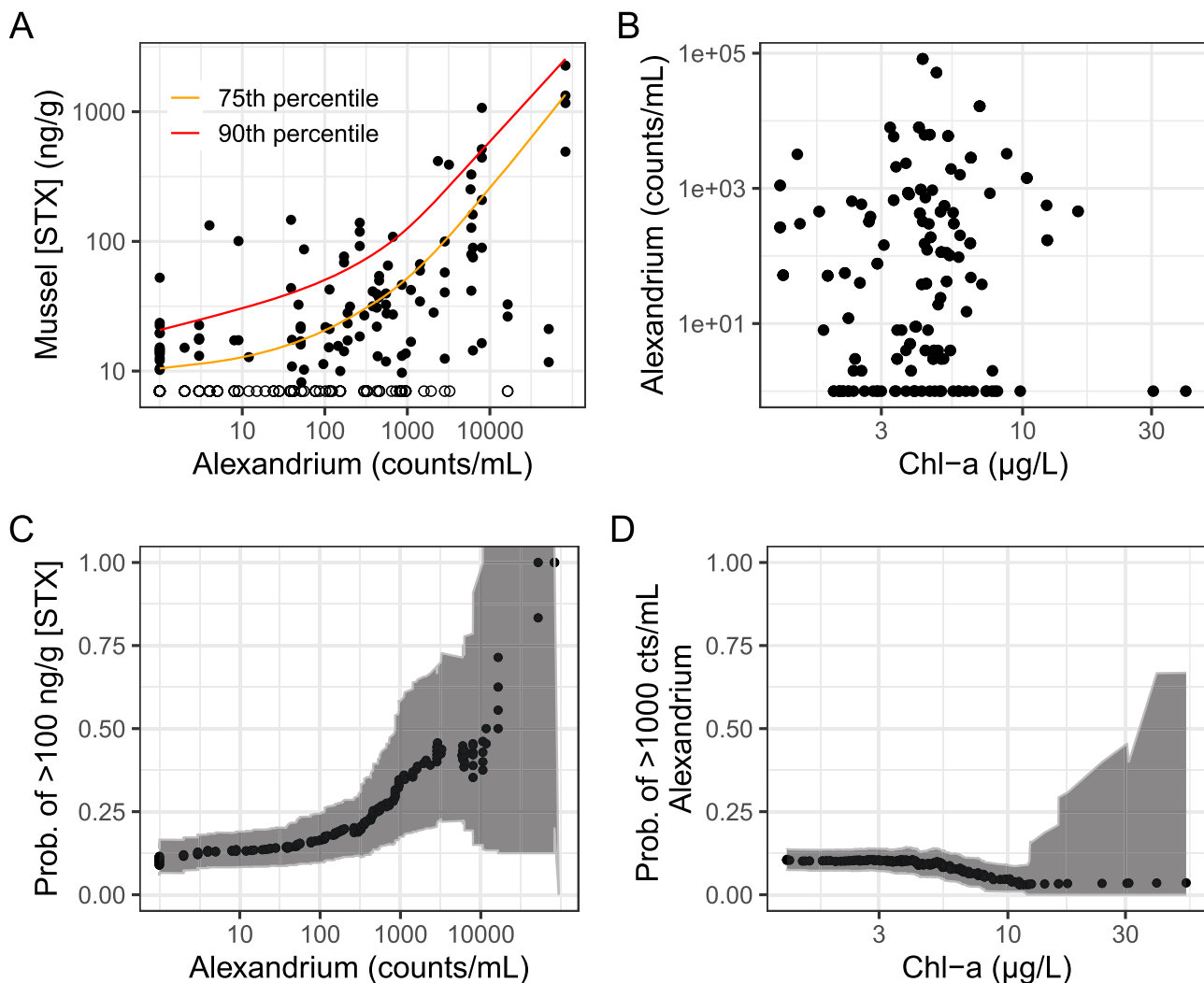


Fig. 4. A: Quantitative polymerase chain reaction (qPCR counts per mL for *Alexandrium* related to saxitoxin. Orange and red lines represent quantile generalized additive models (GAMs) fitted to the 75th and 90th quantiles, respectively. B: Relating chlorophyll-*a* values to the *Alexandrium* qPCR counts. C: Conditional probability analysis (CPA) constructed from qPCR counts per mL, related to likelihood of exceeding 100 ng/g saxitoxin (STX). D: Relating likelihood of *Alexandrium* 1000 counts/mL qPCR threshold being exceeded in relation to chlorophyll *a* (chl-*a*).

3.3. Seasonal probability of HAB taxa exceeding thresholds as a function of chl-*a*

Among the nine HAB taxa investigated, most exceedances of the 87th percentile at higher chl-*a* were observed in the fall, particularly for *Heterosigma*, *Heterocapsa*, *Gonyaulax*, *Dinophysis* and *Gyrodinium* (Fig. 6). At 4.6 µg/L chl-*a*, *Heterosigma* shows an upward inflection in probability of exceeding its baseline abundance of 0.14 %. The other four taxa increased more monotonically. *Heterocapsa*, which had a higher baseline abundance, also had a much higher optimized threshold of 2.55 % abundance, which it approached 75 % probability of exceeding at high fall chl-*a* levels. Summer and fall generally display higher chl-*a* medians than the preceding eras, though fall is distinguishable by concentration appearing more frequently in the 5–10 µg/L range, with the preceding era showing more variability above and below that level.

