Describing presence and abundance of *Alexandrium catenella* with co-occurring environmental conditions in the Puget Sound

Madison Gard

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Marine Affairs

University of Washington

2026

Committee:

Terrie Klinger

Ryan P. Kelly

Elizabeth Andruszkiewicz Allan

Pedro FP Brandão Dias

Program Authorized to Offer Degree:

College of the Environment

University of Washington

Abstract

Describing presence and abundance of *Alexandrium catenella* with co-occurring environmental conditions in the Puget Sound

Madison Gard

Chair of the Supervisory Committee:
Terrie Klinger
School of Marine and Environmental Affairs

Harmful algal blooms (HABs) produced by the marine dinoflagellate *Alexandrium catenella* pose significant risks to public health, marine ecosystems, and shellfish industries in Puget Sound due to the species' production of paralytic shellfish toxins (PSTs). Despite ongoing monitoring efforts, uncertainty remains regarding the environmental drivers and spatio-temporal variability of blooms. Here, I investigated patterns of presence and abundance of *A. catenella* using a multi-year environmental DNA (eDNA) dataset collected by the Washington Ocean Acidification Center (WOAC) from 2019 to 2024. Water samples from 14 stations across five basins were analyzed using droplet digital PCR (ddPCR) to quantify concentrations of *A. catenella*. Environmental associations were evaluated using Bayesian hurdle models and multivariate clustering approaches incorporating temperature, salinity, pH, dissolved oxygen, and dissolved inorganic nitrogen (DIN). Presence of *A. catenella* varied across sampling months, with higher detection probabilities observed in July and September relative to April. Interannual variability was also evident, with increased presence of *A. catenella* in recent years.

Environmental analyses controlling for temporal variability (month and year) indicated that presence of *A. catenella* was positively associated with DIN and dissolved oxygen, while the abundance was most strongly linked to oxygen concentration. Cluster analysis revealed that detections were more likely to occur under nutrient-rich, marine-influenced conditions. Spatially, *A. catenella* was detected more frequently and in higher abundances in northern and central Puget Sound. Findings demonstrate that *A. catenella* population dynamics are driven by multiple interacting environmental and seasonal factors rather than individual drivers. This study highlights the value of eDNA-based monitoring for detecting HAB species and characterizing favorable environmental conditions, with implications for improved management under a changing climate.

TABLE OF CONTENTS

Introduction

Harmful algal blooms (HABs) driven by eutrophication pose an increasing threat to coastal ecosystems, public health, and marine economies worldwide (Glibert et al., 2005). In Washington State, these risks are particularly significant in Puget Sound, where shellfish harvesting supports important subsistence, cultural, recreational, and commercial activities (L. E. Anderson & Plummer, 2017; Lepofsky et al., 2015; Trainer & Hardy, 2015). Marine resources are increasingly threatened by declining water quality and the proliferation of toxin-producing phytoplankton (D. M. Anderson et al., 2002; Howarth et al., 2002; Trainer & King, 2023). Under certain favorable environmental conditions, some algal species can rapidly multiply, forming HABs that can then introduce potent biotoxins into marine food webs (Cornett et al., 2024; Fu et al., 2012). These biotoxins accumulate in filter-feeding organisms such as shellfish and are transferred to higher trophic levels, including humans, through predation and human consumption of seafood (Erdner et al., 2010). Effective monitoring of HABs and their associated toxins is therefore critical for maintaining seafood safety, ecosystem health, and the economic viability of shellfish industries in the region (Hallegraeff, 1993; Moore, Johnstone, et al., 2015; Trainer & King, 2023).

Among the most consequential HAB taxa within Puget Sound is the dinoflagellate *Alexandrium catenella*, a producer of paralytic shellfish toxins (PSTs). PSTs comprise a suite of neurotoxins synthesized by *Alexandrium*, including one of the most potent naturally known occurring toxins, saxitoxin (Anderson et al., 1990). Saxitoxin is produced as a defense to reduce predation by zooplankton such as copepods by inhibiting the nervous system of such grazers (Deng et al., 2025). When these toxic compounds accumulate in shellfish tissues and are consumed they can cause paralytic shellfish poisoning (PSP) in humans, with symptoms ranging

from tingling and gastrointestinal distress to respiratory paralysis and death (Louzao et al., 2022). As a result, blooms of *A. catenella* represent a persistent concern for public health officials, marine resource managers, and shellfish growers.

The ecological success and recurrence of *A. catenella* blooms are linked to its life history strategies and morphological traits. Although *Alexandrium* is often present in Puget Sound at low background abundances, blooms occur under favorable conditions and are characterized by sudden, rapid population growth. Similar to other dinoflagellates, *A. catenella* has a complex life cycle that includes a dormant overwintering cyst stage in benthic sediments (D. M. Anderson et al., 2012; Greengrove et al., 2012). These dormant cysts persist for extended periods under unfavorable conditions and later germinate to initiate blooms once environmental conditions become suitable. Cyst distributions in sediments have been shown to serve as a "seed bank" for bloom initiation and are often spatially correlated with recurrent bloom hotspots (Greengrove et al., 2012; Ruvindy et al., 2018; Tobin & Horner, 2011). In Puget Sound, the interaction between cyst dynamics, local hydrography, and environmental variability plays a key role in structuring bloom timing, intensity, and spatial distribution (Fu et al., 2012; Mudge et al., 2025).

Long-term monitoring programs have been developed to mitigate the risks associated with PSTs. In Washington State, the Department of Health conducts routine biotoxin monitoring using sentinel mussel deployments across Puget Sound (Feifel et al., 2012; Trainer & King, 2023). In this monitoring program, mussels are periodically sampled and tested for PST concentrations, and shellfish harvesting areas are closed when toxin levels exceed regulatory thresholds (Louzao et al., 2022; Trainer & King, 2023). While this system has been fairly effective for protecting public health, it relies on labor-intensive sampling and can involve delays between toxin accumulation and detection, with additional lags between detection and

management actions such as temporary shellfish harvest closures. As HAB frequency and variability increase under changing environmental conditions, there is growing interest in developing more rapid and sensitive monitoring approaches.

Recent advances in molecular techniques, such as environmental DNA (eDNA) analysis and droplet digital PCR (ddPCR), offer promising alternatives for HAB detection and monitoring. These methods allow for the direct detection and quantification of target species from water samples, potentially enabling earlier identification of bloom development before toxin levels in shellfish become hazardous (Cameron et al., 2026; Gallego et al., 2020; Jacobs-Palmer et al., 2021; Te et al., 2015). Integrating molecular approaches with existing monitoring frameworks could enhance early warning capabilities and improve management responses.

Environmental and seasonal factors play a central role in regulating population dynamics of *A. catenella*. In Puget Sound, blooms are typically associated with the warmer temperatures, stratification, and nutrient dynamics of late summer to early fall (Fu et al., 2012; Moore, Johnstone, et al., 2015). In laboratory studies, growth rates of *A. catenella* increased above 17–18°C, and bloom initiation has been linked to temperature thresholds near 13°C (Bill et al., 2016). Climate-driven changes, including ocean warming, enhanced stratification, and ocean acidification are expected to further influence bloom frequency, distribution, and toxicity (Feifel et al., 2012; Greengrove et al., 2012). These changes may exacerbate HAB impacts by promoting conditions favorable for dinoflagellate growth and toxin production (Hallegraeff, 1993; Jacobs-Palmer et al., 2021).

Despite extensive monitoring efforts, important questions remain regarding the spatial and temporal variability of *A. catenella* in Puget Sound along with the environmental conditions that influence its presence and abundance. Puget Sound is a complex fjord-like estuarine system

composed of multiple interconnected basins (e.g., Main Basin, Whidbey Basin, South Sound), each with distinct hydrographic and biogeochemical characteristics that can shape bloom development. Many of Washington's commercially and recreationally important shellfisheries are concentrated in these basin-specific environments, making it critical to understand how bloom dynamics vary across space and time. Although existing monitoring programs provide valuable information for public health protection, resolution is limited for predicting early bloom development, highlighting the need for approaches that can efficiently capture fine-scale variability in *A. catenella* population dynamics.

To address these gaps, this study leverages a multi-year environmental DNA (eDNA) dataset collected by the Washington Ocean Acidification Center (WOAC) to examine patterns of *A. catenella* presence and abundance across Puget Sound. Quantitative molecular methods such as droplet digital PCR (ddPCR) offer a rapid, sensitive, and potentially more cost-effective alternative to traditional mussel-based toxin assays, enabling earlier detection of harmful algal species in the water column. Here, observations of *A. catenella* are analyzed alongside co-occurring environmental conditions to identify key drivers of its presence and abundance throughout Puget Sound. This approach provides a framework for improving HAB monitoring and forecasting in Puget Sound and demonstrates the broader applicability of molecular tools for HAB management in coastal systems.

