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Describing variation in plankton community assemblages using eDNA (COI) metabarcoding in
the Salish Sea

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A thesis

submitted in partial fulfillment of the
requirements for the degree of

Master of Marine Affairs

University of Washington

2024

Committee:

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Program Authorized to Offer Degree:

Marine and Environmental Affairs

University of Washington

Abstract

Describing variation in plankton community assemblages using eDNA (COI) metabarcoding in the Salish Sea

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Understanding marine plankton assemblages is crucial for assessing ecosystem health and predicting impacts of environmental change. This study uses environmental DNA (eDNA) metabarcoding methods to investigate plankton community assemblages in the Salish Sea with respect to spatial patterns, characteristic taxa, responses to environmental variables, and changing presence of harmful algal bloom (HAB) taxa. Five years of data (2017-2021) were collected from 9 sites across 5 oceanographic basins, revealing significant spatial differences in community composition among sites in the Hood Canal basin, sites in the Saratoga/Whidbey Basin, and remaining sampled basins. Additionally, temperature and phosphate represented the environmental variables significantly associated with the difference in these three distinct community assemblages. Identification of characteristic taxa from the Hood Canal basin assemblage revealed fewer and smaller diatoms compared to those identified from distinct

assemblages outside of Hood Canal. The occurrence of HAB taxa likewise varied across basins over time suggesting significant implications future monitoring goals and design. Overall, this study enhances our understanding of plankton assemblage dynamics and highlights the utility of eDNA metabarcoding for long-term monitoring and conservation efforts in marine ecosystems.

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1.0 INTRODUCTION

Understanding marine ecosystems is a complex and difficult task, often hindered by challenges such as expensive vessel costs, invasive methodologies, cryptic study organisms, and potentially laborious taxonomic identification. Molecular methods ameliorate some of those challenges by providing unique field observations of species diversity and adding important resolution to current field methods by assigning species level taxonomy to target DNA, especially valuable at morphologically identical juvenile stages (McManus & Katz, 2009). As marine environments experience the impacts of climate change, modelling efforts suggest that plankton community diversity could decline, leading to unstable ecosystems (Henson et al., 2021a; Ptacnik et al., 2008a). Monitoring this plankton diversity has been a particular focus of eDNA metabarcoding studies as many common taxa can be observed simultaneously, revealing otherwise cryptic community-wide responses (Bilbao et al., 2023; Gallego et al., 2020; Jacobs-Palmer et al., 2021; Loreau et al., 2001; Lv et al., 2023; McManus & Katz, 2009; Specchia et al., 123 C.E.). Understanding plankton community dynamics both enhances our broader understanding of marine ecosystem function and is critical for effective management and conservation efforts.

Plankton community assemblages are a vast and diverse group of largely motile organisms living in water and are often divided into groups based on trophic level, for instance phytoplankton (primarily producers) and zooplankton (secondary producers)(Simon, 2009; Visser, 2007; Wirtz & Smith, 2020). Within these large categories are taxonomic groups (i.e., diatoms, dinoflagellates, coccolithophores, and large or small copepods) and are often used to describe ecological community structures and track changes over time (Hopkins et al., 1993; Litchman & Klausmeier, 2008). All of these taxonomic groups are crucial in supporting primary production , efficient energy transfer through food webs, and biogeochemical cycling (Anderson, 2005; D'Alelio et al., 2016; Harvey et al., 2012; Newton & Van Voorhis, 2002; Quéré et al., 2005), however growth of some individual taxa can cause harmful algal blooms (HABs) resulting in instances of hypoxic conditions, physical irritation and/or potent toxin production leading to human health and recreation concerns, fisheries and aquaculture economic losses, and losses in higher trophic level species (Berdalet et al., 2016; Gilbert et al., 2005; Moore et al., 2008, 2019). As marine environments continue to be impacted by climate change, studies find shifts in plankton size and abundance (Balazy et al., 2021; Cornwell et al., 2020; Daufresne et al., 2009; Morán et al., 2010;

Rice et al., 2015), reduction in diatom diversity due to increased stratification (Nemcek et al., 2023), taxon specific responses (Keil et al., 2021; McLaskey et al., 2019), detrimental impacts to food web function (Ban et al., 1997; Ceballos & Ianora, 2003; D'Alelio et al., 2016; Halsband-Lenk et al., 2005; Lauritano et al., 2012), and the potential for significant genetic variation within species over short distances (Nuwer et al., 2008; Rynearson & Armbrust, 2004, 2000).

The Salish Sea relies on a diverse plankton community to support broader ecosystem function and potential changes in this plankton community could have large impacts farther up the food chain (Ptacnik et al., 2008b). For example, shifts in plankton size and availability are already being linked with declines in local salmonid and forage fish populations suggesting complex trophic interactions in response to changing environmental variables (Cushing, 1990; Keister et al., 2022). Busch et al. (2013) found copepod declines in the Salish Sea due to ocean acidification (OA) could have the largest impact on higher trophic levels and that the coordinated impacts of OA on plankton community assemblages deserve further study. Finally, regional climate models predict spatially heterogeneous environmental changes, suggesting the need for robust routine monitoring methods capable of characterizing the impacts on plankton community assemblages (Moore et al., 2011; Walker et al., 2022). Although many studies have described single or multiple plankton species' responses to changing ocean conditions in the Salish Sea (Apple et al., 2023; Dyrman et al., 2010; Moore et al., n.d.; Winter et al., 1975), fewer have endeavored to continuously track whole plankton communities across taxonomic groups (diatoms, copepods, dinoflagellates, etc.) due to methodological limitations (Stepien et al., 2023).

This study builds on previous work reported by Gallego et al. (2020) and Jacobs-Palmer et al. (2021), seeking to characterize differences in plankton assemblages in relation to changing environmental conditions, thus strengthening our understanding of ecosystem function, and potential future threats, specific to the Salish Sea. Primary objectives of this study were to: a) quantify differences in plankton community assemblages across different locations in the Salish Sea using eDNA metabarcoding, b) describe correlations between environmental variables (temperature, pH, salinity, nutrients) and plankton community assemblages, c) determine the taxa most characteristic of each sub-basin within Puget Sound and d) track the occurrences of HAB-forming taxa over time.