In the other seasons, most HAB taxa show negative relationships with chl-*a* concentration (Fig. 6), though *Heterocapsa* seemed to increase in percent abundance in relationship to chl-*a* across seasons. *Alexandrium* and *Gonyaulax* were more likely to exceed their high thresholds of 1.3 % and 4.4 % in the summer at the highest chl-*a* values.

Investigating the combined probability of any of the studied HAB taxa exceeding their thresholds in different seasons (Fig. 7A), we

observed a rapid increase in probability of any of the studied species exceeding their baseline abundance above 4.6 µg/L in fall. When the five genera with the greatest fall increases identified in Fig. 7 are subset out (Fig. 7B), the slope is steeper, reaching above a 1.75x probability multiplier of encountering any member of the mentioned genera above their baseline abundance at higher chl-*a* levels.

4. Discussion

Using molecular data in a novel analytical approach, we identified a seasonal chl-*a* threshold of 4.6 µg/L corresponding to a disproportionate increase in risk of flagellate mixotrophic HABs. The relative and absolute qPCR-measured abundances of *Alexandrium* both show positive relationships with the probability of saxitoxin observation in nearby mussels, pointing to the utility of molecular thresholds as early warning or screening values for potential shellfish consumption risk, either to humans or aquatic life. This work demonstrates the promise of molecular approaches in disentangling the complex interplay between stressors and phytoplankton/HAB community responses and has the potential to provide better information to guide management of eutrophication at lower cost over the long run than non-molecular methods.

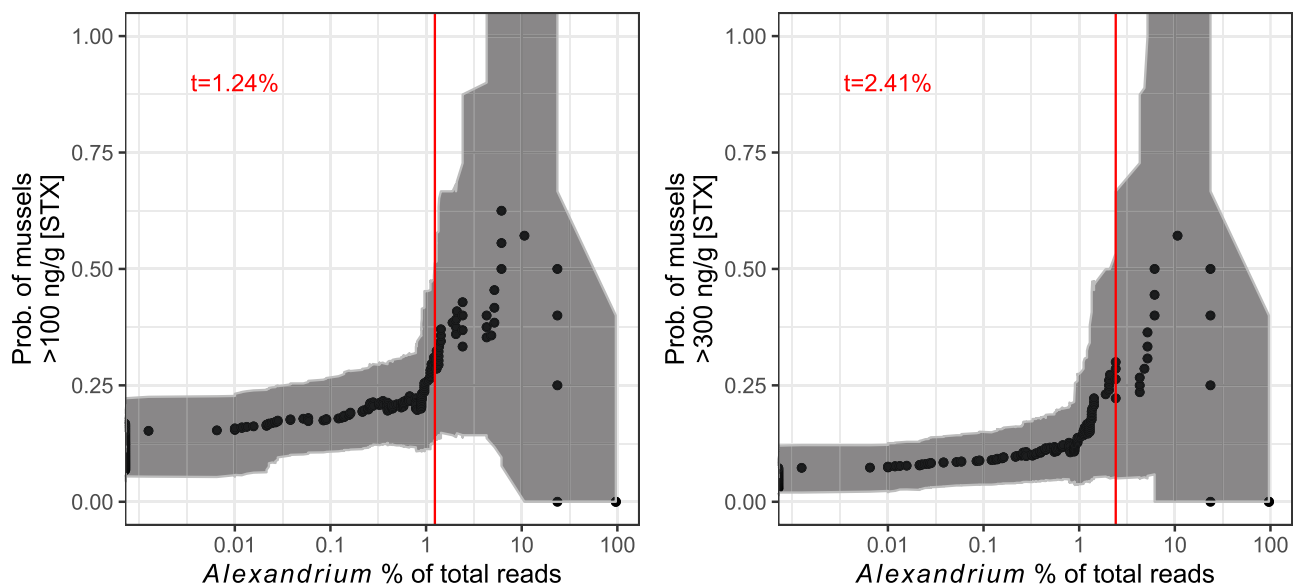


Fig. 5. Conditional probability of saxitoxin (STX) exceeding ecological thresholds as a function of increasing *Alexandrium* abundance. The relative abundance when there is a 50 % likelihood of nearby mussels exceeding the set threshold is shown as a red line. The “ $t=$ ” annotations indicate the percent abundance threshold for each analysis.