Methods

2.1 eDNA Sampling and Environmental Data Collection

Water samples were repeatedly collected by the Washington Ocean Acidification Center (WOAC) during ship-based cruises in April, July, and September from 2019-2024. A CTD-rosette sampler was deployed at each station to collect 1L of water from the surface, middle, and

bottom layers of the water column. Each water sample was filtered through a cellulose acetate filter (0.45 µm pore size, 47 mm diameter) to capture environmental DNA (eDNA). Filters were subsequently preserved and held at room temperature in 1 mL of Longmire's buffer to prevent DNA degradation prior to extraction (Renshaw et al., 2015). Environmental data—including water temperature (°C), salinity (PSU), pH, dissolved oxygen (mg/L), and dissolved inorganic nitrogen (umol/L)—was collected simultaneously with water samples using the conductivity-temperature-depth (CTD) sensor on the rosette. Seawater carbonate chemistry and nutrient analyses were performed by NOAA Pacific Marine Environmental Laboratory (NOAA/PMEL) and the Marine Chemistry Lab at the University of Washington's School of Oceanography (Alin et al., 2024). Sampling was performed at 14 stations (Table 1) across 5 basins (Figure 1) from 2019-2024.

Table 1. Sample stations. Stations were sampled in April, July, and September for 6 years (except for April 2019 and 2020).

Station	Station Name	Latitude	Longitude
P1	Gedney Island	48.0165	-122.3045
P4	Skagit Bay	48.2423	-122.5527
P7	Mutiny Bay	47.9818	-122.6195
P8	Hood Head	47.8970	-122.6050
P10	Hood Canal Sill	47.8007	-122.7185
P11	The Great Bend	47.3708	-123.1330
P12	Hoodsport	47.4232	-123.1092
P14	Hood Point	47.6077	-122.9388
P22	Eastern Bank	48.2708	-123.0207
P28	North of West Point	47.7005	-122.4555
P29	South of Alki Point	47.5563	-122.4422
P36	Devils Head	47.1682	-122.7860
P38	Carr Inlet	47.2750	-122.7067
P402	Sisters Point	47.3567	-123.0235

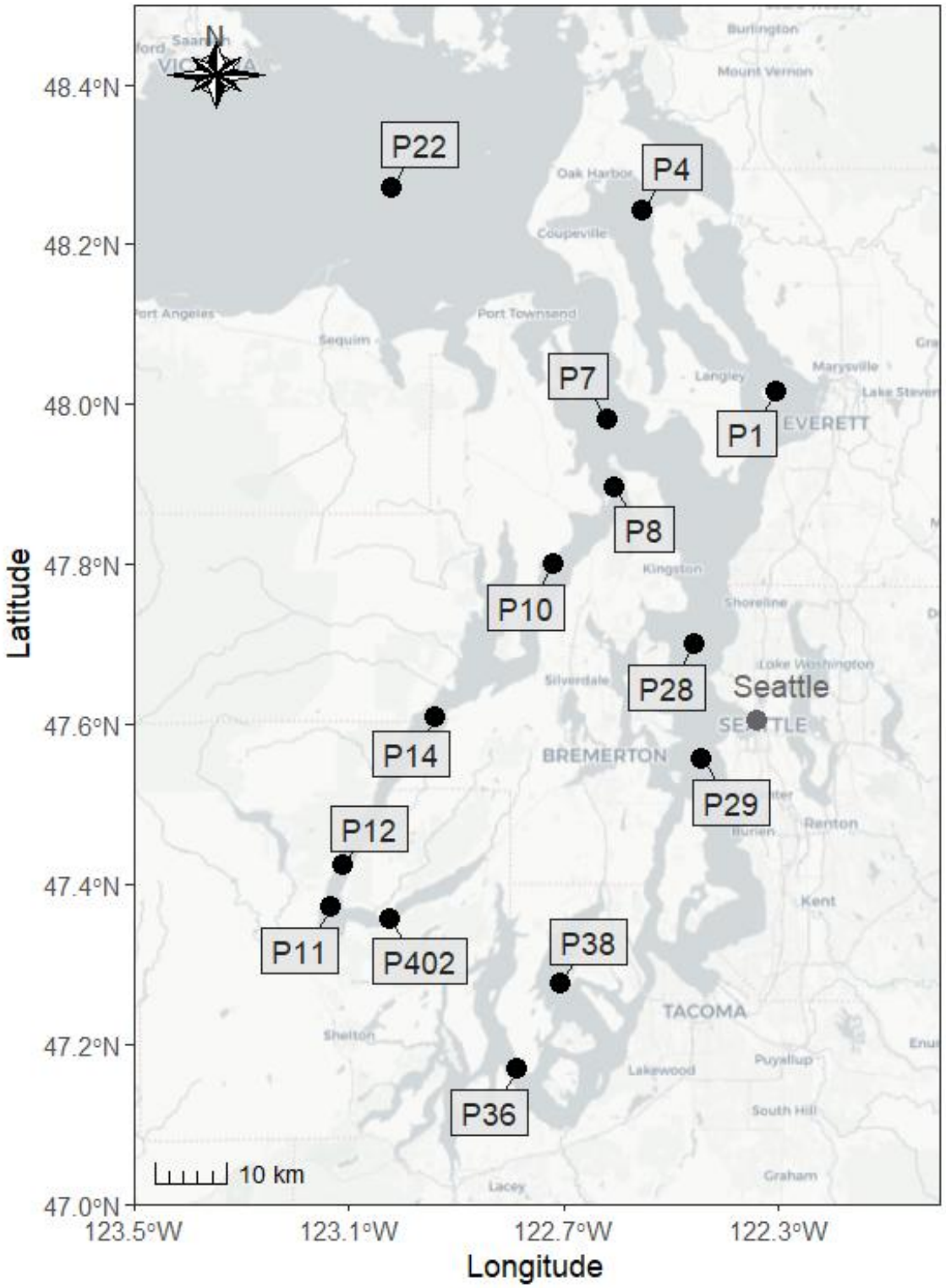


Figure 1. Map showing sampling stations within Puget Sound, located in Washington State.

2.2 Laboratory Methods

2.2.1 eDNA PCI Extraction

eDNA extraction from preserved filters followed the phenol:chloroform:isoamyl (PCI) protocol described in Jacobs-Palmer et al. (2021). Extraction blanks were made with each batch of extractions and served as negative controls throughout the workflow to test for cross contamination between samples. To ensure maximal DNA yield and reduce phenol contamination in the final sample, a phase lock was used to separate organic and aqueous layers with a non-reactive silicone-based grease (Dow Corning, Midland, MI, USA, (Murphy & Hellwig, 1996; Ramón-Laca et al., 2021). The phase lock separated and sealed the organic layer below the grease layer during centrifuge protocol steps which allowed for easy removal of the aqueous layer without contamination. Extracted eDNA (500 µL) was combined with 20 µL of 5 M NaCl and 500 µL of isopropanol, followed by overnight precipitation at −20°C. Samples were eluted in 200 uL of laboratory grade water and stored at -20°C prior to downstream analyses. Total extracted DNA within each sample was quantified using a Qubit 3.0 fluorometer (ThermoFisher Scientific, Waltham, MA, U.S.) to ensure negative controls did not contain cross contamination.

2.2.2 PCR Primers

The primers selected for PCR quantification targeted the 28S, D1-D2 region of the Large Subunit (LSU) rDNA, gene for *A. catenella* (Table 2) (Gao et al., 2015; Kim et al., 2024). The LSU TaqMan probe for *A. catenella* was designed with a dye label (FAM) on the 5′ end and a Black Hole Quencher (BHQ) on the 3′ end. The assay was tested using two WOAC samples known to contain *A. catenella* from April 2021, collected at the surface from stations P4 and P8 (Garber-Yonts, 2024). A gradient of annealing temperatures ranging from 57.5-62.2°C was used to determine optimal thermocycling conditions for amplification.

Table 2. PCR primers targeting the 28S, D1-D2 region of the LSU rDNA, gene for *A. catenella* used in ddPCR quantification.

Target gene	Primers and Probe	Sequence (5' → 3')	Reference
LSU	Atl-F	GCTTGGTGGGAGTGTTGCAC	Gao et al. 2015
	Atl-R	TAAGTCCAAGGAAGGAAGCATC	
	Atl-P	FAM-AGAGCTTTGGGCTGTGGGTGTA-BHQ	

2.2.3 ddPCR Protocol

Droplet Digital PCR (ddPCR) was used for quantification of *A. catenella* in all samples. By partitioning samples into thousands of droplets, absolute quantification is determined by Poisson statistics using the ratio of positive to negative droplets, which removes the variability associated with standard curves. This method is highly sensitive, allowing for precise measurement and detection of low concentration target DNA and greater reproducibility, particularly between laboratories. Furthermore, by measuring target presence using end-point fluorescence instead of the rate of amplification, samples containing inhibitors still yield accurate results.

All pre-PCR sample preparation and post-PCR amplification steps were conducted in physically separated laboratory spaces to minimize contamination risk. Workspaces and equipment were decontaminated with 10% bleach between sample processing steps, and standard molecular biology clean practices were followed throughout to prevent cross-contamination.

Individual samples were amplified in duplicate, and resulting quantifications were averaged for downstream analysis. No target controls (NTCs) were included in each ddPCR reaction. Each ddPCR master mix included ddPCR Supermix for Probes (no dUTP) (Bio-Rad Inc., Hercules, CA), 0.8µM of each primer (Atl-F and Atl-R), 0.2µM TaqMan Probe (Atl-P), and lab grade water to reach 20µL. Lastly, 2µL of template (sample DNA) was added for a final volume of 22µL prior to droplet generation.