I find that there are significant spatial differences in plankton community assemblages revealing three unique assemblages. These distinct community assemblages occur in Hood Canal

basin, Saratoga/Whidbey basin, and a grouping of remaining sampled basins which shared similar a similar plankton assembly (Central Puget basin, Eastern Strait of Juan de Fuca basin, and Admiralty Inlet basin). Moreover, characteristic taxa identified for each of the unique assemblages found that the Hood Canal assemblage was represented by fewer and smaller diatoms and copepods compared to the assemblages outside of the Hood Canal. Of the environmental variables tested, temperature and phosphate were most strongly correlated with differences in the three identified plankton community assemblages. Lastly, HAB taxa occurrence appears to become more common in the Main Basin while Hood Canal and the Saratoga/Whidbey Basin show less clear trends.

2.0 METHODS

2.1 eDNA Sample and Environmental Data Collection

Bulk water samples were collected from ship-based cruises at 9 sites (Table 1) across 5 basins (Figure 1), three times per year (April, June/July, and September) for 5 years (2017-2021) by the Washington Ocean Acidification Center (WOAC). A rosette cage was deployed at each station to collect 1L water samples from 2-meter depths, filtered through a 0.45µM, 47 mm diameter cellulose acetate filter funnel used to capture environmental DNA (eDNA). Filters were preserved and held at room temperature in 1mL of Longmire's buffer to stop DNA degradation prior to extraction (Renshaw et al., 2015). Environmental data used for this analysis was collected alongside water sample collections with a conductivity-temperature-depth (CTD) sensor and carbonate chemistry and nutrient analysis was completed by NOAA Pacific Marine Environmental Lab (NOAA/PMEL) and the Marine Chemistry Lab at the University of Washington's School of Oceanography (Alin et al., 2024). These data from 2008-2018 are part of an ongoing monitoring program funded by the Washington Ocean Acidification Center (WOAC) and US National Oceanic and Atmospheric Association (NOAA) and are published in Alin et al. (2022).

Table 1. WOAC Cruise sample sites. Sites were samples in April, June/July, and September for 5 years (except April of 2020).

Station	Station Name	Latitude	Longitude
P28	North of West Pt	47.7034	-122.4544
P1	Gedney Island	48.0165	-122.3042
P4	Skagit Bay	48.2422	-122.5533
P22	Eastern Bank	48.2717	-123.0189
P8	Hood Head	47.8967	-122.6053
P14	Hood Point	47.6068	-122.9399
P12	Hoodsport	47.4253	-123.1083
P11	The Great Bend	47.3712	-123.1329
P402	Sisters Point	47.3567	-123.0233

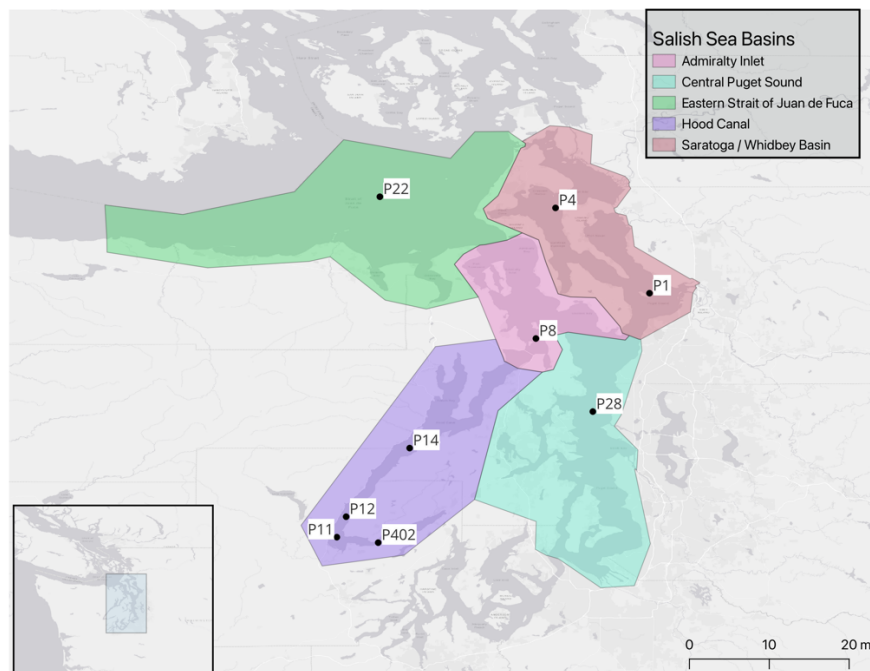


Figure 1. Southern Salish Sea map showing sample sites in relation to their oceanographic basins (WA DNR).

2.2 Laboratory Methods

eDNA extraction from filters was carried out following the phenol:chloroform:isoamyl (PCI) extraction protocol described in Jacobs-Palmer et al. (2021). Extraction blanks were used as negative controls and carried through the workflow for each batch of extractions to test for cross contamination between samples. Additionally, a phase lock was used to ensure maximal DNA

output and reduce phenol contamination in our final sample by creating a non-reactive silicone-based grease (Dow Corning, Midland, MI, USA) to separate organic and aqueous layers (Murphy & Hellwig, 2018; Ramón-Laca et al., 2021). The separation and sealing of the organic layer below the grease allows for easy removal of the top aqueous layer without contamination. Extracted eDNA was precipitated in isopropanol at -20 degrees C overnight and samples were eluted in 200uL of lab grade water. eDNA was then quantified using a Qubit1 3.0 fluorometer (ThermoFisher Scientific, Waltham, MA, U.S.) to ensure negative controls did not contain cross contamination.

This study, in line with previously amplified samples from this time series (Jacobs-Palmer et al., 2021), uses the universal COI primer developed by Leray et al. (2013) (F: mICOLintF, R: jgHCO2198R) amplifying a 315 bp fragment of mt-DNA in each sample. Individual samples were amplified in triplicate to reduce stochastic detections among rare amplicons (Gold et al., 2022; Shirazi et al., 2021); each of these triplicates was sequenced separately but pooled in downstream bioinformatic steps. Additionally, PCR Negative and Positive controls were amplified to ensure successful amplification and to check for cross contamination. PCR mastermix included 2µL of 1X HotStar Buffer, 2 µL of 2.5 mM MgCl₂, 1.25µL 0.5 mM dNTP, 0.6µL each of 0.3 uM primers (F/R), and 0.1µL of 0.5 units of HotStar Taq Polymerase (Qiagen Corp., Valencia, CA, USA) with 1uL of each sample. Total PCR reaction volume per sample was 20uL. PCR cycling protocol included an annealing-temperature touchdown from 62 to 46 degrees C over 15 cycles , with an additional 25 cycles at 46 C, and PCR products were cleaned using 0.8x SPRI magnetic beads (Agencourt AMPure XP, Beckman Coulter, Inc., CA, USA) to amplified remove non-target fragments. Samples were then individually quantified using a Fluoroskan (ThermoFisher Scientific, Waltham, MA, U.S.) to assess amplification success. Samples were also assessed for inhibition using a 10% gel electrophoresis. Inhibited samples were diluted and re-amplified until amplification band was present (1:10-1:100). Uninhibited samples were then normalized to 10ng/uL prior to second step PCR.