4.1. No evidence for “proportionate increase” response with increasing chl-*a*

We used a molecular monitoring approach to investigate evidence for both the “proportionate increase” versus the “disproportionate increase” hypotheses. We found that the “proportionate increase” hypothesis does not appear to be applicable to the HAB-forming taxa common to SFB. In fact, the density of *Alexandrium* and *Pseudo-nitzschia* declined in the qPCR abundance data at the highest chl-*a* concentrations. In SFB, as well as many similar temperate estuaries, the spring phytoplankton bloom is typically dominated by diatoms like *Thalassiosira* or *Skeletonema* (Caffrey et al., 1998; Smayda and Trainer, 2010; Tiselius and Kuylensstierna, 1996) which do not pose known human health risks and are considered an important food source for primary consumers (Glibert et al., 2011). At chl-*a* levels above 10 $\mu\text{g/L}$, such as during the spring bloom, *Alexandrium* appears to be outcompeted by other taxa (Fig. 4). Additionally, higher spring chl-*a* does not always correlate with increased mussel toxin. Saxitoxin is largely uncorrelated with chl-*a*, or even declines at the highest chl-*a* levels (Fig. S4). Therefore, population dynamics of HAB-forming taxa in SFB can be decoupled from the growth or loss trajectory of the overall phytoplankton community.

Alexandrium proliferates at high chl-*a* values in summer, but the summer increase has very large confidence intervals due to the sparse sampling of *Alexandrium* in that season. Saxitoxin concentrations in mussels, meanwhile, were highest mostly in the spring (Fig. S3), but only at low to moderate spring chl-*a* values (Fig. S4). While the dilution effect of increased availability of food as well as greater grazing selectivity of mussels during the spring bloom could explain the lower overall toxin concentration in mussels at the highest spring bloom chl-*a* values (Strohmeier et al., 2005), it is more likely that a genuine decline in *Alexandrium* abundance accounts for this lower toxin concentration. This is evidenced by the fact that qPCR counts for *Alexandrium* decline to near-zero during the spring bloom (Fig. 4B). *Alexandrium* in SF Bay has previously been proposed to thrive in times of higher salinity (Cloern and Dufford, 2005), so it is possible that *Alexandrium* is unable to compete in the lower salinity conditions associated with March and April, when the diatom-dominated spring bloom typically occurs (Cloern, 1984; Cloern and Jassby, 1995).

4.2. Selected SFB HAB taxa show a disproportionate response to increasing chl-*a*, but only during some seasons

Our analysis shows some taxa are disproportionately favored with increasing chl-*a* during some seasons. This study as well as previous work have shown HAB-forming genera such as the raphidophyte *Heterosigma akashiwo* and some dinoflagellates like *Dinophysis*, *Heterocapsa*, *Gonyaulax*, and *Gyrodinium* generally show lower probability of proliferating above their baseline relative abundance at higher chl-*a* levels in the spring, but increase as a function of chl-*a* in the fall, when chl-*a* levels were historically low (Fig. 6)(Cloern et al., 2005; Kudela et al., 2024). Comparing literature expectations of the behavior of higher-order groups, many of the studied dinoflagellates and the raphidophyte *Heterosigma* do follow the patterns expected of their groups, though *Alexandrium* and *Noctiluca* diverge from that pattern (Table 5). Dinoflagellates and raphidophytes share flagellated morphologies and a mixotrophic life history, which may incline them to proliferate in the late summer, a time of high insolation and higher salinity, with less salinity stratification that favors diatoms (Cloern et al., 2017). Under these conditions, flagellated taxa may benefit from being able to swim to remain in the photic zone during the daylight hours and travel throughout the water column at night scavenging nutrients. Additionally, *Heterosigma* and the selected dinoflagellates are all mixotrophic (Burkholder et al., 2008; Jeong, 2011) and thus able to source nutrients from various dissolved phases or by ingesting bacteria or picoplankton at a time of year when nutrients are lower than during the spring bloom interval (Cloern, 1996; Peterson et al., 1985).

These flagellated mixotrophic harmful algae vary in their prevalence, with *Heterosigma*, *Dinophysis* and *Gonyaulax* usually appearing below 1 percent of reads, while *Heterocapsa* and *Gyrodinium* commonly reach 2.5 and 4.4 % of reads respectively (Table 3). However, each taxon poses its own respective risks to the SFB ecosystem risks when environmental conditions present a “window of opportunity” for a bloom to occur (Moore et al., 2008). *Dinophysis*, *Gonyaulax* and *Gyrodinium*, for example, produce known toxins (Gentien and Arzul, 1990; Reguera et al., 2014; Riccardi et al., 2009), while *Heterosigma* and *Heterocapsa*’s known mechanisms of harm are less understood but are still associated with biomass-linked anoxia (Allaf and Trick, 2024; Wu et al., 2022), and *Heterosigma* is believed to produce an as-yet unidentified ichthyotoxin (Cembella et al., 2023). SFB is characterized by high ambient nutrient