Thermocycling protocols were as follows: hold at 4°C for 10 min for droplet stabilization, followed by 95°C for 10 min for initial denaturing; 45 cycles of 94°C for 30 sec, 58°C for 1 min (annealing); followed by 98°C for 10 min, and a 4°C holds. For all ddPCR assays, droplets were generated using the AutoDG Droplet Generator (Bio-Rad Inc.), thermocycling was performed on C1000 Touch Thermal Cyclers (Bio-Rad Inc.), and droplet fluorescence was read using a QX200 Droplet Reader (Bio-Rad Inc.). Amplification thresholds for determining positive droplets were set manually for each plate, consistently within 1350-1400, using QX Manager Software (Version 2.3.1). A total of 430 samples were quantified for *A. catenella* abundance collected during 2019-2024.

2.3 Statistical Analysis

2.3.1 Hurdle Models

To evaluate environmental drivers of *A. catenella* presence and abundance, Bayesian hurdle models implemented in the brms package in R were used (Bürkner, 2018). Hurdle models are well-suited for zero-inflated ecological data because they separately consider variables influencing (1) the probability of presence and (2) the concentration, or abundance, of non-zero observations.

Concentrations of *A. catenella* from duplicate samples collected at the same station and time were retained as individual observations rather than averaged together. Each observation was treated as a draw from the underlying distribution of DNA concentrations within the sample extract for a given sampling event, allowing estimation of within-site variability while avoiding inappropriate loss of information. Selected environmental predictors included pH, salinity, temperature, dissolved oxygen, and dissolved inorganic nitrogen (DIN). Month and year were accounted for in different ways (see below) to consider seasonal and interannual variability, and

station was included to account for spatial structure. All models were fit using a hurdle lognormal distribution, with the conditional (mu) component modeling concentration of *A. catenella* when present, and the hurdle (hu) component modeling the probability of "zero" observations (absence of *A. catenella*). Four candidate hurdle models were evaluated:

1. H1: Environmental predictors with random intercepts for station and year
2. H2: Environmental predictors with random intercepts for station, year, and month
3. H3: Environmental predictors with month as a fixed effect and random intercepts for station and year
4. H4: Reduced model including only predictors whose 95% credible intervals did not overlap zero (oxygen for abundance; oxygen, DIN, and month for presence), with random intercepts for station and year

Models were run with four Markov chains with 2,000 warmups and 4,000 sampling iterations in each. Convergence was assessed using $\hat{R}$ values and effective sample sizes. Model performance was compared using approximate leave-one-out cross-validation (LOO), which compares models based on their ability to predict new observations. Differences in expected log predictive density (Δelpd) were used to evaluate relative model support, with differences < 2 considered negligible.

2.3.2 Environmental Clusters

With the majority of positive detections of *A. catenella* occurring in surface water samples, these were grouped into three environmental clusters based on water properties (pH, salinity, temperature, dissolved oxygen, and dissolved inorganic nitrogen (DIN)). Prior to clustering, all variables were standardized (z-scores) to ensure equal weighting across different units and scales. K-means clustering was then applied to the standardized dataset to identify

groups of samples with similar environmental conditions. The number of clusters (k = 3) was selected based on evaluation of within-cluster sum of squares (elbow method) and cluster separation. This provided the most ecologically interpretable partitioning of surface water masses without over-segmentation of the data. The resulting clusters were interpreted as distinct surface water masses based on their mean environmental characteristics.

To evaluate drivers of presence for *A. catenella* (binary response: presence/absence), we fit a series of logistic regression models. First, an environmental model assessed the influence of continuous predictors (pH, salinity, temperature, oxygen, DIN). Next, a categorical model evaluated whether cluster membership (chem_cluster) alone explained positive detection (presence). Finally, I fit a model incorporating cluster identity along with temporal covariates (Month and Year) to account for seasonal and interannual variability:

$$\text{logit}(P(\text{presence})) = \beta_0 + \beta_1(\text{chem\_cluster}) + \beta_2(\text{Month}) + \beta_3(\text{Year})$$

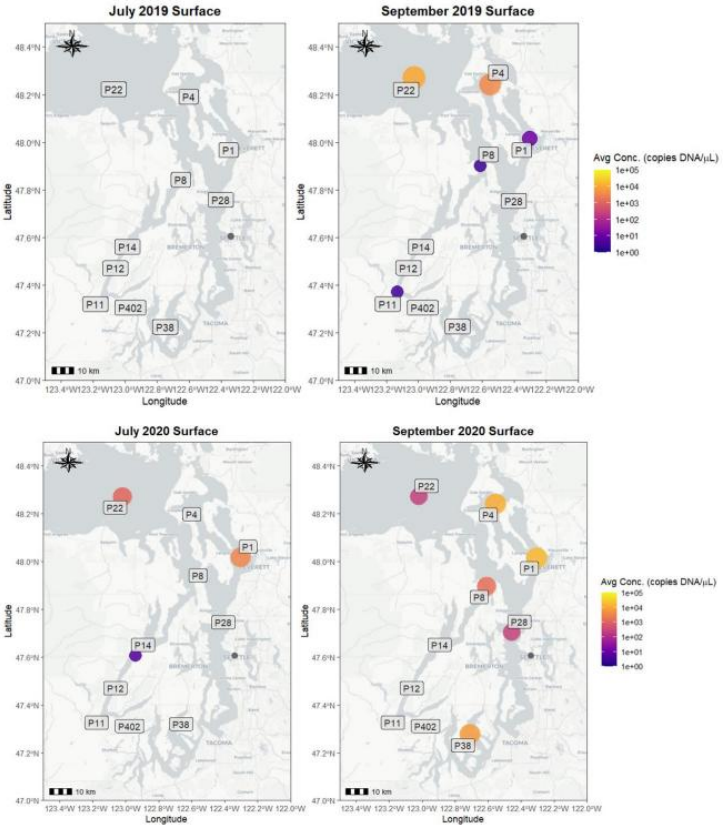
Model performance was compared using Akaike's Information Criterion (AIC), with lower values indicating better fit. Cluster-specific environmental conditions were summarized using mean values of each variable to support ecological interpretation.

Results

3.1 Presence and abundance of *A. catenella* in the Salish Sea

Spatial and temporal patterns in *A. catenella* indicated recurring late summer to early fall seasonal trends, as well as interannual variability captured by the statistical models (Figure 2). Maps display limited presence in spring (April) with progressively greater spatial extent and abundance in July and September (Figure 2). These observations are consistent with model results indicating significantly higher detection probability in July ($\beta = 1.25 \pm 0.33$ SE, $p < 0.001$; OR ≈ 3.49) and September ($\beta = 1.70 \pm 0.35$ SE, $p < 0.001$; OR ≈ 5.47) relative to April.

Interannual variability was also evident in the spatial distribution of positive detections, with more widespread and higher-abundance detections occurring in 2022, 2023, and 2024, aligning with model results showing significantly elevated probability of presence in these years (2022 ($\beta = 2.08 \pm 0.52$ SE, $p < 0.001$; OR ≈ 8.00); 2023 ($\beta = 1.31 \pm 0.49$ SE, $p = 0.007$; OR ≈ 3.71); and 2024 ($\beta = 1.70 \pm 0.49$ SE, $p < 0.001$; OR ≈ 5.48)). In contrast, earlier years (2019–2021) exhibited more limited and patchier distributions. In addition to temporal patterns, spatial patterns emerged across the study region. Positive detections and higher concentrations of *A. catenella* occurred more frequently at northern stations, suggesting a potential influence of oceanic water masses or nutrient inputs associated with this region (Figure 2). Together, these spatial observations reinforce the model-based findings that presence and abundance are shaped by strong seasonal signals, interannual variability, and underlying environmental gradients.