A secondary PCR attached unique 10bp Nextera adapter (Illumina, San Diego, CA, USA) tags at both 5' and 3' ends marking individual samples as discrete. The secondary PCR consisted of 12.5uL Kapa HiFi HotStart Ready mastermix (KAPA Biosystems, Wilmington, MA, USA), 1.25uL of unique Nextera adapter per sample, and 11.25µL of normalized sample DNA. All samples, including positive controls, went through 8 subsequent PCR cycles at 56 C annealing

temperature. All samples were again cleaned using 0.8x SPRI beads and quantified. Individual samples were quantified, and samples were pooled according to final DNA concentrations. Final libraries included controls and triplicate barcoded samples and were loaded at 6-8 pM with ~20% phiX due to relatively low diversity runs. Sequencing was conducted on an Illumina MiSeq machine using v3 600-cycle kits. Samples from 2019-2021 were split across 4 total runs.

2.3 Bioinformatics and Taxonomic Assignment

Sequencing results from 2017-2021 were collated and put through a bioinformatic pipeline in R (R Core Team, 2023.09 *Desert Sunflower*, 2023). Cutadapt (Version 1.18, Martin 2011) first removes unique Nextera adapters from all sequences before DADA2 (Koster and Callahan 2021, version 1.2) trims, filters, merges sequences. Prior to merging, trim length specified in DADA2 is determined by quality scores of forward and reverse reads over the course of sequencing cycles (Quality Score cutoff >30). DADA2 reports the unique amplicon sequence variants (ASVs) detected in each sample along with the number of reads each ASV received. Triplicate PCR replicate results were then pooled to combine all observed ASVs in each replicate into one sample. Illumina Miseq sequencing resulted in 29.8 million reads over four years covering 14 sampling events. Within those reads, there were 10,225 unique ASVs.

Taxonomy was determined by matching ASVs to NCBI GenBank database using BLAST+ (v2.13.0)(Camacho et al., 2009). MEGAN (Huson et al., 2007) takes BLAST+ output, including multiple possible taxonomic IDs per ASV, and determines a singular ID based on lowest common ancestor. Ongoing work in the realm of taxonomic assignment means that previously assigned ASVs can be reassigned revealing higher resolution results as reference databases become more robust. ASVs were kept for analysis if MEGAN was able to identify taxonomy to genus or species level, for a total of 233 unique identified organisms. To account for potential amplification bias and rare taxa, I retain only assigned taxa which are “common” in our data meaning each ASV had to occur in >10% of total pooled samples. Final output included 58 unique “common” taxa and each taxon was evaluated for presence of absence in each sample resulting in a binary matrix.

2.5 Statistical Analysis

a) Community Assemblages

All statistical analysis was performed in R (R Core Team, 2023). Assemblage dissimilarity among all samples was measured with Jaccard distances using *vegan*. Cluster analysis using *hclust* was used to visualize site groupings in relation to basin delineations. Groupings were further assessed with a PERMANOVA multilevel pairwise comparison using *pairwiseadonis2* to determine if sites sampled within basins were significantly different from each other. These basin clusters are then used throughout the analysis to differentiate unique plankton assemblages.

To understand if measured environmental variables including temperature, salinity, pH, and phosphate were significantly correlated with the observed differences in our assemblages I used a constrained ordination. Samples with missing environmental data were omitted from the constrained ordination. A distance-based redundancy analysis model (*capscale*, *vegan* package) was performed using the Jaccard distance matrix with the *capscale* function in the *vegan* R package. This model was fit using a stepwise procedure (*ordistep*, in the *vegan* package) to remove collinear environmental variables and plotted to visualize variable importance. The significance of each environmental constraint was determined using permutational multivariate analysis of variance (PERMANOVA) via the *adonis2* function in *vegan* (permutations = 999).

Individual taxa driving between-cluster differences were identified using the *multipatt* function in the *indicspecies* package. Taxa selected as associated with a cluster are defined using an Indicator Species Analysis (ISA) (Dufrene & Legendre, 1997). A diagnostic statistic value is reported as the square root of the product of a taxon's specificity to a grouping (A) and its fidelity across samples within that grouping (B). Stat values larger than 0.5 are considered significantly associated with that grouping and have a p-value < 0.5 (Dufrene & Legendre 1997).

b) Individual HAB Taxa

Presence or absence in response to environmental variables was measured for 5 Harmful Algal Bloom taxa; *Alexandrium* spp., *Heterosigma akashiwo*, *Karlodinium veneticum*, *Phaeocystis globosa*, *Pseudochattonella farcimen*. A stepwise model selection, using the Akaike Information Criteria (AIC) from *MASS* package in R, was used to fit a generalized linear model and I used an ANOVA to evaluate the predictive power of environmental variables for to each HAB taxon. Equations are reported and significant environmental variables plotted for

visualization. Additionally, the probability of occurrence of all 5 HAB taxa were analyzed over time using a generalized linear model with the explanatory variable as year and displayed by basin grouping.

3.0 RESULTS

3.1 Spatial Patterns in the Salish Sea

An unconstrained nMDS plot (*stress* = 0.213) showed Jaccard dissimilarities of community assemblages across all sites at all time points and initial visual analysis showed separation of centroids among sample assemblages found in Hood Canal and those found in basins outside Hood Canal (Figure 2). A PERMANOVA confirmed significant differences in Jaccard dissimilarity distances among assemblages when grouped into the five oceanographic basins regardless of year or month ($df = 4$, $R^2 = 0.19$, $F = 3.5$, $p\text{-value} = .00001$).