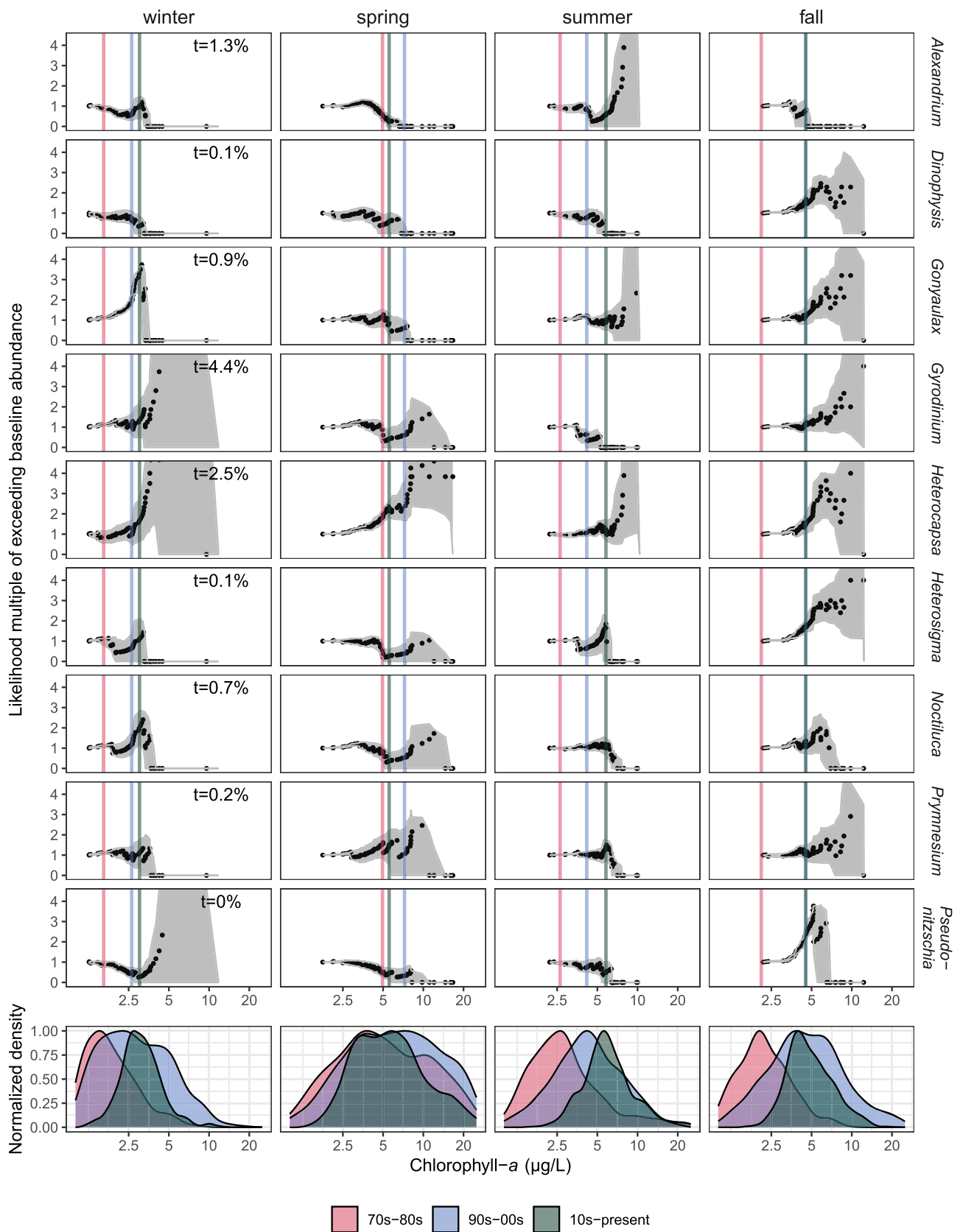


Fig. 6. Conditional probability curves for nine harmful algae of concern in San Francisco Bay, faceted by season. The abundance thresholds are set at the 87th percentile of relative abundance for each taxon. The percent abundance represented by the 87th percentile is listed for each row as “t=”. Below, the distributions of chlorophyll a (chl-a) concentration for different time periods are shown as kernel density functions, grouped into 1970s-1980s, 1990s-2000s, and 2010s-present, sourced from the long-term cruise monitoring data collected since 1969. Colored vertical lines extending above indicate the medians of each group for comparison to the conditional probability plots.

