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Macroalgae host pathogenic *Vibrio* spp. in a temperate estuary

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Anthropogenic climate change is altering coastal systems globally, affecting macroalgae abundance and composition. These macroalgae host diverse microbiomes, including pathogenic bacteria. Of particular concern are *Vibrio* species, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, which are linked to human disease and impact public health, the economy, and recreation in coastal areas. This study examined the presence and abundance of pathogenic *Vibrio* spp. across seven genera of macroalgae in a temperate estuary (Narragansett Bay, RI, USA). Using colony-forming unit (CFU) counts and multiplex qPCR, we quantified *V. parahaemolyticus* and *V. vulnificus* abundance to assess if pathogenic *Vibrio* abundance varied by macroalgae genus and morphology. We also examined potential environmental factors influencing pathogenic *Vibrio* prevalence. We demonstrate that both *V. vulnificus* and *V. parahaemolyticus* were present on all macroalgae genera, with *V. vulnificus* showing higher average abundance. Environmental factors like temperature, salinity, and nutrient concentrations did not strongly correlate with *V. vulnificus* or *V. parahaemolyticus* abundance, suggesting that macroalgae might offer a protective microhabitat for these pathogens. Macroalgae with opportunistic life strategies had the highest abundance of *V. vulnificus* and *V. parahaemolyticus*, highlighting their potential role as reservoirs for pathogenic *Vibrio* spp. Future research should explore broader environmental contexts and macroalgae–*Vibrio* spp. interactions to better understand and forecast pathogen dynamics.

KEYWORDS

***Vibrio parahaemolyticus*, *Vibrio vulnificus*, marine pathogens, qPCR, Narragansett Bay, *Ulva* spp., *Gracilaria* spp., *Fucus* spp.**

1 Introduction

Marine macroalgae are a diverse group of photosynthetic eukaryotic primary producers who perform vital services for ecosystems worldwide. In fact, marine macroalgae are considered the most productive and extensive organisms of all coastal vegetated ecosystems on the planet (Ji and Gao, 2021; Duarte et al., 2022). They live in every ocean and along

every continent, with extensive ranges of morphology, physiological requirements, and lifestyle types (e.g., attached and slower growing vs. free floating and fast growing) (Gattuso et al., 2006; Duarte et al., 2022). The highly productive habitats formed by macroalgae support both local and global food webs and important commercial and recreational fisheries (Krumhansl and Scheibling, 2012; Pessarrodona et al., 2018; Queirós et al., 2019; Duarte et al., 2022).

Marine macroalgae abundance, composition, and distribution are changing due to human activities at global (e.g., anthropogenic climate change) and local (e.g., nutrient loading) scales. Globally, changes in ocean conditions such as acidification and warming have led to widespread replacement of slower-growing perennial macroalgae with more opportunistic species, causing fundamental changes in ecosystem function worldwide (Connell and Russell, 2010; Ji and Gao, 2021). On a local scale, nutrient-rich waters can fuel large macroalgae blooms (Valiela et al., 1997; Wiencke and Bischof, 2012; Ji and Gao, 2021). These macroalgae blooms are increasing in frequency and scale in regions across the world and are positively correlated with human-caused nutrient loading, with hotspots in western Europe, North America, Central America, and China (Joniver et al., 2021). Macroalgae blooms have significant impacts on ecosystem services and cause changes in the ecology of coastal systems spanning trophic levels (Wang et al., 2020).