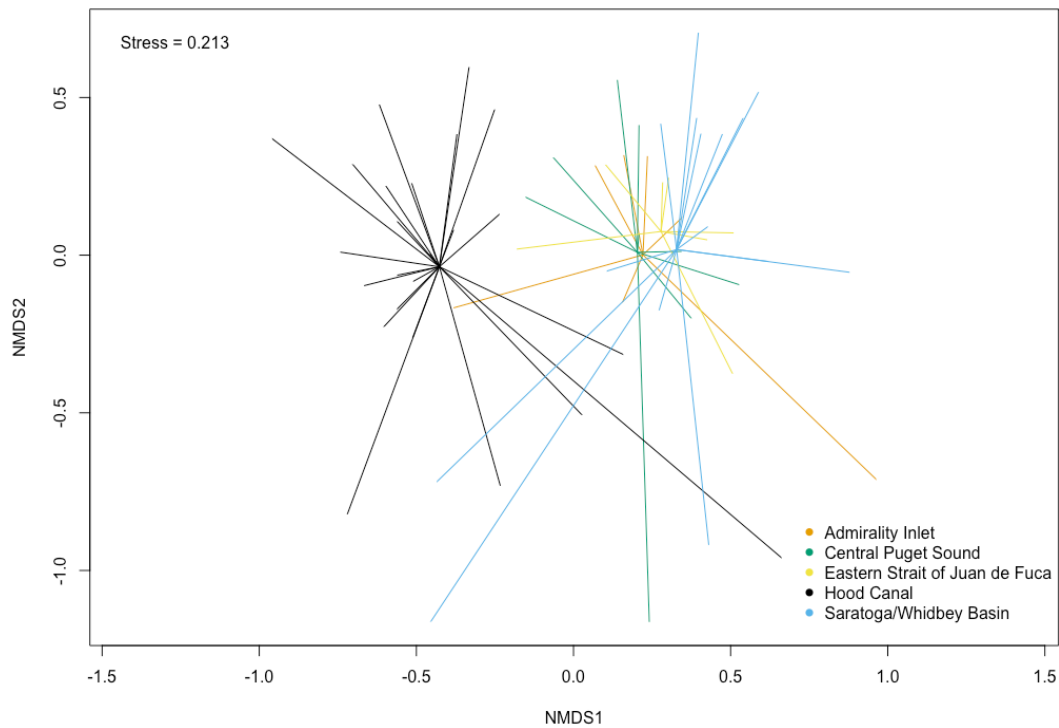


Figure 2. Unconstrained NMDS plot with each centroid consisting of Jaccard dissimilarity distances for all sampled assemblages across time and space for each basin.

A multilevel pairwise PERMANOVA comparing basin community assemblages finds that the plankton assemblage in Hood Canal basin is significantly different from all other basins and that the Saratoga/Whidbey basin is most significantly different from Hood Canal and Eastern Strait of Juan de Fuca basins (Table 2).

Table 2. A multilevel pairwise PERMANOVA comparing community assemblages in five Salish Sea basins. Hood Canal is significantly different than all other basins ($p < 0.01$). Saratoga/Whidbey basin is significantly different than Hood Canal and Eastern Strait of Juan de Fuca ($p < 0.05$)

	Admiralty Inlet	Central Puget Sound	Eastern Strait of Juan de Fuca	Hood Canal
Central Puget Sound	0.744	NA	NA	NA
Eastern Strait of Juan de Fuca	0.721	0.188	NA	NA
Hood Canal	0.001	0.001	0.001	NA
Saratoga / Whidbey Basin	0.072	0.079	0.028	0.001

These results show three distinct community assemblages present in all samples and will be referred to as the Hood Canal basin assemblage, the Saratoga/Whidbey basin assemblage, and the Main basin assemblage (includes samples from sites in Central Puget, Eastern Strait of Juan de Fuca, and Admiralty Inlet basins). A test of the three community assemblage dispersion from the centroid was not significant (Distance to centroid: Hood Canal = 0.4412, Main Basin (Central Puget Sound, Eastern Strait of Juan de Fuca, and Admiralty Inlet) = 0.4145, Saratoga/Whidbey Basin = 0.4557, ANOVA p -value = 0.405) meaning that distance from the centroid between groups is not different, indicating that no assemblage is significantly more homogenous than any other.

3.2 Constrained Ordination by Environmental Factors

A constrained ordination identified the differentiation between the distinct community assemblages as explained by selected measured environmental variables. Model selection was determined using *ordistep*, revealing the model of best fit to included temperature, salinity, and phosphate. Salinity was removed from final ordination due to collinearity with temperature. (community distance \sim temperature + phosphate; ANOVA $F = 1.5926$, $p < 0.05$, $R^2_{adj} = 0.06$) (Figure 3). CAP1 is most explained by the temperature values and CAP2 is explained most by Phosphate values thus the Hood Canal assemblage is most correlated with higher temperatures and higher phosphate concentrations, Saratoga and Whidbey basin assemblage is correlated with

relatively lower phosphate concentrations and temperatures and the Main Basin assemblage is correlated with low temperatures and relatively moderate levels of phosphate. The adjusted R-squared value suggests that environmental variables alone explain little of the observed variability in community assemblages.