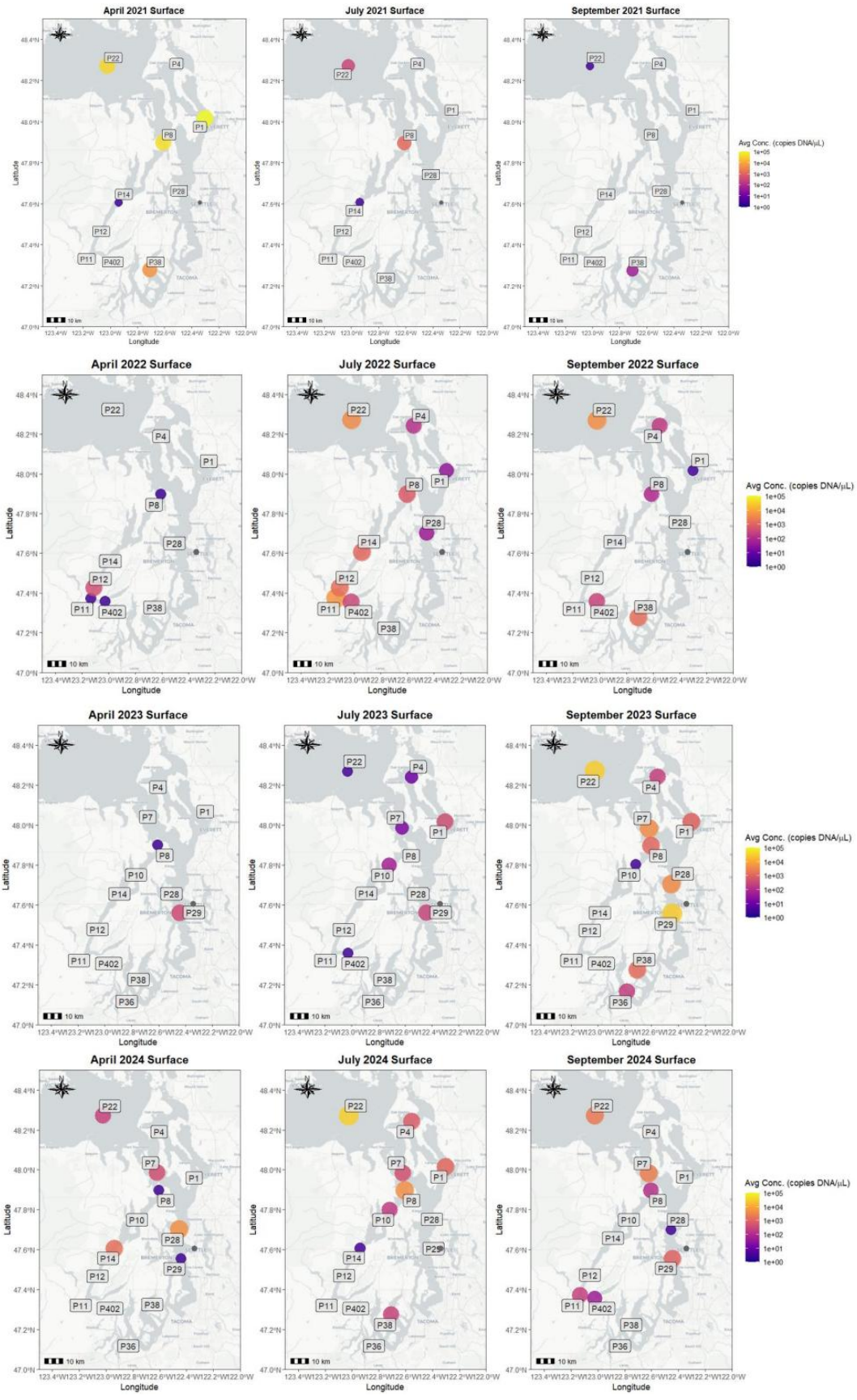

April 2021 Surface
July 2021 Surface
September 2021 Surface
April 2022 Surface
July 2022 Surface
September 2022 Surface
April 2023 Surface
July 2023 Surface
September 2023 Surface
April 2024 Surface
July 2024 Surface
September 2024 Surface
Avg Conc. (copies DNA/µL)
1e+05
1e+04
1e+03
1e+02
1e+01
1e+00
Latitude
Longitude
10 km

Figure 2. Spatial distribution of surface concentrations of *A. catenella* across sampling stations from 2019–2024. Panels show April, July, and September for each year. Point size and color represent average concentration (copies DNA μL^{-1}; log-scaled), with consistent scales across all panels to allow direct comparison.

3.2 Association of *A. catenella* with environmental factors

Environmental conditions across surface samples fell into three distinct clusters that captured gradients in nutrient availability, temperature, salinity, and oxygen. Cluster 1 was characterized by warm, stratified, low-nutrient waters (highest mean temperature ≈ 16.6°C; moderate oxygen ≈ 9.6 mg L^{-1}; lowest DIN ≈ 2.32 µM); Cluster 2 by cool, mixed, oxygenated waters (lowest salinity ≈ 26.3 PSU, highest oxygen ≈ 11.4 mg L^{-1}; temperature ≈ 12.7°C); and Cluster 3 by marine-influenced, nutrient-rich waters (highest salinity ≈ 29.9 PSU; highest DIN ≈ 18.24 µM; lowest oxygen ≈ 7.49 mg L^{-1}) (Figure 3). Together, these clusters represent a spectrum from warm, nutrient-poor surface waters to higher-salinity, nutrient-rich marine conditions.

Logistic regression models indicated that environmental conditions partially explained the presence of *A. catenella* (AIC = 474.22). Among individual variables, dissolved inorganic nitrogen (DIN) and oxygen were significant positive drivers ($\beta = 0.10$, $p < 0.001$; $\beta = 0.25$, $p = 0.003$, respectively). In contrast, pH, salinity, and temperature were not statistically significant predictors in the environmental model. A model using cluster identity alone performed similarly (AIC = 481.51), suggesting that that grouped environmental regimes captured comparable ecological structure to individual variables. Within this model, Cluster 3 (nutrient rich, low-oxygen, marine-influenced waters) was strongly associated with presence of *A. catenella* ($\beta = 1.05 \pm 0.26$ SE, $p < 0.001$), corresponding to a nearly threefold increase in the odds of detection (Odds Ratio (OR) ≈ 2.86). Cluster 2 also showed a significant positive association with presence ($\beta = 0.55 \pm 0.27$ SE, $p = 0.044$) (Figure 4).

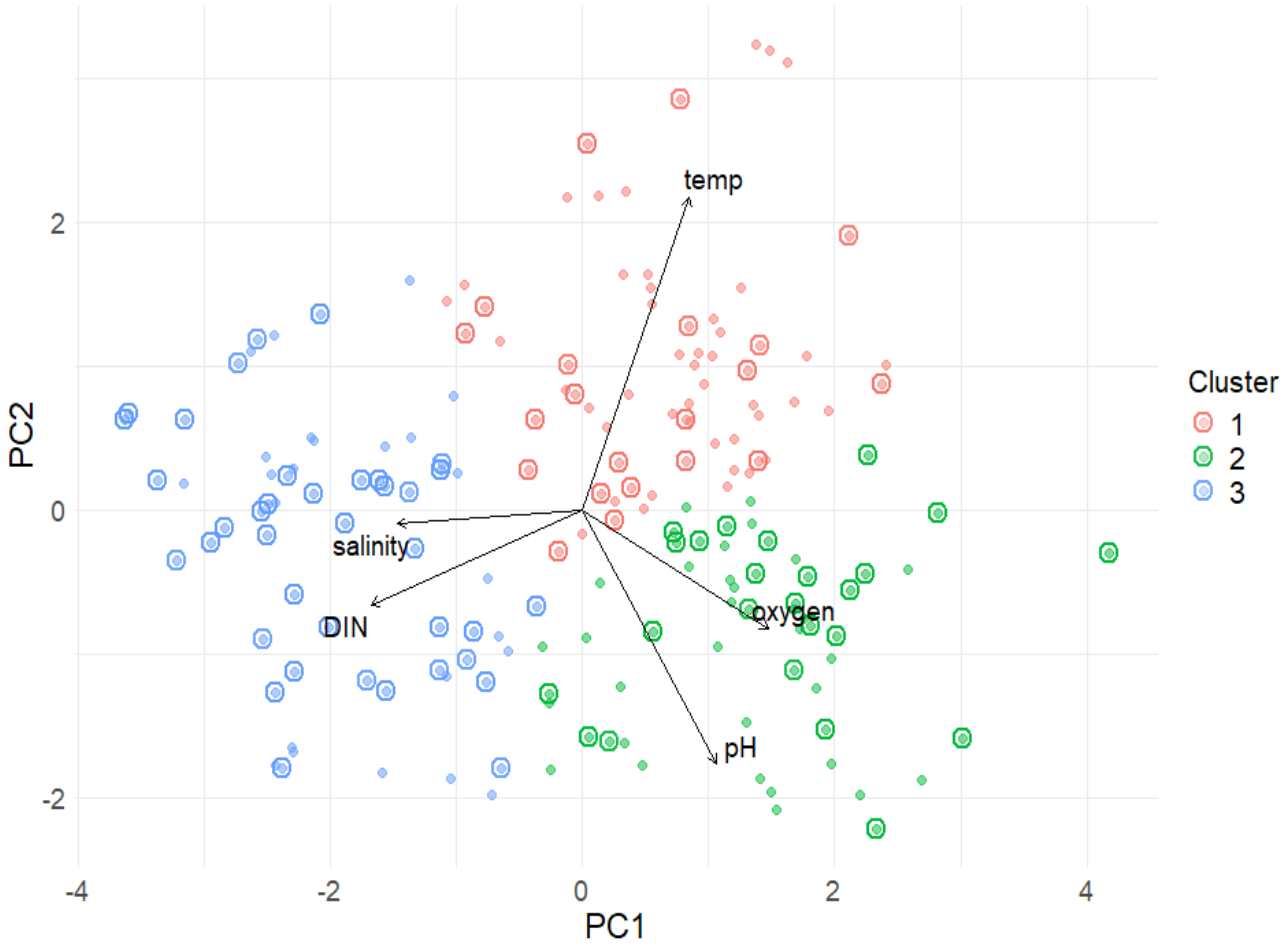


Figure 3. Principal components analysis (PCA) plot of scaled surface water properties (pH, salinity, temperature, dissolved oxygen, and dissolved inorganic nitrogen). PC1 (54% variance) represents a gradient from nutrient-rich, more saline conditions to more oxygenated, higher-pH waters, while PC2 (26% variance) is primarily driven by temperature, separating warmer samples from cooler conditions. Points are colored by k-means cluster, and samples with positive detections of *A. catenella* are highlighted with circle outlines. Arrows indicate the direction and relative contribution of each environmental variable.