Expanding macroalgae habitat and increasing macroalgae growth can be positive as humans use macroalgae in a wide variety of ways, such as in nutrition-dense food products, manufacturing, fertilizer, pharmaceuticals, and animal feed (Garcia-Vaquero and Hayes, 2016; Peñalver et al., 2020; Duarte et al., 2022). Macroalgae also represent an important global carbon sink (Pessarrodona et al., 2018) and a tool for the bioremediation of anthropogenically impacted waters (Kumar and Sudhakar, 2024). However, macroalgae blooms can lead to deleterious ecosystem consequences. For example, macroalgae can outcompete seagrasses for resources (Zribi et al., 2023), macroalgae senescence and decomposition encourages hypoxia and anoxia (Castel et al., 1996; Viaroli et al., 1996; Senga et al., 2021), and large blooms are associated with losses of ecosystem biodiversity (Ji and Gao, 2021). Another emerging potential negative impact of excess macroalgae is that they can host pathogenic bacteria (e.g., *Vibrio* spp.) (Noorian et al., 2023). Macroalgae are regularly colonized by diverse microbial communities, with the genera *Vibrio* spp. being a commonly detected member (Akrong et al., 2023). The characteristics of the macroalgae (e.g., growth style, tissue differentiation, morphology, and physiological requirements) play a major role in determining the composition of their microbial community (Littler and Littler, 1980; Steneck and Watling, 1982; Staufenberger et al., 2008; Barott et al., 2011; Lachnit et al., 2011; Lemay et al., 2021). It remains largely unknown if macroalgae could be a host for the human pathogen *Vibrio* spp., specifically *V. vulnificus* and *V. parahaemolyticus*. Few studies have quantified the abundance of specifically pathogenic *Vibrio* on macroalgae; however, those that have suggest that macroalgae may provide a habitat for pathogenic *Vibrio* spp. (Mahmud et al., 2007; Mahmud et al., 2008; Gonzalez et al., 2014).

Vibrio spp. are halophilic, heterotrophic bacteria that occur naturally in marine and estuarine environments worldwide. They can account for as much as 40% of the readily culturable bacteria population in estuaries (Thompson and Polz, 2014; Urakawa and Rivera, 2014). Of the over 100 species contained within the *Vibrio* genus, the two non-cholera species of most interest are *V. vulnificus* and *V. parahaemolyticus*. *V. vulnificus* has been detected across a wide range of salinities (4‰ to 37‰) and temperatures (7°C–36°C) and found to grow optimally at salinities between 10‰ and 25‰ and at a temperature of 20°C (Noorian et al., 2023). *V. vulnificus* is an emerging pathogen which can cause necrotizing fasciitis and primary septicemia when an open wound or an immunocompromised individual is exposed (Tison and Kelly, 1984). *V. vulnificus* infections account for 95% of all *Vibrio* spp.-related deaths in the United States and are considered pathogenic whenever present (Morris and Acheson, 2003). *V. parahaemolyticus* preferentially grows in warm (>15°C), low salinity <25‰ waters. *V. parahaemolyticus* is the most common pathogenic bacterium associated with raw or undercooked seafood consumption and typically leads to self-limiting gastroenteritis (Baker-Austin et al., 2018). Rarely, *V. parahaemolyticus* can also cause necrotizing fasciitis and septicemia in immunocompromised individuals (Letchumanan et al., 2014).

While not all *V. parahaemolyticus* strains are pathogenic, those that are possess a complex array of factors that control their virulence. Pathogenic *V. parahaemolyticus* strains are typically identified by the presence of two key genes: thermostable direct hemolysin (*tdh*) and thermostable direct-related hemolysin (*trh*) (Nishibuchi et al., 1992; Xu et al., 1994; Yeung and Boor, 2004; Raghunath, 2015). However, strains carrying both *tdh* and *trh* are less common in environmental isolates, with their prevalence generally lower compared to clinical strains (e.g., those isolated from hospitalized patients) (Nishibuchi et al., 1992; DePaola et al., 2000; DePaola et al., 2003). In contrast, *V. vulnificus* is always considered pathogenic and is commonly identified by the presence of the *toxR* gene, a crucial marker for its virulence (Warner and Oliver, 2008; Baker-Austin et al., 2018; U.S. Food and Drug Administration (FDA) and Center for Food Safety and Applied Nutrition, 2024).