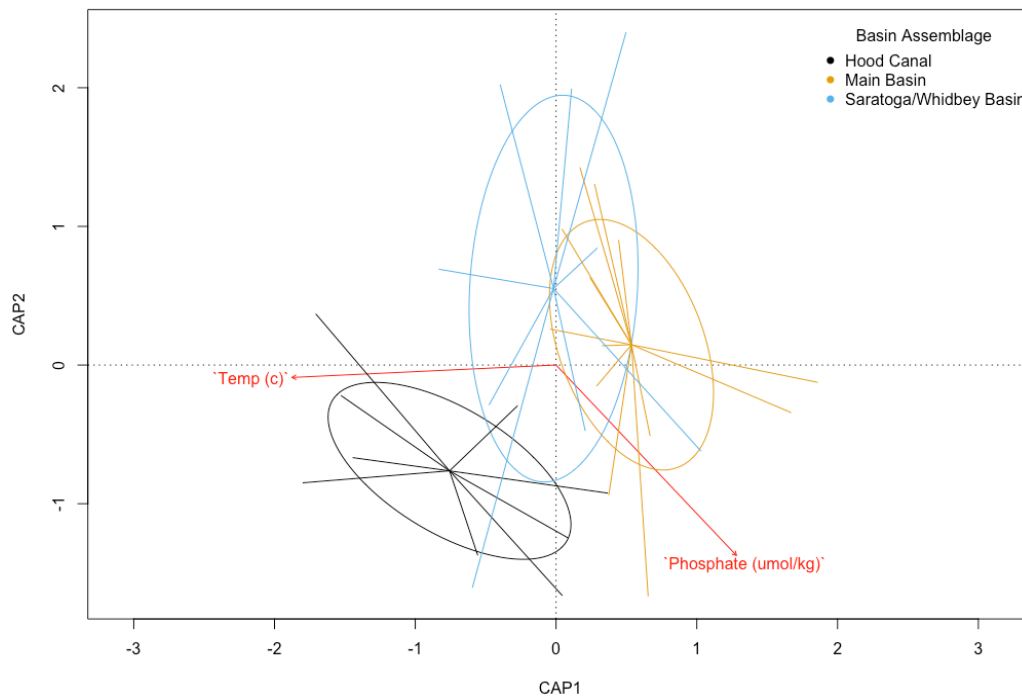


Figure 3. Constrained ordination of community distance matrix using a distance-based redundancy analysis model and model significance tested with an ANOVA (community distance ~ temperature + phosphate; ANOVA $F = 1.5926$, $p < 0.05$, $R_{\text{square.adj}} = 0.06$).

3.3 Individual Taxa Characterizing Assemblages

Given that basin clusters appear to host different community assemblages, I asked whether there are specific taxa are most responsible for the difference among assemblages. An analysis of the community assemblages reveals 8 taxa characteristic of Hood Canal, 12 taxa characteristic of the Salish Sea grouped basins, and just 3 taxa characteristic of Saratoga/Whidbey Basin. Stat A is a probability representing the probability a taxon occurred more often in that basin than in any

other basin. Stat B represents the ubiquity with which that taxon was found across all samples in that basin. Samples were omitted if $p < 0.05$. P-values were calculated using test statistic $stat = \sqrt{(A + B)}$ (Dufrêne & Legendre, 1997). In Hood Canal, *Karlodinium veneficum* and *Oithona similis* were found most often only in Hood Canal while *Bathycoccus prasinos* and *Pycnococcus provasolii* were found in 100% and 70% of all samples, respectively, from Hood Canal. In the Main Basin *Paralia sulcata*, *Clytia gregaria*, and *Acartia longiremis* occurred most often in the Salish Sea while *Phaeocystis globosa* and *Heterocapsa rotundata* were found in >90% of samples in this basin. Lastly, *Pseudo-nitzschia pungens* commonly occurred in Saratoga/Whidbey basin and *Attheya longicornis* is found in 80% of samples there. Taxonomic groups are assigned in Table 3.

Table 3. Taxa determined as characteristic of each clustered grouping defined as any taxa either more commonly found in the basin than outside the basin and/or ubiquitous across samples within the basin grouping. Taxonomic groups further characterize identified taxa.

	A	B	stat	Taxonomic Group
Hood Canal				
<i>Pycnococcus provasolii</i>	0.66	0.71	0.68	Green Alga
<i>Minutocellus polymorphus</i>	0.66	0.67	0.66	Diatom
<i>Emiliana huxleyi</i>	0.58	0.71	0.64	Coccolithophore
<i>Bathycoccus prasinos</i>	0.40	1.00	0.64	Green Alga
<i>Aureococcus anophagefferens</i>	0.67	0.54	0.60	Brown Alga
<i>Oithona similis</i>	0.70	0.46	0.57	Copepod
<i>Karlodinium veneticum</i>	0.81	0.38	0.55	Dinoflagellate
<i>Coscinodiscus granii</i>	0.65	0.42	0.52	Diatom
Main Basin (CP, ESJdF, and AI)				
<i>Paralia sulcata</i>	0.81	0.74	0.77	Diatom
<i>Pseudocalanus newmani</i>	0.72	0.70	0.71	Copepod
<i>Phaeocystis globosa</i>	0.47	0.96	0.67	Haptophyte
<i>Coscinodiscus wailesii</i>	0.66	0.61	0.64	Diatom
<i>Skeletonema dohrnii</i>	0.51	0.78	0.63	Diatom
<i>Skeletonema japonicum</i>	0.49	0.78	0.62	Diatom
<i>Thalassiosira nordenskiöldii</i>	0.57	0.61	0.59	Diatom
<i>Clytia gregaria</i>	0.89	0.35	0.56	Ciliate
<i>Pseudo-nitzschia delicatissima</i>	0.51	0.61	0.56	Diatom
<i>Acartia longiremis</i>	0.84	0.35	0.54	Copepod
<i>Hemiaulus sinensis</i>	0.62	0.43	0.52	Diatom
<i>Heterocapsa rotundata</i>	0.43	0.93	0.63	Dinoflagellate
Saratoga/Whidbey				
<i>Attheya longicornis</i>	0.47	0.80	0.61	Diatom
<i>Calanus pacificus</i>	0.51	0.67	0.58	Copepod
<i>Pseudo-nitzschia pungens</i>	0.65	0.40	0.51	Diatom

Average sizes of characteristic taxa found in each assemblage for identified in the published literature are visualized in a boxplot (Figure 4) and were found to be significantly different (Kruskal-Wallis Test, $p < 0.05$) and a post hoc Dunn's test found Hood Canal sizes to be significantly different from the Main Basin assemblage. This study found that the assemblage

identified in Hood Canal basin consisted of fewer and smaller diatoms and copepods.