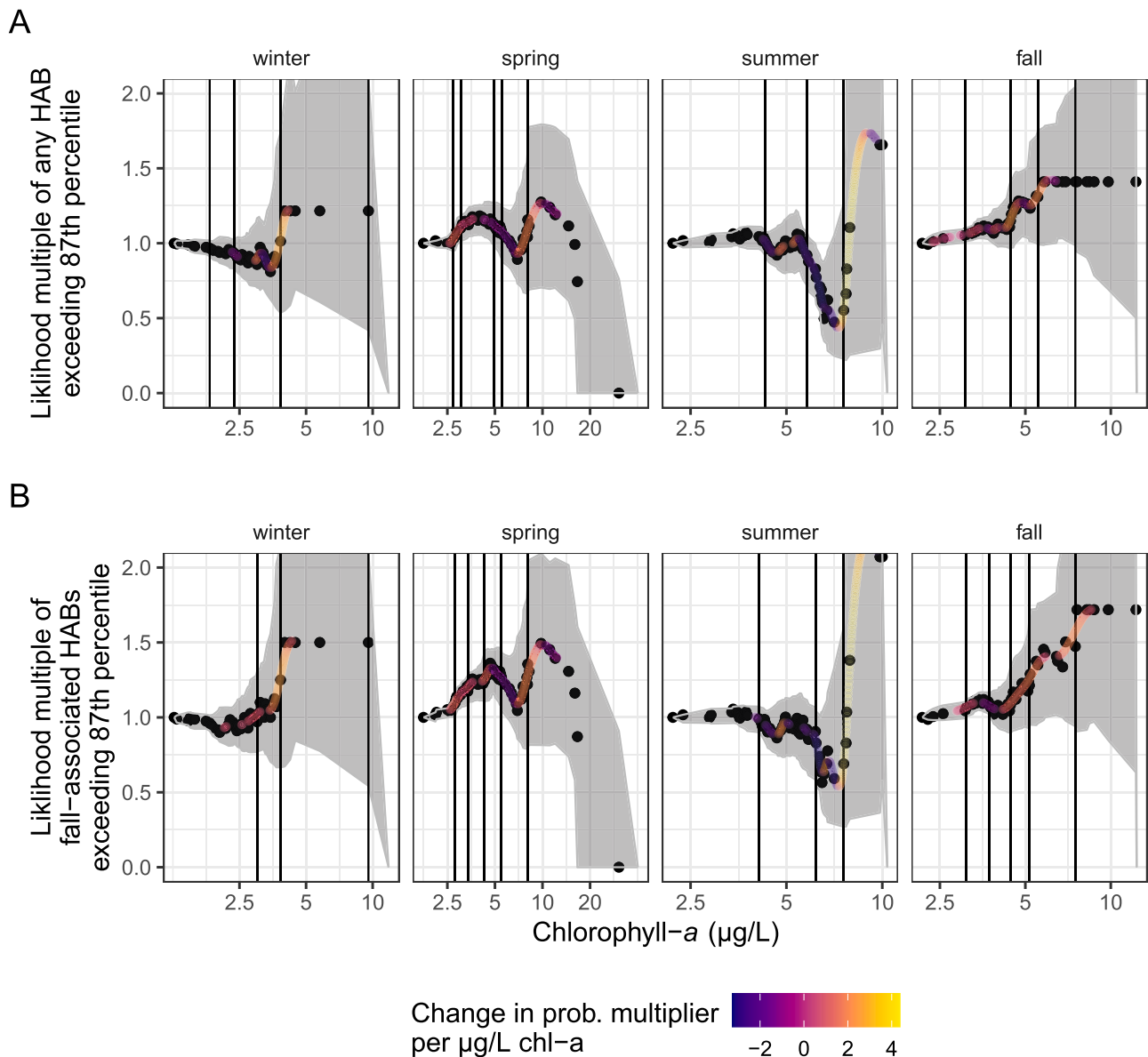


Fig. 7. A. Change in likelihood of any of the studied HAB-forming taxa increasing above its baseline (87th percentile) abundance as a function of chlorophyll a (chl-a) concentration in fall. Vertical black lines indicate statistically significant changepoints. The list includes *Alexandrium*, *Pseudo-nitzschia*, *Heterosigma*, *Dinophysis*, *Gonyaulax*, *Gyrodinium*, *Heterocapsa*, *Prymnesium* and *Noctiluca*. The colored line through the points represents a generalized additive model (GAM) function relating the likelihood to the chl-a value, with the color corresponding to the instantaneous slope of the GAM function at that chl-a value. Fall is the only season where the probability increases consistently across the chl-a distribution. B. Change in likelihood of fall-associated harmful taxa including *Heterosigma*, *Dinophysis*, *Gonyaulax*, *Gyrodinium*, and *Heterocapsa* increasing above 87th percentile as a function of chl-a.

concentrations, (e.g., 2014–2019 median dissolved inorganic nitrogen concentrations in excess of 30 μM in South Bay and Lower South Bay), indicating incomplete nutrient assimilation by phytoplankton and the potential to support additional phytoplankton biomass (Cloern et al., 2020; Sutula et al., 2017), a response that would have significant water-quality implications. This scenario occurred in the summer of 2022, when ideal conditions resulted in the exponential, uncontrolled proliferation of *Heterosigma akashiwo* in SFB, causing widespread ecological impacts including events of low dissolved oxygen and mass fish kills (Kudela et al., 2024; Nardelli et al., 2026). The release of constraining factors such as mixing and benthic grazing may change abruptly over time, leading to surprisingly large increases or decreases of biomass as a phenomenon that has occurred in other estuaries (Raimonet and Cloern, 2017). This heightened risk for HAB biomass accumulation in SFB points to the benefits of establishing chl-a thresholds (Sutula et al., 2017).