Increases in the abundance of *V. vulnificus* and *V. parahaemolyticus* are associated with climate change, and they are undergoing a global expansion typically related to warming of coastal waters (Baker-Austin et al., 2017; Almagro-Moreno et al., 2023; Noorian et al., 2023). Reports of *Vibrio* infections are on the rise (Baker-Austin et al., 2010; Baker-Austin et al., 2013). In addition to increasing the abundance of *Vibrio* spp. in the water, elevated water temperatures may also increase the virulence and transmission of marine pathogens widely by increasing the pathogen's metabolism and fitness (Karvonen et al., 2010; Burge et al., 2014; Lam et al., 2014; Billaud et al., 2022; Roncarati et al., 2024). The prevalence of *Vibrio* spp. is predicted to make a significant expansion into the higher latitudes of the northern hemisphere in the next century as temperatures rise and expand their habitable range (Baker-Austin et al., 2010; Baker-Austin et al., 2013; Almagro-Moreno et al., 2023). The increasing risk for *Vibrio*

spp. infection has made it essential to understand the ecology, drivers, and reservoirs for pathogenic *Vibrio* spp. in the environment.

The purpose of this study was to assess whether the abundance and composition of pathogenic *V. vulnificus* and *V. parahaemolyticus* varied across space, time, and macroalgae genera. To do so, we quantified the abundance of these pathogenic strains on seven dominant macroalgae genera, representing all three classes of marine macroalgae, over the growing season in a temperate estuary (Narragansett Bay, RI, USA). We also measured environmental characteristics commonly linked to *Vibrio* spp. abundance, such as temperature, salinity, and total suspended particles. We had two hypotheses: 1) *V. vulnificus* and *V. parahaemolyticus* would be present on all macroalgae, even in small quantities, with more complex morphologies and opportunistic species exhibiting higher abundance due to their larger surface area-to-volume ratio (Ji and Gao, 2021). 2) Both *V. vulnificus* and *V. parahaemolyticus* would show a clear peak in abundance across all sites and genera, coinciding with higher temperatures, as previous research suggests these *Vibrio* spp. prefer warmer waters (Baker-Austin et al., 2018). To test these hypotheses, *V. vulnificus* and *V. parahaemolyticus* abundance was quantified through colony-forming unit counting, followed by species identification using qPCR and assays to assess pathogenicity.

2 Materials and methods

2.1 Study sites and characteristics

Narragansett Bay is a well-mixed, shallow (~8 m mean depth) estuary located in the state of Rhode Island on the east coast of the United States. Narragansett Bay broadly follows a north–south gradient in salinity, nutrient loading, and productivity driven by freshwater inputs at the north end of the Bay. While typically described as a phytoplankton-based system, it also supports a robust macroalgae community of over 90 documented species (Villalard-Bohnsack and Harlin, 1992). We selected three sites for this study (Figure 1): site 1 is the northern most and shallowest site; site 2 is a small, shallow subestuary (called Greenwich Bay) that branches off along the northwest side of Narragansett Bay; and site 3 is the southernmost site, in a sheltered embayment with long-term poor water quality (Lorraine and Lucht, 2000; Oakley et al., 2012).