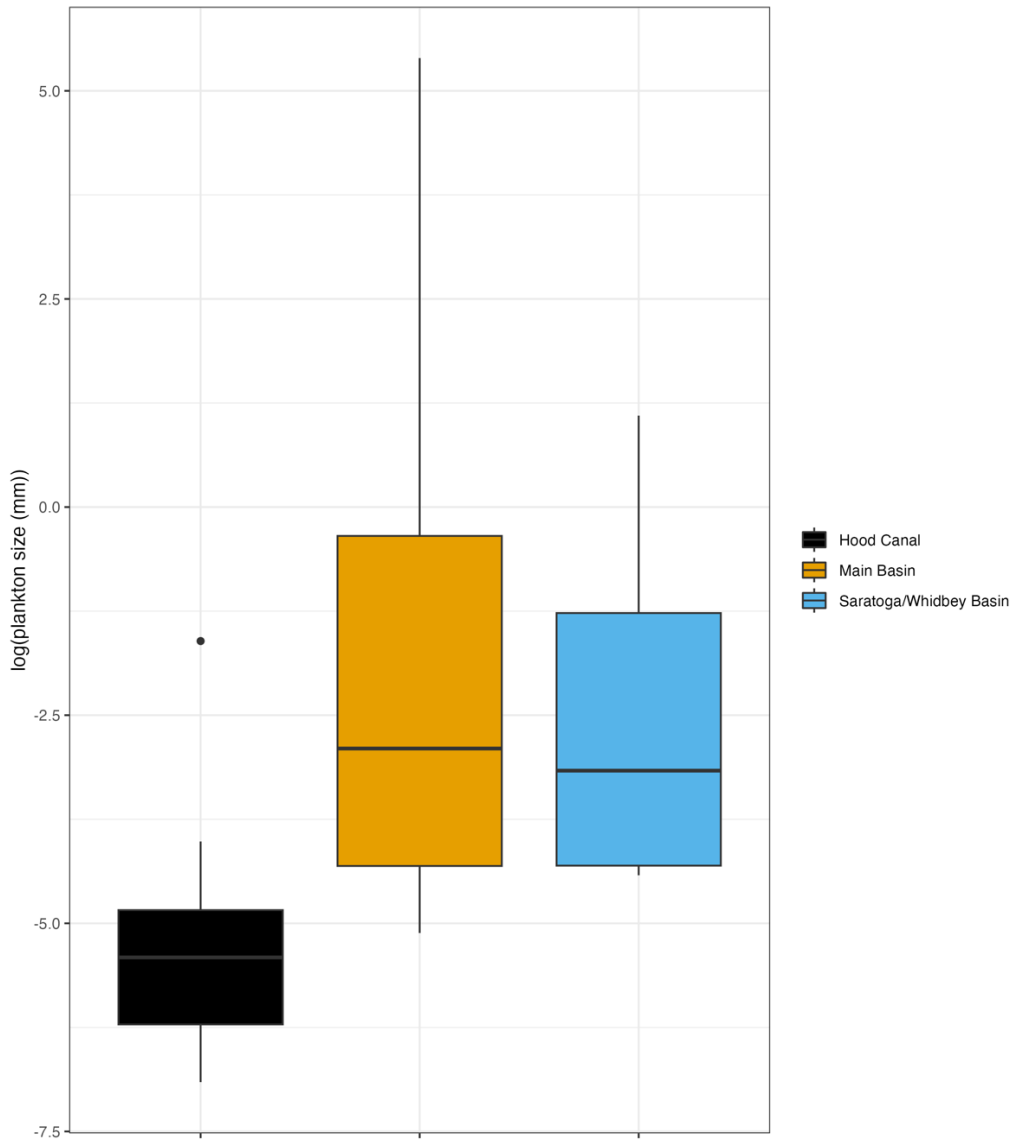


Figure 4. Boxplot shows the differences in sizes of identified characteristic taxa from each basin assemblage group. Size was transformed to log-scale for visual comparison. Color denotes basin group.

3.4 HAB taxa response to Environmental Variables

To understand if certain environmental variables were significant predictors of HAB taxa occurrence, a stepwise model selection chose suitably explanatory environmental variables for

each HAB taxon. Of 5 HAB taxa, only 3 could be predicted by environmental covariates (Figure 5a). Temperature was a significant predictor for *P. globosa* showing a negative correlation, salinity was positively correlated while phosphate (umol/kg) was negatively correlated with *P. farcimen*, and while phosphate alone was a significant predictor for *H. akashiwo* showing a negative correlation. GLM models were then plotted to visualize identified covariate influence on occurrence probability (Figure 5b-d).