4.3. Evidence for a chl-a threshold associated with fall occurrence of flagellated mixotrophs

Our findings suggest a relationship between chl-a values above 4.6 $\mu\text{g/L}$ in fall and elevated relative abundance of *Heterosigma*, *Dinophysis* and other flagellated, mixotrophic harmful algae. This chl-a concentration is low in the context of year-round variability, but for fall, it was historically in the upper quantile of observed values. As chl-a concentrations have gradually increased in SFB (Beck et al., 2022; Cloern et al., 2020), those values have become more commonly observed in the late summer/early fall, and now represent the mode, with the majority of observed chl-a values clustering around that value in the last 20 years (Fig. 6). As these chl-a levels have become more common in recent years, it is therefore unsurprising that an unprecedented baywide high biomass event occurred in SFB in 2022, causing impacts to ecosystem health (Kudela et al., 2024). Looking at the combined probability of any of the

Table 5

Comparing the dominant season identified from literature for different high level groups in temperate estuaries from previous literature, to the results from this study on which genera follow those expectations as measured by molecular monitoring.

Taxon	Dominant season	HAB genera that follow/diverge from dominant season	
		Follows	Diverge
Diatoms	Spring (Caffrey et al., 1998; Smayda and Trainer, 2010)		<i>Pseudo-nitzschia</i>
Dinoflagellates	Late Summer/Fall (Caffrey et al., 1998; Smayda and Trainer, 2010)	<i>Dinophysis</i> , <i>Gyrodinium</i> , <i>Gonyaulax</i> , <i>Heterocapsa</i>	<i>Alexandrium</i> , <i>Noctiluca</i>
Raphidophytes	Late Summer/Fall (Li and Smayda, 2000)	<i>Heterosigma</i>	
Haptophytes	Inconsistent/lack of seasonality (Jordan and Chamberlain, 1997)	<i>Prymnesium</i>	

studied HAB taxa exceeding their baseline abundance, we see the probability increases consistently as a function of chl-*a* concentration in fall, but we see no consistent up or down trend in probability in other seasons (Fig. 7A). When this combined probability is computed for only taxa which individually showed increases in exceeding their baseline abundance in the fall, such as *Heterosigma*, *Dinophysis*, *Heterocapsa*, *Gyrodinium* and *Gonyaulax*, the slope is more drastic and converges on a higher multiple of baseline probability (Fig. 7B).

The threshold discussed in this paper (4.6 µg/L in fall) is lower than the previous year-round level of 13 µg/L (Sutula et al., 2017). That prior threshold was intended to be protective against biomass-related HAB hazards like low dissolved oxygen, and included spring data, which as this study discusses, is dominated by diatoms not associated with HAB risk. So instead of being a threshold of concerning conditions, the fall threshold discussed in this study of 4.6 µg/L is intended as a seasonally-specific indicator of conditions associated with above baseline abundance of HAB-forming taxa.

4.4. Thresholds of relative metabarcoding and qPCR molecular HAB abundances in relation to mussel toxin concentration

The highest mussel saxitoxin concentrations corresponded with the highest relative abundances of *Alexandrium* in the dataset as measured by 18S metabarcoding (Fig. 3). These high mussel saxitoxin concentrations occurred over several months in 2018, and mussel concentrations were in excess of 300 ng/g, a level associated with neurotoxic effects in the mussels themselves, including reduced feeding and slowed ability to close their valves (Lavaud et al., 2021). This 2018 window also was the only time point when saxitoxin in our dataset surpassed the human health advisory threshold of 800 ng/g, which was also observed in ongoing mussel monitoring by the CDPH and triggered a public health advisory at that time (Egel, 2018).

These data highlight the importance of identifying early warning or screening level thresholds of qPCR abundance or relative abundance. The relative (as measured by metabarcoding) and qPCR abundances of *Alexandrium* both show positive relationships with the probability of saxitoxin observation in nearby mussels. This points to the utility of thresholds like 1.24 % of total reads derived via metabarcoding (Fig. 5) or 1000 counts per ml derived via qPCR as early warning or screening values for potential shellfish consumption risk, either to humans or aquatic life (Fig. 4C).

A subset of observations had high *Alexandrium* relative abundances, when mussel toxin was below thresholds (Fig. 5). This may have resulted from the occurrence of non-toxic *Alexandrium* species that were not captured by our genus-level analyses but are known to occur in SFB (Cloern and Dufford, 2005), or due to variability in toxin production in

relation to other environmental/ecological factors such as nutrient availability, temperature, and salinity, which can increase or decrease its comparative toxicity (Murray et al., 2021). Additionally, the gene copies present per cell for dinoflagellates like *Alexandrium* can vary in time (Ruvindy et al., 2023), and during times of high dinoflagellate population, there can be dynamics of cross-reactivity during PCR between closely related groups (Coyne et al., 2022). Lastly, there is the possibility that during the interval of greatest *Alexandrium* relative abundances, which occurred in February 2020, the mussels, which were sampled only a day later at nearby sites, had simply not yet accumulated saxitoxin. All of these factors point out that further species-focused research into *Alexandrium*-mussel interactions, ideally using corroborating data like qPCR, is warranted, as well as testing for saxitoxin genes.

An *Alexandrium* qPCR abundance of 1000 counts per ml could be of potential future use as an early warning threshold. Previous studies found that ingested intracellular saxitoxin represents 99 % of the toxin fraction present in mussels (Li et al., 2024), so it is likely that the association between *Alexandrium* 18S metabarcoding detections and toxin in mussels results from higher *Alexandrium* cell counts in the environment rather than high extracellular toxin concentrations. When mussels feed on phytoplankton assemblages with low relative abundance of *Alexandrium*, they would be expected to accumulate lower concentrations of toxin in their tissues, due to the dilution effect of other non-toxic phytoplankton in their diet.