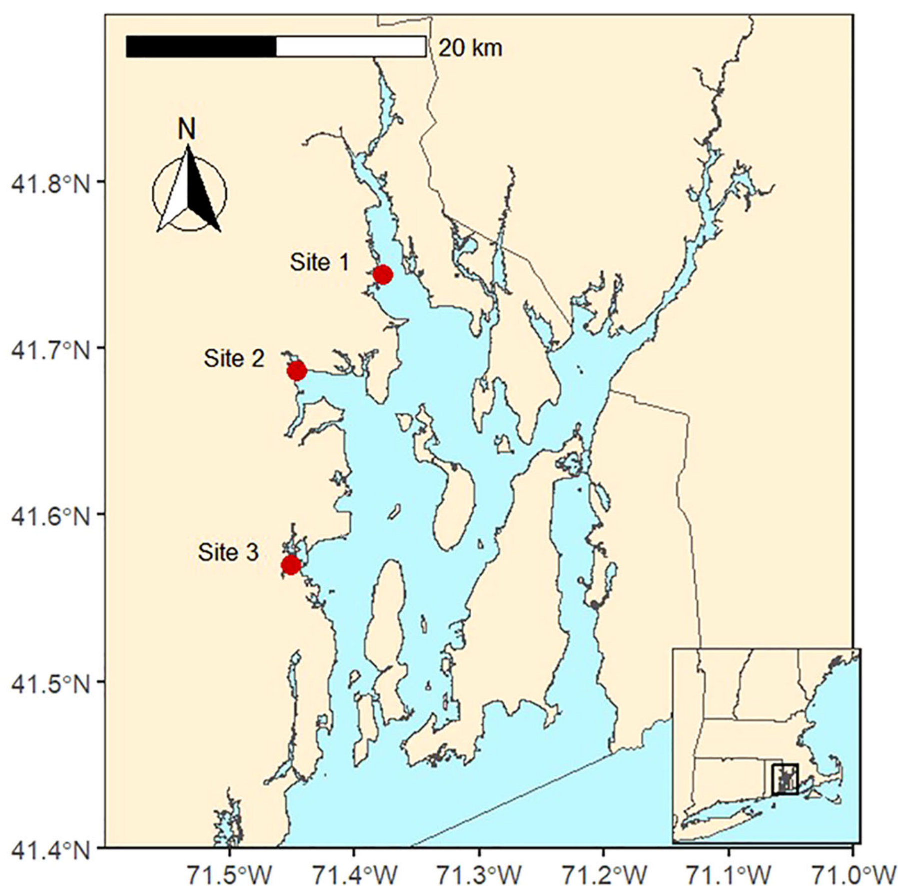


FIGURE 1
The three sampling locations (shown with red dots) in Narragansett Bay, Rhode Island, USA. Samples of macroalgae, water column nutrients, chlorophyll-a, and physiochemical parameters were collected at each site between June and October 2022.

Overall, during this study, the sites had similar physiochemical conditions, with some differences in nutrient and water column chlorophyll concentrations (Table 1). Environmental conditions in Narragansett Bay during the study period (June–October 2022) were typical for late spring through early fall. Water temperatures ranged from 21.2°C to 27.4°C, with the highest temperatures recorded in August. Salinity remained relatively stable, varying between 25‰ and 31.6‰. Dissolved inorganic nitrogen (DIN) concentrations fluctuated across sites, with the highest levels observed in August. Total suspended solids (TSS) was variable, with the highest levels in July. These environmental factors provide context for interpreting *Vibrio* spp. abundance patterns (please see Supplementary Table S2 for more details).

2.2 Sample collection

We collected samples twice monthly from June 2022 to September 2022 and once in October 2022 for a total of nine sampling events at each site. We chose this sampling scheme to capture the warmest yearly temperatures, which should facilitate pathogenic *Vibrio* spp. growth (Baker-Austin et al., 2018).

We collected samples from the shore either off the beach (site 1; Figure 1) or off floating docks (sites 2 and 3; Figure 1). At each sampling event, triplicates of the three most abundant genera of live macroalgae were collected by hand in sterile Whirl-Pak™ bags to determine the concentration of pathogenic *Vibrio* spp. on the thalli. We placed the bags on ice in a dark cooler and transported them back to the laboratory at Boston University for processing within 3 h of collection. Macroalgae with visible epibiont growth (e.g., bryozoans, filamentous fouling organisms) were excluded from sampling to minimize confounding effects of epibionts on *Vibrio* spp. abundance.