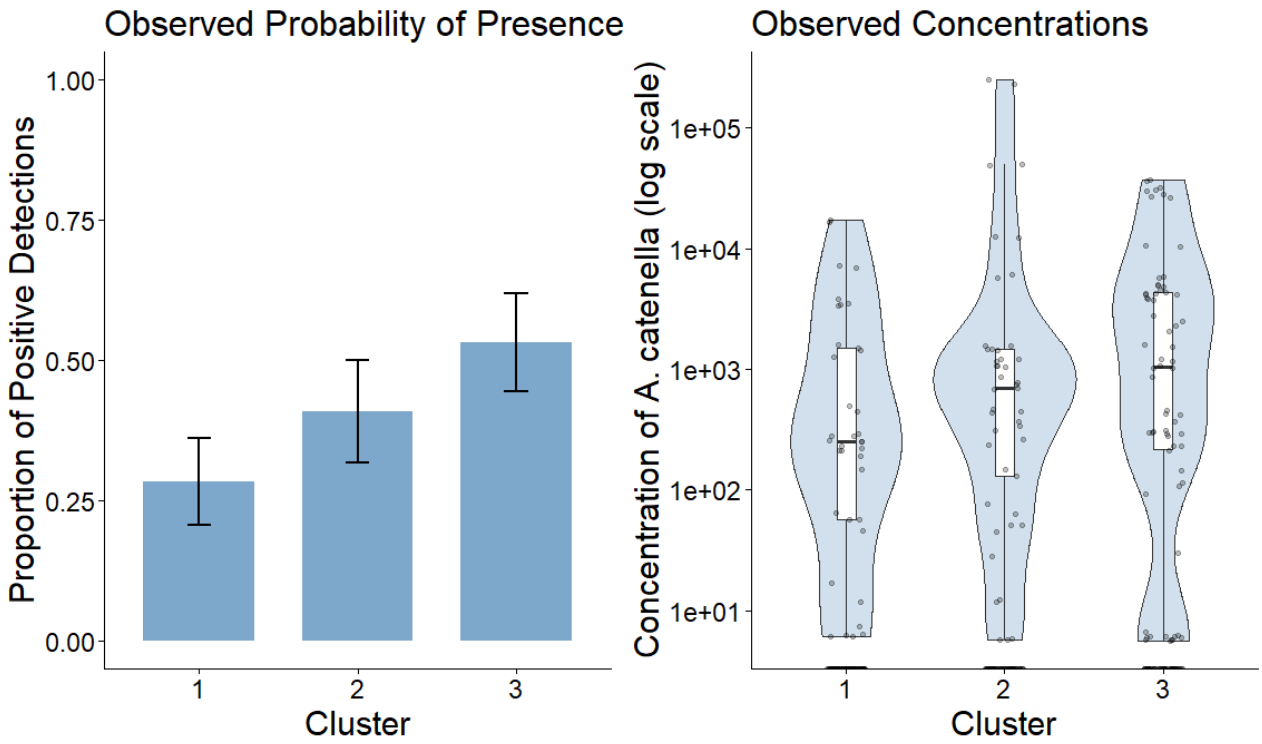


Figure 4. Bar plots (left) show the observed proportion of samples with positive detections (CONC > 0) within each cluster, with error bars representing 95% confidence intervals around the observed proportions. Violin plots (right) display the distribution of concentrations (log_{10}-scaled) within each cluster, highlighting differences in abundance of *A. catenella*. Together, these panels illustrate variation in presence and abundance among environmentally distinct surface water clusters. Clusters were defined using k-means clustering of surface water properties.

Incorporating seasonal and interannual variability substantially improved cluster model performance. The full model including cluster identity, month, and year had the lowest AIC (452.64), indicating the best fit among candidate models. Within the logistic regression model incorporating cluster identity and temporal covariates (Month and Year), cluster identity was an important predictor of presence of *A.* catenella. Cluster 2 was associated with significantly higher detection probability relative to Cluster 1 ($\beta = 1.22 \pm 0.35$ SE, $p < 0.001$; OR ≈ 3.39), while Cluster 3 showed an even stronger positive association ($\beta = 1.49 \pm 0.30$ SE, $p < 0.001$; OR ≈ 4.43). These results indicate that both Cluster 2 and 3 water masses are associated with elevated

probability of detection of *A. catenella* relative to Cluster 1, with the strongest effects observed in Cluster 3 (Figure 4).

Strong seasonal patterns in the presence of *A. catenella* were evident. Compared to April as the model's reference month, the probability of detection of *A. catenella* was significantly higher in July ($\beta = 1.25 \pm 0.33$ SE, $p < 0.001$; OR ≈ 3.49) and even more pronounced in September ($\beta = 1.70 \pm 0.35$ SE, $p < 0.001$; OR ≈ 5.47). These results indicate a distinct late summer-to-early fall peak in probability of positive detections.

Interannual variability was also substantial. Probability of detection was significantly elevated in 2020 ($\beta = 1.46 \pm 0.55$ SE, $p = 0.008$), 2022 ($\beta = 2.08 \pm 0.52$ SE, $p < 0.001$); 2023 ($\beta = 1.31 \pm 0.49$ SE, $p = 0.007$), and 2024 ($\beta = 1.70 \pm 0.49$ SE, $p < 0.001$). Earlier years within the time series (2019 and 2021) exhibited weaker, non-significant effects and served as the baseline for comparison (Figure 5). These patterns indicate that probability of detection is strongly shaped by both seasonal timing and interannual environmental variability.

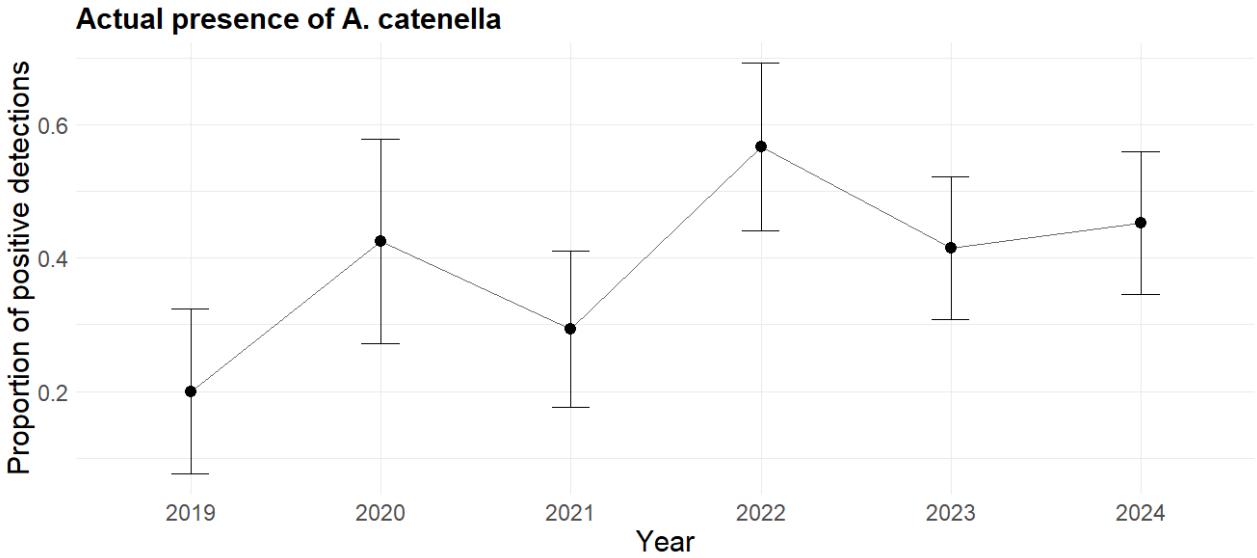


Figure 5. Annual proportion of positive detections of *Alexandrium catenella* in Puget Sound. Points represent the proportion of samples with positive detections in each year, with error bars showing ±1.96 standard errors. The plot summarizes observed presence/absence across all sampling stations and months, highlighting interannual variation in detection frequency over the study period.

Hurdle models incorporating both the presence and concentration of *A. catenella* were fit to 858 observations and showed good convergence overall ($\hat{R} \approx 1.00$). Models with more complex random effects exhibited divergent transitions, particularly the H2 model including station, year, and month as random effects (89 divergences), indicating overparameterization (H1: 1 divergence, H3: 2 divergences, H4: 1 divergence).

Model comparison using leave-one-out cross-validation indicated that the reduced hurdle model (H4) demonstrated the best predictive performance among candidate models, although differences between H2 and H3 were small ($\Delta elpd < 3$). In contrast, H1 showed lower predictive performance ($\Delta elpd = -9.9 \pm 6.8$). Since H4 achieved improved predictive accuracy with fewer parameters and better model convergence, it was retained as the final model for interpretation. The final hurdle model included random intercepts for station and year, dissolved oxygen as a predictor for higher concentrations of *A. catenella*, with dissolved oxygen, dissolved inorganic nitrogen (DIN), and month as predictors of presence probability.