4.5. Setting thresholds of baseline relative abundance: the value of probabilistic approaches for interpreting molecular data

Metabarcoding approaches can be powerful for the detection of rare taxa, which are elusive to other monitoring methods. The methods are also reproducible and the resulting sequences can be reanalyzed as taxonomic classifiers improve. Despite this, they have not been as extensively used for monitoring harmful algae as microscopy because of certain limitations inherent in the technique. Because of the amplification of rRNA genes from the sample, the data can only be used to interpret relative abundance, rather than absolute abundance. There are also sources of uncertainty that complicate efforts to interpret trends in metabarcoding relative abundance. Imperfect taxonomic databases lead to potential clade jumping (Cirtwill and Hambäck, 2021), the multiple 18S gene copies observed in many taxa can inflate their relative abundance (Marinich et al., 2023), different amounts of source genetic material might be sourced from filters on different days, and the process of polymerase chain reaction itself is subject to chance processes leading to some taxa being preferentially amplified (Bachy et al., 2013; Logares et al., 2014). One preferable way to control this noise is to search for thresholds of relative abundance, seeing if the probability that taxa exceed that threshold is a function of chl-*a* or other continuous explanatory variables. In effect, this discretizes the dependent variable (Lucena-Moya et al., 2015) and allows probabilistic analysis of a binary output, rather than a continuous variable, which is subject to numerous sources of noise. In the case of this study, the threshold can be set to represent a taxon's baseline abundance, with any observations above that baseline being potentially indicative of increased population growth. The challenge is understanding how to set the baseline threshold in an ecologically relevant and standardized way. The threshold could be set through iterative testing of abundance thresholds for each taxon, finding the thresholds resulting in the greatest slope as a function of chl-*a*, but that risks amplifying biases in detection, such as the possibility that rare taxa are inordinately less likely to be observed during times of low chl-*a*.

In the case of the risk modeling of this study, we selected *Alexandrium*'s relative abundance as the standard measure by which to compare the proliferation of the other HABs. *Alexandrium* is known to be regularly present in SFB (Cloern and Dufford, 2005), its toxin is measured as part of our monitoring, and most importantly, its toxin accumulates to levels known to be harmful to biota and humans. The

relative abundance level of 1.2 % of reads represented the level at which 100 ng/g toxin was >50 % likely to be detected in nearby mussels. That abundance corresponded to the 87th percentile of all *Alexandrium* abundances in the dataset, which was used as a threshold by which to assess if other taxa were above or below their respective baseline abundances. This 87th percentile relative abundance threshold serves as an indicator of proliferation above baseline relative abundance, meriting a monitoring response, but the approach of this study could be expanded as new data is gathered. As techniques quantifying other taxa of concern in SFB are developed, such as a targeted assay for *Heterosigma* absolute abundance, those can be used to expand the approach to set abundance thresholds individualized to those taxa. In that way, this probabilistic approach can continue to improve in terms of relating broad-spectrum metabarcoding measures of abundance to the results of targeted but less scalable monitoring approaches.

There is uncertainty in the specific risk relationships produced by this approach, both due to the bootstrapping-based confidence intervals, but also the specific risk thresholds identified, which were a function of the years analyzed. The metabarcoding abundances of some taxa do not relate well to microscopy-based estimates of relative abundance, such as -for *Pseudo-nitzschia* (Fig. S1A), despite showing relationships with the qPCR-based analyses (Fig. S1C). This parallels prior comparative studies, which found that microscopy and molecular surveillance approaches could be complementary (Chen et al., 2024), but with weaknesses in sensitivity and specificity leading each method being insufficient on its own to fully understand phytoplankton population dynamics. This may be particularly true for rare or cryptic taxa like some of the dinoflagellate HAB-forming taxa mentioned in this study, and also *Pseudo-nitzschia* (Turk Dermastia et al., 2023). As additional data are collected and compiled for SFB and quantified with molecular or microscopy data, new taxa of concern could be identified, since phytoplankton can wax and wane over decadal intervals (Cloern, 1996; Harding et al., 2016), and their bloom events can be ephemeral and thus rarely observed even over multiple year monitoring programs. As new bloom events are measured, different thresholds of concern may be identified, and our *Alexandrium*-based threshold could be supplanted by an alternative measure of HAB-related risk. Thus, the type of analysis this paper demonstrates could serve as a framework for managers to use to re-evaluate the relative risk of HABs in an estuary where drivers and responses change over time.

4.6. Implications for future analysis and risk assessment in SFB and other systems

While this study focused on assessing risk of HAB proliferation as a function of chl-*a* in SFB, this approach could be applied to other explanatory variables of interest in this setting and other systems. This approach could also be used to investigate the conditional probability of phytoplankton proliferating in SFB in response to other variables also measured during the channel cruises, such as temperature, light availability, salinity and nutrient concentrations. These analyses could be combined in a unified “growth index” integrating such factors and relating them to the conditional probability of phytoplankton growth. Such relationships have often been investigated via empirical approaches and models (Cloern, 1999; Ralston and Moore, 2020), but the benefit of the probabilistic approach is that it allows for modeling of the increased probability of target taxa proliferating above baseline relative abundance.