Along with the macroalgae samples, we collected water samples for concentrations of dissolved inorganic nitrogen and phosphorus, chlorophyll-a and phaeophytin, and total suspended solids. In the field, we filtered duplicate water samples using a 60-mL acid-washed polypropylene syringe with glass fiber filters (Whatman GF/F, 0.70 micron pore size) into 30 mL of acid-washed and deionized water-leached polyethylene bottles. The filter samples were stored on ice in a dark cooler until being returned to the lab and then were stored in a freezer (−20°C) until analysis for dissolved inorganic nitrogen [DIN: ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-)] and dissolved inorganic phosphate (DIP) (Foster and Fulweiler, 2014). Chlorophyll-a and phaeophytin measurements were also collected in duplicate at each site using a deionized water cleaned 60 mL polypropylene syringes (Arar and Collins, 1997; Fagherazzi et al., 2014; Ray et al., 2020). Filters were stored in the dark, on ice, until being returned to the lab, and then at −80°C until analysis. During each macroalgae collection, we also measured water column temperature, salinity, and dissolved oxygen using a Hach sensor (LDO101).

2.3 Macroalgae overview

Over the duration of this study, 233 macroalgae samples were collected and analyzed for the presence of pathogenic *Vibrio* spp. bacteria. Out of the 233 total macroalgae samples, phylum *Rhodophyta* accounted for 112 samples, phylum *Chlorophyta* for 112 samples, and phylum *Phaeophyceae* accounted for the rest. The majority (n = 210) of the macroalgae were opportunistic. Of the seven macroalgae genera we studied, three genera were classified as a thin blade morphology, two had a finely branched morphology, and two were coarsely branched (further sample information may be found in Supplementary Table S1) (Hurd, 2000).

2.4 *Vibrio* spp. laboratory processing

Macroalgae was identified to the genus level and other characteristics (e.g., morphology type) were identified using surface area to volume (SA:V) ratios reported by (Littler and Littler, 1980). Note that while the genera of *Enteromorpha* and *Ulva* contain multiple synapomorphies and the nomenclature is altered, for the purpose of this study, we chose to keep them separated due to the large differences in morphology (and therefore SA:V) (Hayden et al., 2003; Ning et al., 2022). We processed the macroalgae samples as outlined by (Gonzalez et al., 2014). Briefly, we weighed 10 g (wet weight) of the macroalgae sample, then combined it with 100 mL of phosphate-buffered saline (PBS) and shook the sample for 5 min to dislodge the microbial biofilm on the macroalgae where *Vibrio* spp. may be present (Figure 2). Immediately after shaking, samples from the resulting liquid were serially diluted with PBS and then plated. Specifically, we plated all macroalgae samples on CHROMagar™ *Vibrio* medium (CHROMagar™, Paris, France) to determine the counts for *V. vulnificus* and *V. parahaemolyticus* (Supplementary Figure S1). To control for the variable initial water content of the macroalgae, we determined the dry weight of each sample by

TABLE 1 Site characteristics during this study (June–October 2022).

Site	Temperature (°C)	Salinity (‰)	DO (mg L^{-1})	NH_4 (μmol L^{-1})	NO_x (μmol L^{-1})	DIN (μmol L^{-1})	DIP (μmol L^{-1})	Chl-a (μg L^{-1})	Phaeophytin (μg L^{-1})	TSS (mg L^{-1})
1	22.4 ± 0.91	25 ± 0.92	7.5 ± 0.32	3.1 ± 0.69	3.3 ± 1.51	6.4 ± 2.54	2.4 ± 0.38	8.5 ± 1.71	7.3 ± 1.47	0.26 ± 0.11
2	23.4 ± 1.13	29 ± 0.31	4.9 ± 0.48	3.3 ± 1.05	0.9 ± 0.47	4.6 ± 2.02	1.8 ± 0.21	15 ± 1.29	4.9 ± 0.63	0.21 ± 0.12
3	23.4 ± 1.00	29 ± 0.54	5.1 ± 0.50	4.9 ± 1.09	2.9 ± 0.91	7.9 ± 1.85	1.8 ± 0.15	6.2 ± 0.68	2.9 ± 0.41	0.18 ± 0.15

Mean (± standard error) is reported for each parameter as measured on the day of pathogenic *Vibrio* spp. sample collection. For more detailed data on site conditions for each sampling period, please see Supplementary Table S2.