Results from the hurdle models reinforced environmental patterns observed in the cluster analysis. Dissolved oxygen was an important predictor of presence of *A. catenella*, with higher oxygen concentrations associated with an increased probability of detection (H4 posterior mean = −0.51, 95% credible interval: −0.71 to −0.33), indicating that positive detections were associated with higher oxygen conditions. DIN concentration was also positively associated with probability of *A. catenella* detection (H4 posterior mean = −0.08, 95% credible interval: −0.12 to −0.03). In the simpler H1 model, temperature was associated with the probability of detection (H1 posterior mean = −0.22, 95% CI: −0.34 to −0.10), indicating higher likelihood of *A. catenella* detection under warmer conditions. However, this relationship weakened once seasonal structure (month effects) was included, suggesting that the apparent temperature effect is largely

explained by seasonal timing rather than a direct independent influence. Seasonal effects were again pronounced, with September showing the strongest increase in the detection probability of *A. catenella* (H4 posterior mean = −2.04, 95% CI: −2.69 to −1.39).

The conditional component of the hurdle model describes variation in concentrations of *A. catenella* conditional on presence (i.e., when concentrations are > 0). Within this component, dissolved oxygen was the only environmental variable with a consistent and statistically supported effect on abundance. In the H4 model, oxygen had a positive effect on *A. catenella* concentration (H4 posterior mean = 0.37, 95% CI: 0.26 to 0.48), indicating that higher oxygen concentrations were associated with greater abundance. Other environmental variables including pH, salinity, DIN, and temperature showed no consistent relationships with abundance of *A. catenella* with credible intervals overlapping zero across all models.

Month effects in the final hurdle model (H4) indicated strong seasonal variability in detection of *A. catenella*. Relative to April (reference month), the probability of detection was substantially higher in both July and September, with the strongest effect observed in September (July: H4 posterior mean = −1.05, 95% credible interval: −1.61 to −0.51; September: H4 posterior mean = −2.04, 95% credible interval: −2.72 to −1.40). The hurdle component models the probability of non-detection, with negative coefficient estimates corresponding to increased probability of detecting *A. catenella*. These results indicate that probability of detecting *A. catenella* was considerably higher during later sampling months relative to spring sampling in April.

Substantial spatial variability was indicated by the random effects included in the final hurdle model, with moderate to high variation among stations in both the conditional ($\sigma = 1.10$) and presence/absence components ($\sigma = 0.96$). Interannual variability was also evident, though

slightly weaker (σ = 0.72 conditional, σ = 0.69 presence/absence). These results suggest that both local-scale processes and broader annual variability influence presence and abundance of *A. catenella.*

Together, the environmental cluster analysis and hurdle modeling reveal a consistent picture of *A. catenella* population dynamics structured by interacting environmental and temporal drivers. Nutrient-rich, marine-influenced conditions (Cluster 3) and higher dissolved oxygen levels were associated with increased likelihood of detection, while warm, stratified, low-nutrient waters (Cluster 1) were associated with reduced probability of detection. Seasonal timing exerted a strong influence, with detection probability peaking in late summer to early fall. Once *A. catenella* is present, its abundance is primarily associated with dissolved oxygen concentration, suggesting that oxygen may reflect underlying processes such as water column structure or productivity conditions that also favor growth and accumulation. Overall, these results indicate that presence and abundance emerge from the combined effects of nutrient conditions, water mass properties, dissolved oxygen, and seasonal forcing.

Discussion

4.1 Temporal and spatial distribution of *A. catenella*

The distinct late summer to early fall seasonal signal observed in this study aligns with long-term observations of *A. catenella* in Puget Sound (Feifel et al., 2012; Moore, Johnstone, et al., 2015; Trainer et al., 2003). Blooms in this region are consistently reported to peak between August and October, coincident with peak sea surface temperatures in the Salish Sea (Khangaonkar et al., 2019; Tobin & Horner, 2011). Seasonality is due to multiple overlapping processes. Warmer water temperatures and stronger stratification during summer create favorable growth conditions for dinoflagellates while simultaneously structuring nutrient availability

within the water column (D. M. Anderson et al., 2012; Fu et al., 2012; Horner et al., 1997; Moore, Bill, et al., 2015). The life cycle of *A. catenella* reinforces seasonal signals. Spring transitions are associated with cyst germination and initial population growth, with increased germination rates under favorable temperature and oxygen conditions (Fischer et al., 2018; Fu et al., 2012; Tobin & Horner, 2011).

The spatial distribution observed in this study reflects well-documented geographic patterns in Puget Sound, particularly in the higher frequency of detections and elevated abundances at northern stations. Northern basins, including regions closer to the Strait of Juan de Fuca, have historically exhibited more frequent and intense blooms of *A. catenella* (Moore, Johnstone, et al., 2015; Trainer et al., 2003). This is most likely attributed to both hydrographic conditions and the distribution of benthic cyst beds. Previous surveys have shown that *Alexandrium* cysts tend to occur in "hotspots" that correspond to areas of recurring bloom activity (Fu et al., 2012; Moore, Bill, et al., 2015). The alignment between northern station detections in this study and previously identified cyst-rich regions suggests that sediment cyst beds play a critical role in structuring not only bloom timing but also spatial patterns of presence (Feifel et al., 2012; Greengrove et al., 2012).

Hydrodynamic processes further reinforce spatial patterns. Circulation in Puget Sound is strongly influenced by estuarine exchange with the coastal ocean, with northern regions experiencing greater connectivity to offshore waters through the Strait of Juan de Fuca (Khangaonkar et al., 2019; MacCready et al., 2021; Sutherland et al., 2011). Previous studies have shown that this exchange regulates nutrient delivery, stratification, and the transport of planktonic organisms through the Salish Sea system, all of which can influence harmful algal bloom development (Giddings et al., 2014; Winter et al., 1975). Increased coastal connectivity

may therefore facilitate the introduction or retention of *A. catenella* populations in northern basins, where oceanic inflow and frontal circulation patterns can promote bloom formation and persistence (Moore, Johnstone, et al., 2015; Trainer et al., 2003). In contrast, the complex fjord-like morphology of Puget Sound also produces long residence times and restricted circulation in some sub-basins, conditions that may allow populations to persist and intensify once established (MacCready et al., 2021; Stang & Allen, 2025). Together, growing evidence suggests bloom dynamics in Puget Sound reflect interactions between seasonal environmental suitability and physical transport processes operating across multiple spatial scales.

4.2 Associated environmental conditions

Availability of dissolved inorganic nitrogen was positively associated with the presence of *A. catenella*. Nutrient availability sustains populations and has been shown to regulate bloom intensity and toxicity (D. M. Anderson et al., 2012). Within Puget Sound, nutrient dynamics are strongly mediated by upwelling through the Strait of Juan de Fuca and by seasonal water column structure. During the summer, coastal upwelling intensifies, driven by prevailing coastal winds. Surface stratification is also enhanced, with surface waters becoming nutrient-depleted due to uptake by phytoplankton (Fu et al., 2012; Moore, Johnstone, et al., 2015). Although dinoflagellates such as *A. catenella* are well adapted to stratified environments through diel vertical migration, allowing access to both deeper nutrient pools and surface light conditions (Smayda, 1997), the present results suggest that presence was less likely under the warmest and most strongly stratified surface conditions (Cluster 1). Instead, presence was most strongly associated with cooler, nutrient-rich, marine-influenced water masses (Cluster 3), suggesting that *A. catenella* may depend more strongly on nutrient replenishment than on peak stratification. This pattern indicates that nutrient availability is an important constraint on growth and

accumulation of the species even under strongly stratified conditions. Low surface nutrient concentrations during some positive detections indicate that rather than abundances reflecting instantaneous surface conditions, presence of *A. catenella* likely depends on nutrient availability across depth and time, mediated by physical structure in the water column and migration behaviors.

In this study, dissolved oxygen emerged as one of the strongest predictors of both the presence and abundance of *A. catenella*. Elevated oxygen concentrations typically are associated with high primary productivity and surface waters that support phytoplankton growth. The relationship with *A. catenella* is likely complex because the species itself contributes to oxygen production through photosynthesis. As a result, dissolved oxygen may reflect broader environmental conditions favorable for *A. catenella* occurrence and accumulation, as well as biological activity associated with high-density populations. Oxygen also plays an important role in the life history of *A. catenella*, as cyst germination in spring requires oxygenated benthic conditions (Fischer et al., 2018; Tobin & Horner, 2011). Together, these relationships suggest that dissolved oxygen may function as an integrated indicator of both environmental conditions and biological processes associated with the population dynamics of *A. catenella*.

The euryhaline nature of *A. catenella* allows it to tolerate a wide range of salinities (Fu et al., 2012; Moore, Bill, et al., 2015; Vandersea et al., 2018). While salinity did not emerge as a significant predictor of presence or abundance, it remains ecologically important as an indicator of water mass origin and mixing processes. The higher probability of *A. catenella* presence in more saline, marine-influenced waters suggests oceanic upwelling supplies nutrients and potentially transports algal cells within Puget Sound (Trainer et al., 2003). Moreover, stratified estuarine systems characterized by salinity gradients can enhance retention of dinoflagellate

populations, promoting bloom development (Smayda, 1997). Thus, while salinity may not directly regulate growth, it helps explain the physical structure of the estuarine system and define the environmental niche in which *A. catenella* populations occur.