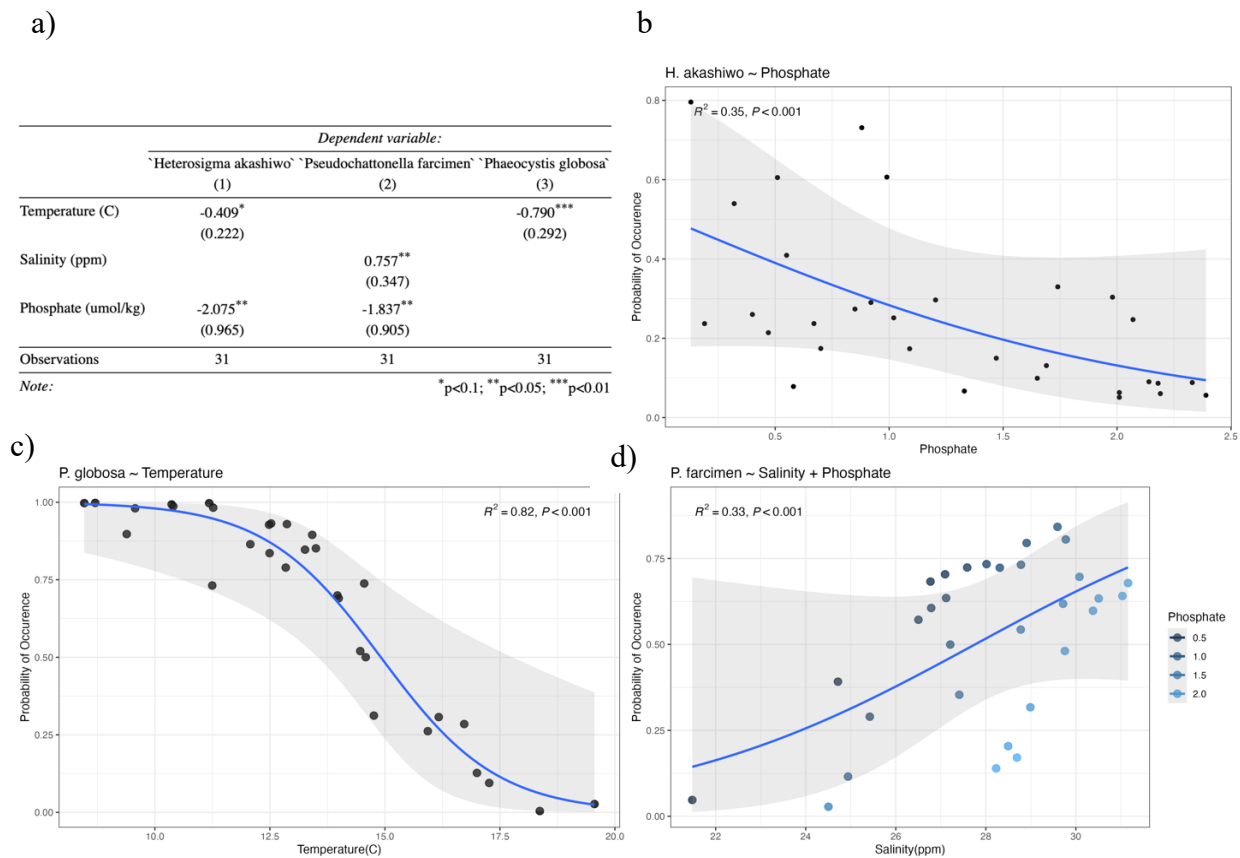


Figure 5. a) Fitted generalized linear model outputs for each HAB taxon using environmental covariates. b-d) Logistic regressions showing the probability of occurrence for *H. akashiwo* ~ Phosphate ($r^2 = 0.35$, p -value < 0.001), *P. globosa* ~ Temperature ($r^2 = 0.82$, p -value < 0.001), and *P. farcimen* ~ Salinity + Phosphate ($r^2 = 0.33$, p -value < 0.001).

Additionally, a generalized linear model was used to visualize potential trends in the probability of occurrence of HAB taxa probability in each basin grouping selected all months and samples from 2017-2021 (Figure 6). Hood Canal shows all taxa declining except for *P. farcimen*

with *H. akashiwo* completely absent from the samples. Salish Sea samples (Eastern Strait of Juan de Fuca, Central Sound, and Admiralty Strait) all 5 measured taxa occurring more often over time while Saratoga/Whidbey is split with *H. akashiwo* and *Alexandrium* spp. Increasing, declines in *P. globosa* and *P. farcimen* with *K. veneficum* absent.

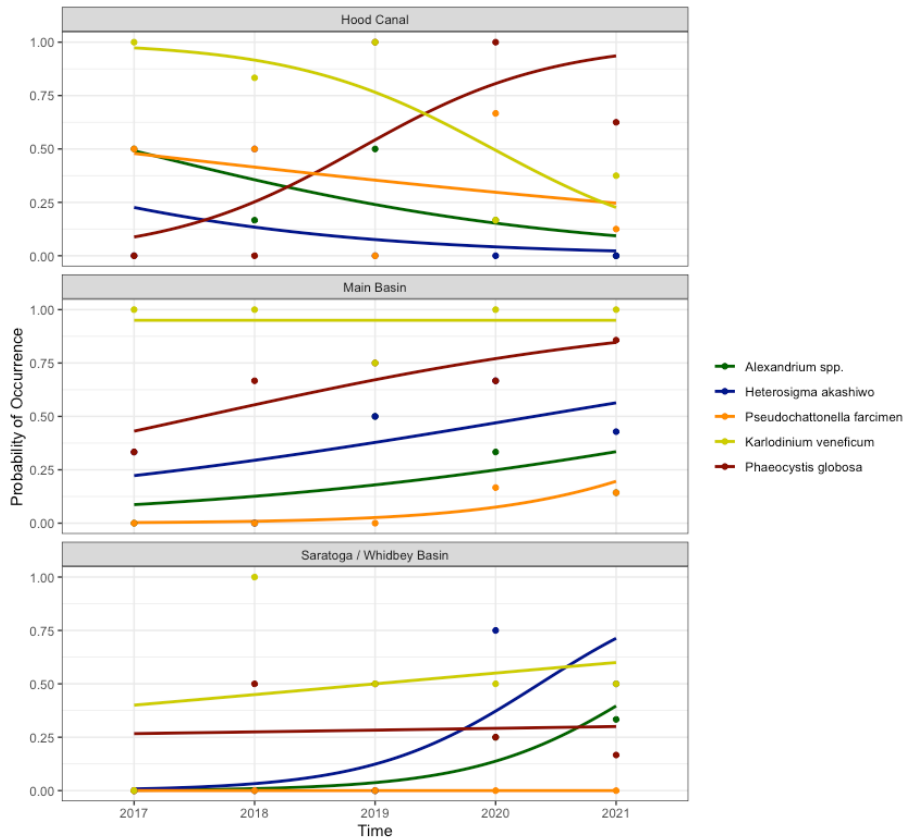


Figure 6. Generalized Linear Model of the probability of occurrence of HAB taxa as a function of time. Data points probability of occurrence (0-1) of each taxon for each year for each assemblage group in all months and sites sampled.

4.0 DISCUSSION

4.1 Spatial Distribution of Plankton Assemblages

Using eDNA metabarcoding, this study identified 58 common taxa from surface samples in the Salish Sea from 2017-2021. Results from both constrained and unconstrained ordination clearly show difference in community assemblage among Hood Canal basin, Saratoga/Whidbey basin, and remaining the basins. Plankton community assemblages at each site were shown to cluster significantly into 3 major basin groupings consisting of Hood Canal basin, Saratoga/Whidbey basin, and Main Basin (Eastern Strait of Juan de Fuca, Central Puget, and Admiralty Inlet basins). These differences are consistent with basin-specific differences in zooplankton diversity associated with differences in environmental processes (Keister et al. 2022).

To understand the underlying causes driving the differences in community assemblages, this study found that environmental variables such as temperature and phosphate were significant predictors however, they accounted for only 6% of the total observed variation in community assemblages suggesting that there are important physical processes not captured in this analysis. One hypothesis is that oceanographic conditions unique to these groupings are most likely significant in determining community characteristics. Water residency time in the Salish Sea varies significantly by basin: for example, seawater in the Hood Canal Basin exchanges between 64-121 days, contributing to stratification, high temperatures, and low oxygen levels, while basins outside of Hood Canal exchange seawater roughly every 33-44 days (Babson et al, 2006, Newton et al. 2007, Sutherland et al. 2011). Moreover, the Saratoga/Whidbey Basin receives input from three major rivers and the most freshwater of any basin during the winter and late spring snow melt (Babson et al. 2006). Additionally, Phosphate was found to be most significantly correlated with community assemblage differences which suggests that it may be having a presence/absence effect in a different way than other important nutrients such as Nitrogen which tends to be a limiting factor in abundance (Burkholder et al., 2006). More research is needed to understand the role of oceanographic processes overlaid with changing environmental conditions in the region on shifts in plankton community assemblages.