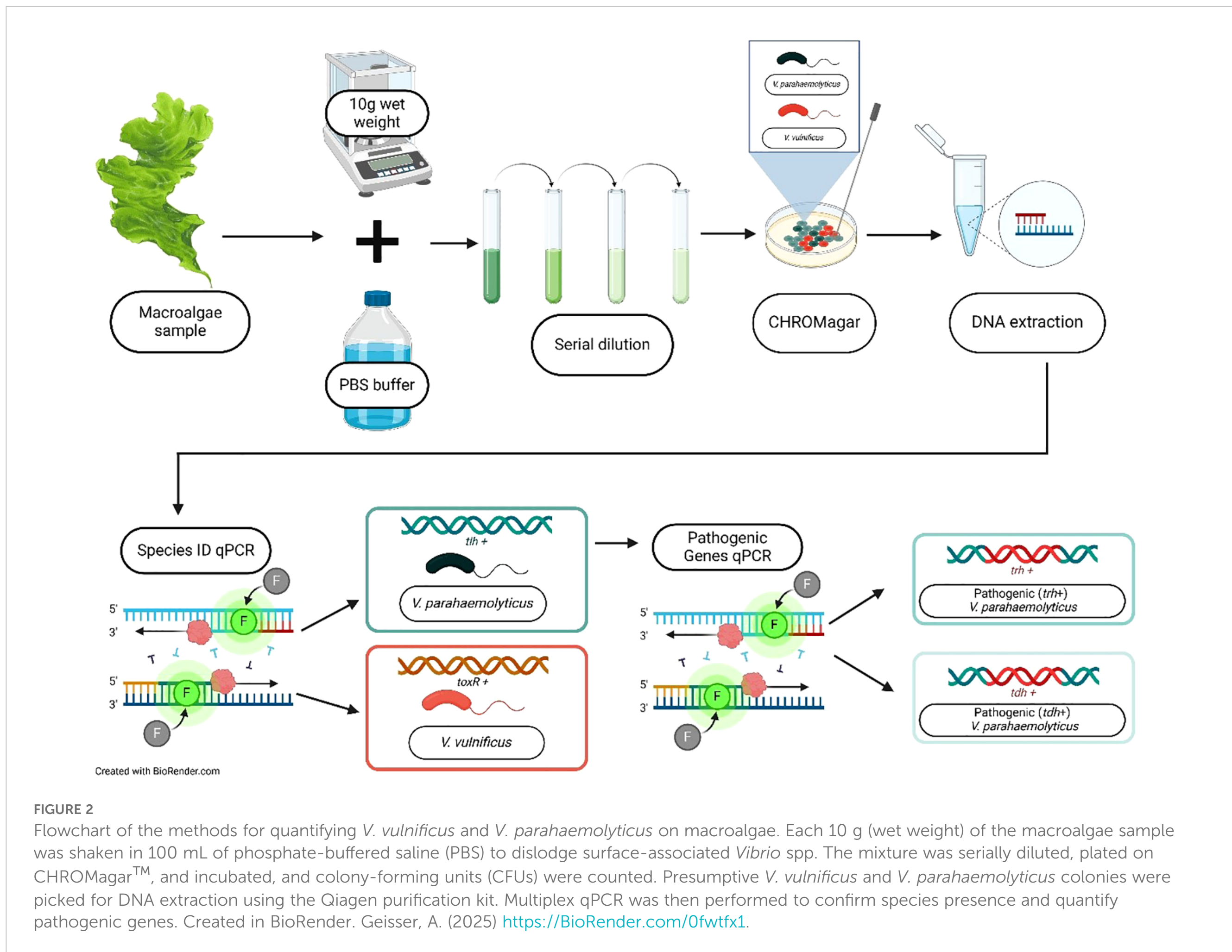


FIGURE 2
Flowchart of the methods for quantifying *V. vulnificus* and *V. parahaemolyticus* on macroalgae. Each 10 g (wet weight) of the macroalgae sample was shaken in 100 mL of phosphate-buffered saline (PBS) to dislodge surface-associated *Vibrio* spp. The mixture was serially diluted, plated on CHROMagar^{TM}, and incubated, and colony-forming units (CFUs) were counted. Presumptive *V. vulnificus* and *V. parahaemolyticus* colonies were picked for DNA extraction using the Qiagen purification kit. Multiplex qPCR was then performed to confirm species presence and quantify pathogenic genes. Created in BioRender. Geisser, A. (2025) https://BioRender.com/0fwtfx1.

drying the initial 10 g of the samples in preweighed tins in a 60°C oven for a minimum of 48 h (or until constant weight) and reweighed.

We first confirmed *Vibrio* species type using previously published duplex qPCR assays (Scro et al., 2019). We confirmed the presence of *V. parahaemolyticus* by using specific primers for the gene encoding for thermostable hemolysin (*tlh*) that is found in all *V. parahaemolyticus*. *V. vulnificus* was confirmed by the *toxR* gene, which codes for the transmembrane virulence regulator gene present in this species (Warner and Oliver, 2008; Scro et al., 2019). The primers and probes for both *Vibrio* species and *V. parahaemolyticus* pathogenic gene identification in a secondary multiplex qPCR can be found in Supplementary Table S1 (Scro et al., 2019).

Next, we performed another multiplex qPCR assay on any sample positive for *V. parahaemolyticus* to determine the presence of pathogenic genes (Supplementary Table S3). Genes that encode for thermostable direct hemolysin (*tdh*) and thermostable direct hemolysin-related hemolysin (*trh*) are strongly representative of the virulence of *V. parahaemolyticus* (Nishibuchi et al., 1992; Gutierrez West et al., 2013). All *V. vulnificus* species were considered pathogenic, so no further genetic identification was needed.