Water bodies in other regions have been found to display long-term trends in chl-*a*, such as Narragansett Bay, Rhode Island, which has had a long-term decline in biomass, with chl-*a* decreasing by 49 % since 1959 (Thibodeau et al., 2024). The ‘proportionate increase’ hypothesis would suggest that the risk of all HABs would have declined during that interval, but instead, a new hazard of *Pseudo-nitzschia* and associated domoic acid has emerged in summer, which researchers have proposed was decoupled from chl-*a* and largely linked to nitrogen limiting

conditions (Sterling et al., 2022). While this study used chl-*a* to estimate the relative risk of harmful algae proliferation, other settings like Narragansett Bay could similarly use nutrient concentrations or other explanatory variables to predict HAB risk. Other examples include Baffin Bay, Texas, where elevated salinity correlates with increased abundance of *Aureoumbra lagunensis* (Cira et al., 2021), and the West Florida shelf, where there are links between *Karenia* and the abundance of other phytoplankton groups like haptophytes (Dixon et al., 2014). Indices combining multiple factors could also be related to the conditional probability of harmful algal taxa exceeding abundance thresholds to improve the ability to estimate risk of target harmful algae proliferating.

In SFB, the association between higher chl-*a* and increased relative abundance of key HABs in fall could be used to identify when relative abundances of harmful algae have the potential to begin increasing nonlinearly as well as recurrence intervals of bloom events. Chl-*a* has increased through time at several of the channel cruise monitoring stations (Beck et al., 2022). While changes in the summer chl-*a* value have not tracked directly to climate change related indicators in SFB, there have been changepoints identified in the seasonality of bloom events, such as a long-term increase in summer chl-*a*, which has been linked by some researchers to the abundance of grazing invasive clams (Cloern et al., 2023). Generally, the probabilistic approach can be used to extrapolate the future probability of harmful algae increasing as a function of variables that are more easily monitored, such as chl-*a* concentration.

5. Conclusion

Seasonal variations in chl-*a* were associated with unequal changes in the abundance of a variety of harmful algae. Several harmful dinoflagellate and raphidophyte taxa increased disproportionately in the fall and usually declined during the annual spring bloom event. In SFB in fall, the probability that certain harmful taxa are abundant increased when chl-*a* exceeded approximately 4.6 µg/L, a concentration that has been observed with increasing frequency during ship-based cruises as summer/fall blooms have become more common.

The molecular surveillance approach described here shows that the raphidophyte *Heterosigma* and flagellated, mixotrophic dinoflagellates with similar traits have a higher potential for growth during summer/fall. Combining molecular metabarcoding surveillance with mussel-based toxin monitoring allows us to set risk thresholds, which can be used to construct risk-based models based using parameters commonly measured during ship-based surveys, such as chl-*a*. This work shows the advantages of integrating traditional methods of water quality assessment like chl-*a* with markers of ecological status such as metabarcoding data. A probabilistic approach can be used to inform a framework to assess the risk for proliferation of a broad assemblage of harmful taxa, with capabilities continuing to improve as molecular libraries expand and new, more sensitive measurement techniques are developed. Future work could refine risk assessments by using multiple combined factors to quantify HAB probabilities, with the goal to quantify the risk of bloom events harmful to human and ecosystem health and inform development of policies to reduce the risk.

CRedit authorship contribution statement

Daniel Killam: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Keith Bouma-Gregson:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Martha Sutula:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Raphe Kudela:** Writing – review & editing, Resources, Methodology, Investigation. **James Hagy:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Stephanie Anderson:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **David**

Senn: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, 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.

Acknowledgments

Erica Nejad, Joel Fritsch, and other U.S. Geological Survey staff collected samples during long-term R/V Peterson channel monitoring cruises. Timothy Otten and staff (Bend Genetics) conducted 18S and qPCR analyses. Kristin Art (San Francisco Estuary Institute), Chris Ikeda (San Francisco State University, Cochlan Lab) and others collected mussels from docks. Kendra Negrey and others (UC Santa Cruz) conducted toxin analyses on mussels. Funding was provided by the San Francisco Bay Nutrient Management Strategy, NOAA MERHAB Award NA23NOS4780288, and the U.S. Geological Survey Priority Ecosystems Science Program. The views expressed in this manuscript are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Any mention of trade names, products, or services does not imply an endorsement by the U.S. Government or the U.S. Environmental Protection Agency. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.hal.2026.103086](https://doi.org/10.1016/j.hal.2026.103086).

Data availability

Data are available on the Environmental Data Initiative (Killam et al., 2025), an open access repository, and analysis code is archived on Zenodo (Killam, 2026). Metabarcoding reads are deposited in the National Center for Biotechnology Information (NCBI) Sequencing Read Archive (SRA) (Sayers et al., 2025) under bioproject PRJNA1425045.

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