Together, these results reveal that population dynamics of *A. catenella* within Puget Sound are governed by the interaction of seasonal factors, nutrient availability, and water column structure. Seasonal transitions regulate temperature, light, and stratification, which in turn influence nutrient distributions and biological processes such as cyst germination and vertical migration. Nutrients support growth and sustain blooms. Oxygen reflects primary productivity and favorable surface conditions. Salinity describes water mass structure and potential transport pathways of *A. catenella* cells. The relatively weak effects of individual variables compared to those captured by clustering approaches underscore the importance of considering these drivers in combination to best represent the environmental conditions that structure population dynamics of *A. catenella*.

4.3 Implications for climate change-related ocean acidification and warming

The sensitivity of *A. catenella* to environmental variability has important implications for climate change in Puget Sound. Projected increases in temperature, altered freshwater inputs, and changes in nutrient loading are expected to modify bloom dynamics with potential to expand the spatial and temporal extent of harmful algal blooms in coastal ecosystems (Hallegraeff, 1993; Wells et al., 2015). Previous studies have suggested that warming conditions could contribute to earlier bloom initiation, longer bloom seasons, and increased bloom frequency (D. M. Anderson et al., 2012; Feifel et al., 2012; Jacobs-Palmer et al., 2021). Although stronger stratification is often expected under climate change and may favor dinoflagellates relative to other phytoplankton groups such as diatoms (Gallego et al., 2020; Khangaonkar et al., 2019), the

relationship between stratification, oxygen dynamics, and blooms of *A. catenella* is likely complex. In this study, presence and abundance were associated with elevated dissolved oxygen concentrations, suggesting that hypoxic conditions may not directly favor growth and accumulation of this species. Instead, future bloom responses will likely depend on the interacting effects of warming, circulation, nutrient availability, and ecosystem productivity. Improving harmful algal bloom predictions will therefore require accounting for the interacting effects of physical, chemical, and biological drivers.

Anthropogenic nutrient loading represents an additional pressure on bloom dynamics. Increased inputs of nitrogen from wastewater discharge, agricultural runoff, and urbanization have been linked to eutrophication in parts of Puget Sound, particularly in restricted basins such as Hood Canal (Erdner et al., 2010; Khangaonkar et al., 2019). Because dissolved inorganic nitrogen was positively associated with presence of *A. catenella* in this study, increasing nutrient availability may contribute to conditions favorable for rapid growth in some regions of Puget Sound. However, bloom dynamics are likely shaped by interactions among nutrient supply, circulation, water mass exchange, and seasonal environmental variability rather than nutrient enrichment alone (D. M. Anderson et al., 2012).

While pH was not found to be a significant driver of presence or abundance in this study, ocean acidification in Puget Sound has previously been predicted to enhance the growth and toxicity of some harmful algal bloom species, including *A. catenella* (Fu et al., 2012). Harmful algal blooms in Puget Sound are increasingly influenced by human-driven environmental change operating across multiple scales. Improving monitoring and management will therefore require integrated approaches that consider how warming, nutrient availability, and regional

hydrodynamics interact to shape oxygen dynamics and bloom-favorable conditions under future climate scenarios.

Conclusion

This study demonstrates that population dynamics of *A. catenella* in Puget Sound are shaped by the interaction of seasonal variability, spatial structure, and environmental conditions. Presence and abundance were most strongly associated with late summer to early fall conditions and occurred more frequently in northern, marine-influenced regions of Puget Sound. Dissolved inorganic nitrogen and dissolved oxygen emerged as the strongest environmental predictors of presence and abundance, highlighting the importance of nutrient availability and hydrodynamic exchange in structuring favorable environmental conditions. Multivariate analyses further demonstrated that positive detections were associated with distinct environmental water mass characteristics rather than any single environmental variable in isolation, underscoring the complexity of harmful algal bloom dynamics in estuarine ecosystems.

These findings also demonstrate the value of molecular approaches such as eDNA and ddPCR for harmful algal bloom monitoring. Compared to toxin-based monitoring alone, molecular tools provide sensitive, scalable, and species-specific methods for detecting and quantifying harmful algal species across broad spatial and temporal scales. As climate change continues to alter temperature, nutrient dynamics, circulation, and carbonate chemistry in Puget Sound, integrated monitoring approaches will become increasingly important for improving bloom prediction and supporting adaptive management. Together, this work advances understanding of the environmental drivers of *A. catenella* and highlights the importance of ecosystem-based monitoring strategies for managing harmful algal bloom risk in a changing coastal ocean.

Bibliography

Alin, S. R., Newton, J. A., Feely, R. A., Greeley, D., Curry, B., Herndon, J., & Warner, M. (2024). A decade-long cruise time series (2008–2018) of physical and biogeochemical conditions in the southern Salish Sea, North America. *Earth System Science Data*, *16*(2), 837–865. https://doi.org/10.5194/essd-16-837-2024

Anderson, D. M., Alpermann, T. J., Cembella, A. D., Collos, Y., Masseret, E., & Montresor, M. (2012). The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae, Harmful Algae--The Requirement for Species-Specific Information*, *14*, 10–35. https://doi.org/10.1016/j.hal.2011.10.012

Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries*, *25*(4), 704–726. https://doi.org/10.1007/BF02804901

Anderson, L. E., & Plummer, M. L. (2017). Recreational Demand for Shellfish Harvesting Under Environmental Closures. *Marine Resource Economics*, *32*(1), 43–57. https://doi.org/10.1086/688975

Bill, B. D., Moore, S. K., Hay, L. R., Anderson, D. M., & Trainer, V. L. (2016). Effects of temperature and salinity on the growth of Alexandrium (Dinophyceae) isolates from the Salish Sea. *Journal of Phycology*, *52*(2), 230–238. https://doi.org/10.1111/jpy.12386

Bürkner, P.-C. (2018). Advanced Bayesian Multilevel Modeling with the R Package brms. *The R Journal*, *10*(1), 395–411. https://doi.org/10.32614/RJ-2018-017

Cameron, H., Fernández, D., Riquelme, C., & Vera-Villalobos, H. (2026). Standardization of A Method for the Detection of the Genus Prorocentrum, A Dinoflagellate Present in Harmful Algae Blooms (habs) in San Jorge Antofagasta Bay by Digital Droplet Pcr (ddPCR). *Marine Biotechnology*, *28*(2), 43. https://doi.org/10.1007/s10126-026-10575-9

Cornett, J. C., Cates, R. J., Ledger, K. J., Pinger, C. W., Hart, C. E., Laboda, K. R., Larson, W. A., & Hollarsmith, J. A. (2024). Assessing methods for detecting Alexandrium catenella (Dinophyceae) and paralytic shellfish toxins in Southeast Alaska. *Integrated Environmental Assessment and Management*, *20*(6), 2189–2202. https://doi.org/10.1002/ieam.4944

Deng, H., Shang, X., Zhu, H., Huang, N., Wang, L., & Sun, M. (2025). Saxitoxin: A Comprehensive Review of Its History, Structure, Toxicology, Biosynthesis, Detection, and Preventive Implications. *Marine Drugs*, *23*(7), 277. https://doi.org/10.3390/md23070277

Erdner, D. L., Percy, L., Keafer, B., Lewis, J., & Anderson, D. M. (2010). A quantitative real-time PCR assay for the identification and enumeration of *Alexandrium* cysts in marine sediments. *Deep Sea Research Part II: Topical Studies in Oceanography, Phytoplankton Life-*

Cycles and Their Impacts on the Ecology of Harmful Algal Bloom, *57*(3), 279–287. https://doi.org/10.1016/j.dsr2.2009.09.006

Feifel, K. M., Moore, S. K., & Horner, R. A. (2012). An Alexandrium Spp. Cyst Record from Sequim Bay, Washington State, USA, and its Relation to Past Climate Variability. *Journal of Phycology*, *48*(3), 550–558. https://doi.org/10.1111/j.1529-8817.2012.01175.x

Fischer, A. D., Brosnahan, M. L., & Anderson, D. M. (2018). Quantitative Response of *Alexandrium catenella* Cyst Dormancy to Cold Exposure. *Protist*, *169*(5), 645–661. https://doi.org/10.1016/j.protis.2018.06.001

Fu, F. X., Tatters, A. O., & Hutchins, D. A. (2012). Global change and the future of harmful algal blooms in the ocean. *Marine Ecology Progress Series*, *470*, 207–233. https://doi.org/10.3354/meps10047

Gallego, R., Jacobs-Palmer, E., Cribari, K., & Kelly, R. P. (2020). Environmental DNA metabarcoding reveals winners and losers of global change in coastal waters. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1940), 20202424. https://doi.org/10.1098/rspb.2020.2424

Gao, Y., Yu, R.-C., Murray, S. A., Chen, J.-H., Kang, Z.-J., Zhang, Q.-C., Kong, F.-Z., & Zhou, M.-J. (2015). High Specificity of a Quantitative PCR Assay Targeting a Saxitoxin Gene for Monitoring Toxic Algae Associated with Paralytic Shellfish Toxins in the Yellow Sea. *Applied and Environmental Microbiology*, *81*(20), 6973–6981. https://doi.org/10.1128/AEM.00417-15

Garber-Yonts, M. (2024). *Describing Variation in Plankton Community Assemblages Using eDNA (COI) Metabarcoding in the Salish Sea—ProQuest*. University of Washington. https://www.proquest.com/docview/3081517325?pq-origsite=gscholar&fromopenview=true&sourcetype=Dissertations%20&%20Theses

Giddings, S. N., MacCready, P., Hickey, B. M., Banas, N. S., Davis, K. A., Siedlecki, S. A., Trainer, V. L., Kudela, R. M., Pelland, N. A., & Connolly, T. P. (2014). Hindcasts of potential harmful algal bloom transport pathways on the Pacific Northwest coast. *Journal of Geophysical Research: Oceans*, *119*(4), 2439–2461. https://doi.org/10.1002/2013JC009622

Glibert, P. M., Seitzinger, S., Heil, C. A., Burkholder, J. M., Parrow, M. W., Codispoti, L. A., & Kelly, V. (2005). The Role of Eutrophication in the Global Proliferation of Harmful Algal Blooms: NEW PERSPECTIVES AND NEW APPROACHES. *Oceanography*, *18*(2), 198–209.