2.5 Environmental laboratory processing

We used standard colorimetric techniques and a high-resolution digital colorimetry on a SEAL AutoAnalyzer 3 with segmented flow injection to analyze water samples for dissolved inorganic nitrogen and phosphorus (Grasshoff et al., 1999; Solórzano, 1969; Johnson and Petty, 1983). The detection limits for these analyses were 0.080 μM for NH_4^+, 0.013 μM for NO_x, 0.013 μM for NO_3^-, 0.006 μM for NO_2^-, and 0.010 μM for PO_4^{3-}. We analyzed filters for chlorophyll-a and pheophytin using standard acetone extraction and a Turner Designs TD-700 fluorometer (Sigma-Aldrich, St. Louis, MO, USA) calibrated with pure chlorophyll-a (Solórzano, 1969; Johnson and Petty, 1983; Hansen and Koroleff, 2007; Foster and Fulweiler, 2014).

2.6 Statistical analysis

All statistical analyses were conducted in R version 4.3.3 (R Core Team, 2024). The results were considered statistically significant when $p < 0.05$. Both *V. vulnificus* and *V. parahaemolyticus* count data were found to best fit a negative binomial distribution, using the *fitdistruplus* package (Delignette-

Muller and Dutang, 2015). The data for both *V. vulnificus* and *V. parahaemolyticus* met all criteria for a zero-inflated negative binomial (ZINB) regression model, meaning the data had excess zeros compared to a standard Poisson or negative binomial distribution, variance greater than the mean which indicates overdispersion, and heterogeneity and justification in both the count and zero-generating facets of the process. Because standard models cannot capture the underlying structure of these data, we employed a ZINB regression model. We used the R package *nyiuab/NBZIMM*, built on top of the commonly used R packages *nlme* and *MASS*, to evaluate the potential environmental drivers of *V. vulnificus* and *V. parahaemolyticus* bacteria counts (Zhang et al., 2017).