The ability to describe these community assemblage differences using molecular techniques will add powerful resolution by differentiating among taxa with nearly identical morphology. Rynearson and Armbrust (2000) hypothesized that there was significantly more

genetic diversity among diatoms than previously thought, allowing them to flourish under variable environmental conditions. With these new molecular techniques monitoring programs and researchers are capturing, at a finer scale, the genetic differences or similarities among previously morphologically cryptic plankton assemblages (O'Donnell et al., 2017; Specchia et al. 2023, Lv et al. 2022, o et al. 2020). Ultimately, molecular methods can further enhance our understanding of plankton community responses to rapidly changing environmental conditions.

4.2 Taxonomic Identification and Characteristic Taxa

In this study, taxonomy was assigned to unique amplification sequence variants by matching DNA base pairs to a reference data base resulting in a taxonomic assignment at the genus or species level that covers a broad cross-section of plankton. There is a significant body of literature discussing the relative costs and benefits of different methods of taxonomic identification (cite). As a result, taxonomy was conservatively retained keeping only ASVs with close matches to our reference data base and that were commonly occurring in our dataset. Occurrence of common taxa was treated as a binary presence or absence rather than quantitatively analyzing sequencing read counts. Future work could re-analyze these data with a focus on quantitative abundance.

This study identified individual taxa highly associated with each grouping of basins to understand how the most common taxa in each grouping may be functioning in the food chain. The Main Basin (Eastern Strait of Juan de Fuca, Central Puget, and Admiralty Inlet basins) assemblage hosts a significant number of diatoms such as the *Paralia sulcata*, *Thalassiosira nordenskiöldii*, *Coscinodiscus wailesii*, *Skeletonema* spp., as well as the bloom forming *Phaeocystis globosa*. Similarly, Saratoga/Whidbey basin includes a prevalent diatom *Attheya longicornis*. Additionally, common copepods include *P. newmani*, *A. longiremis*, and *C. pacificus*. Diatoms and copepods characteristic of the Hood Canal include *Minutocellus polymorphus*, *C. granii*, and *O. similis*. Basins with a diversity of diatoms may provide an important food source for copepod grazers and benefits for higher trophic levels, however lab studies have found species specific responses to toxic and non-toxic diatoms suggesting that not all diatoms confer equal energetic benefits (Lauritano et al., 2012; Halsband-Lenk et al., 2005; Ban et al, 1997; Ceballos and Ianora, 2003).

Taxa found to be characteristic in these different groupings also suggest a size difference with Hood Canal taxa appearing smaller than those found in the other basin groups. Work by Keister et al. (2022) hypothesizes that smaller oceanic taxa such as diatoms and copepods could be correlated with poor salmon outcomes. Additionally, climate models and field observations find an increase in the abundance of smaller phytoplankton in response to climate change (Daufresne et al., 2009; Henson et al., 2021; Roman & Pierson, 2022). Although this study did not specifically measure size of observed taxa, further research is warranted as shifts towards smaller plankton could cause deleterious effects on food web functioning (Rice et al., 2015).

4.3 HAB Taxa Response to Environmental Factors

Harmful algal taxa are of significant concern to managers in the Salish Sea due to their impacts on human health, the local economy, recreational opportunities, and ecosystem function (Horner et al 1997, Moore et al. 2009, Moore et al 2019). Other studies have worked to model how climate change impacts, such as warming sea surface temperatures, pH, and salinity are interacting to increase or decrease abundance of toxic HABs in the Salish Sea (Moore et al., 2011; Anderson et al., 2021). Additionally, toxin producing HABs such as *Alexandrium spp.* and *Pseudo-nitzschia spp.*, may be present or even bloom but produce no toxins suggesting that there are multiple mechanisms driving blooms and/or toxin production (Dyhrman et al., 2010; Lelong et al., 2012). The complicated interaction of environmental conditions on growth and toxin production in the field can confound interpretations of laboratory results (Keil et al. 2021, Lelong et al., 2012). eDNA, along with traditional methods, can serve to increase observational power and test additional hypothesis.

Metabarcoding results reliably identified 5 common HAB taxa and found that, over 5 years, there are differences in the probability of occurrence between basin clusters. Notably, all 5 HAB taxa appear to be increasing in occurrence in the main Basin of the Salish Sea compared to Hood Canal, where *P. farcimen* is decreasing and *H. akashiwo* is absent. *Alexandrium spp.* and *H. akashiwo* appear to show the sharpest increase overtime in the Saratoga/Whidbey basin, which is of significant management concern due to their negative impacts on human health (cite). Additionally, the clear negative relationship of *P. globosa* to temperature is highlighted both in the

predictive linear model and its decline in Hood Canal. This finding is supported by other studies which suggest there are differing genotypes within this species that occur in cold water (Niu et al., 2022; Wang et al., 2010; Smith and Trimborn, 2023). The differential trends in probability of occurrence between basins suggest that there are complex interactions between environmental conditions and spatial properties. The ability to track these taxa over time and make field observations relating environmental variables to occurrence is crucial to understanding the compounding impacts of a changing environment of distribution and occurrence trends.

5.0 CONCLUSION

All together, these results describe spatial differences in community assemblages as detected by eDNA metabarcoding of COI primers. Assemblages from Hood Canal differed from assemblages found in the Saratoga/Whidbey basin and the assemblages in both these basins were significantly different than the assemblage found in the Eastern Strait of Juan de Fuca, Central Sound, and Admiralty Inlet basins. A constrained distance-based redundancy analysis revealed temperature as the most important explanatory factor driving distance between grouped community assemblages, suggesting that future warming conditions may be the largest environmental predictor of differences between these community assemblages. Specific taxa were identified as having high fidelity and occurrence in each of the basin groupings suggesting differences in ecosystem functioning and production at the base the food chain in Hood Canal, Saratoga/Whidbey basin, and the grouped Eastern Strait of Juan de Fuca, Central Sound, and Admiralty Inlet basins. Lastly, 5 HAB taxa of concern were shown to be increasing in Eastern Strait of Juan de Fuca, Central Sound, and Admiralty Inlet basin sites over time. Hood Canal basin sites HAB taxa were declining over time except for *P. farcimen* while in Saratoga/Whidbey basin sites significant taxon of concern, *Alexandrium spp.* Is shown to be increasing.

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