Greengrove, C. L., Masura, J. E., Moore, S. K., Bill, B. D., Hay, L. R., Eldred, K. C., Banas, N. S., Salathé, E. P., Mantua, N. J., Johnstone, J. A., Anderson, D. M., Trainer, V. L., & Stein, J. E. (2012). Alexandrium cyst distribution and germination in Puget Sound WA, USA. *Harmful Algae, Proceedings of the 15th International Conference on Harmful Algae International Society for the Study of Harmful Algae.*

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase: Phycologia: Vol 32 , No 2 - Get Access. *Phycologia*. https://www.tandfonline.com/doi/abs/10.2216/i0031-8884-32-2-79.1

Horner, R. A., Garrison, D. L., & Plumley, F. G. (1997). Harmful algal blooms and red tide problems on the U.S. west coast. *Limnology and Oceanography*, *42*(5part2), 1076–1088. https://doi.org/10.4319/lo.1997.42.5_part_2.1076

Howarth, R. W., Sharpley, A., & Walker, D. (2002). Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries*, *25*(4), 656–676. https://doi.org/10.1007/BF02804898

Jacobs-Palmer, E., Gallego, R., Cribari, K., Keller, A. G., & Kelly, R. P. (2021). Environmental DNA Metabarcoding for Simultaneous Monitoring and Ecological Assessment of Many Harmful Algae. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.612107

Khangaonkar, T., Nugraha, A., Xu, W., & Balaguru, K. (2019). Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads. *Journal of Geophysical Research: Oceans*, *124*(6), 3876–3904. https://doi.org/10.1029/2018JC014670

Kim, H.-S., Kim, T., Park, J., Park, T. G., & Ki, J.-S. (2024). Development of saxitoxin biosynthesis gene *sxtB*-targeted qPCR assay for the quantification of toxic dinoflagellates *Alexandrium catenella* (group I) and *A. pacificum* (group IV) occurring in the Korean coast. *Harmful Algae*, *134*, 102603. https://doi.org/10.1016/j.hal.2024.102603

Lepofsky, D., Smith, N. F., Cardinal, N., Harper, J., Morris, M., White), G. (Elroy, Bouchard, R., Kennedy, D. I. D., Salomon, A. K., Puckett, M., & Rowell, K. (2015). Ancient Shellfish Mariculture on the Northwest Coast of North America. *American Antiquity*, *80*(2), 236–259. https://doi.org/10.7183/0002-7316.80.2.236

Louzao, M. C., Vilariño, N., Vale, C., Costas, C., Cao, A., Raposo-Garcia, S., Vieytes, M. R., & Botana, L. M. (2022). Current Trends and New Challenges in Marine Phycotoxins. *Marine Drugs*, *20*(3), 198. https://doi.org/10.3390/md20030198

MacCready, P., McCabe, R. M., Siedlecki, S. A., Lorenz, M., Giddings, S. N., Bos, J., Albertson, S., Banas, N. S., & Garnier, S. (2021). Estuarine Circulation, Mixing, and Residence Times in the Salish Sea. *Journal of Geophysical Research: Oceans*, *126*(2), e2020JC016738. https://doi.org/10.1029/2020JC016738

Moore, S. K., Bill, B. D., Hay, L. R., Emenegger, J., Eldred, K. C., Greengrove, C. L., Masura, J. E., & Anderson, D. M. (2015). Factors regulating excystment of *Alexandrium* in Puget Sound, WA, USA. *Harmful Algae*, *43*, 103–110. https://doi.org/10.1016/j.hal.2015.01.005

Moore, S. K., Johnstone, J. A., Banas, N. S., & Salathé, E. P. (2015). Present-day and future climate pathways affecting *Alexandrium* blooms in Puget Sound, WA, USA. *Harmful Algae*, *48*, 1–11. https://doi.org/10.1016/j.hal.2015.06.008

Mudge, M. C., Riffle, M., Chebli, G., Plubell, D. L., Rynearson, T. A., Noble, W. S., Timmins-Schiffman, E., Kubanek, J., & Nunn, B. L. (2025). Harmful algal blooms are preceded by a predictable and quantifiable shift in the oceanic microbiome. *Nature Communications*, *16*(1), 3986. https://doi.org/10.1038/s41467-025-59250-y

Murphy, N. R., & Hellwig, R. J. (1996). Improved Nucleic Acid Organic Extraction Through Use of a Unique Gel Barrier Material. *BioTechniques*, *21*(5), 934–939. https://doi.org/10.2144/96215pf01

Ramón-Laca, A., Wells, A., & Park, L. (2021). A workflow for the relative quantification of multiple fish species from oceanic water samples using environmental DNA (eDNA) to support large-scale fishery surveys. *PLOS ONE*, *16*(9), e0257773. https://doi.org/10.1371/journal.pone.0257773

Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, *15*(1), 168–176. https://doi.org/10.1111/1755-0998.12281

Ruvindy, R., Bolch, C. J., MacKenzie, L., Smith, K. F., & Murray, S. A. (2018). qPCR Assays for the Detection and Quantification of Multiple Paralytic Shellfish Toxin-Producing Species of Alexandrium. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.03153

Smayda, T. J. (1997). Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography*, *42*(5part2), 1137–1153. https://doi.org/10.4319/lo.1997.42.5_part_2.1137

Stang, C., & Allen, S. E. (2025). Seasonably Variable Estuarine Exchange Through Interconnected Channels in the Salish Sea. *Journal of Geophysical Research: Oceans*, *130*(5), e2024JC022003. https://doi.org/10.1029/2024JC022003

Sutherland, D. A., MacCready, P., Banas, N. S., & Smedstad, L. F. (2011). A Model Study of the Salish Sea Estuarine Circulation. *Journal of Physical Oceanography*, *41*(6), 1125–1143. https://doi.org/10.1175/2011JPO4540.1

Te, S. H., Chen, E. Y., & Gin, K. Y.-H. (2015). Comparison of Quantitative PCR and Droplet Digital PCR Multiplex Assays for Two Genera of Bloom-Forming Cyanobacteria, Cylindrospermopsis and Microcystis. *Applied and Environmental Microbiology*, *81*(15), 5203–5211. https://doi.org/10.1128/AEM.00931-15

Tobin, E. D., & Horner, R. A. (2011). Germination characteristics of *Alexandrium catenella* cysts from surface sediments in Quartermaster Harbor, Puget Sound, Washington, USA. *Harmful Algae*, *10*(2), 216–223. https://doi.org/10.1016/j.hal.2010.10.002

Trainer, V. L., Eberhart, B. T. L., Wekell, J. C., Adams, N. G., Hanson, L., Cox, F., & Dowell, J. (2003). Paralytic shellfish toxins in Puget Sound, Washington State. *Journal of Shellfish Research*, *22*, 213–223.

Trainer, V. L., & Hardy, F. J. (2015). Integrative Monitoring of Marine and Freshwater Harmful Algae in Washington State for Public Health Protection. *Toxins*, *7*(4), 1206–1234. https://doi.org/10.3390/toxins7041206

Trainer, V. L., & King, T. L. (2023). SoundToxins: A Research and Monitoring Partnership for Harmful Phytoplankton in Washington State. *Toxins*, *15*(3), 189. https://doi.org/10.3390/toxins15030189

Vandersea, M. W., Kibler, S. R., Tester, P. A., Holderied, K., Hondolero, D. E., Powell, K., Baird, S., Doroff, A., Dugan, D., & Litaker, R. W. (2018). Environmental factors influencing the distribution and abundance of *Alexandrium catenella* in Kachemak bay and lower cook inlet, Alaska. *Harmful Algae*, *77*, 81–92. https://doi.org/10.1016/j.hal.2018.06.008

Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson, B. S. O., Trick, C. G., Kudela, R. M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D. M., & Cochlan, W. P. (2015). Harmful algal blooms and climate change: Learning from the past and present to forecast the future. *Harmful Algae*, *49*, 68–93. https://doi.org/10.1016/j.hal.2015.07.009

Winter, D. F., Banse, K., & Anderson, G. C. (1975). The dynamics of phytoplankton blooms in puget sound a fjord in the Northwestern United States. *Marine Biology*, *29*(2), 139–176. https://doi.org/10.1007/BF00388986