To determine which predictor variables were driving pathogenic *Vibrio* counts on macroalgae, we created two sets of ZINB models using predictor variables of interest that did not covary (determined by first calculating a Pearson correlation, Supplementary Figure S2). These predictor variables are the environmental parameters we collected at each sampling date to correspond with *Vibrio* spp. abundance. Each environmental parameter was included in the model as an individual measurement corresponding to each sampling date rather than as a seasonal average. To assess which predictor variables to include in the final model, we assessed a combination of statistical significance, theoretical relevance, and ZINB model considerations. For a predictor variable to be included in the final model, the count model (negative binomial part) for each parameter needed to have a *p*-value ($Pr(>|z|)$) less than the significance level of 0.05, signifying a statistically relevant association between the predictor variable and the *Vibrio* spp. count. Temporal variation was inherently captured by using environmental data from each sampling event rather than aggregated seasonal values. If significant seasonal patterns were present, they would be reflected in the relationships identified by the ZINB model. Once the variables of interest were selected, we performed backwards stepwise variable elimination to find the best fit model using the function *be.zeroinfl* in the package *mpath* (Wang et al., 2015). The model for each *Vibrio* species that best explained the variation in the data and had the least number of parameters was selected based on the lowest Akaike's information criterion (AIC) (Sakamoto et al., 1986). To quantify the explanatory power of the ZINB model, we calculated Nagelkerke's adjusted R^2 value (Foster et al., 2005; Martin and Hall, 2016). Due to the complex nature of ZINB models, we based our analysis and interpretation of predictor variable importance on Nagelkerke's adjusted R^2 due to this approach being scaled to resemble the R^2 from linear regression more closely (Foster et al., 2005). We tested whether *V. vulnificus* and *V. parahaemolyticus* abundances were significantly different between sites, genera, class, morphology, and lifestyle type of macroalgae using pairwise least-square means tests through the *emmeans* package (Lenth, 2017).

3 Results

V. vulnificus and *V. parahaemolyticus* were quantified using CFU counts, and the two species were then confirmed using qPCR multiplex assays to target genes present in each respective species. *V. vulnificus* is always considered pathogenic, while *V. parahaemolyticus* needs to be further analyzed for the presence of pathogenic genes using a qPCR multiplex assay with samples that tested positive for initial detection of the species (Scro et al., 2019). We first give background on macroalgae samples tested, then describe pathogenic *Vibrio* spp. abundance on macroalgae via CFUs of each species that have been confirmed using molecular methods. We do this because molecular methods paired with traditional counting methods (CFU and MPN) are more sensitive and precise in enumerating pathogenic *Vibrio* spp. Then, we describe the pathogenic gene abundance in the context of all samples taken, as well as further identifying the presence of pathogenic genes within *V. parahaemolyticus*.

3.1 Quantification of pathogenic *Vibrio* spp. on macroalgae

We acknowledge that while CHROMagar™ *Vibrio* is a widely used selective medium, there is a potential for misidentification due to the metabolic overlap among *Vibrio* species (Yeung and Thorsen, 2016; Parveen et al., 2020). However, we followed the FDA-BAM protocol for enumeration and species confirmation, which is based on statistical likelihood rather than exhaustive molecular confirmation of all colonies. To enhance confidence in our identifications, we tested as many colonies as we could identify, beyond the minimum subset required. This approach aligns with previous studies using similar methods (Gonzalez et al., 2014; Barberi et al., 2020, Givens et al., 2014; Parveen et al., 2008; Brumfield et al., 2023). While we did not adjust CFU estimates based on PCR confirmation rates, our methodological consistency with FDA protocols and prior research suggests that our reported *Vibrio* spp. abundances are robust. Future work using whole-genome sequencing or MALDI-TOF could further validate species assignments and refine culture-based methods for *Vibrio* identification (Moussa et al., 2021). We found that pathogenic *Vibrio* spp. were present across all genera, with mean counts of 1.12×10^5 CFU g^{-1} for *V. parahaemolyticus* and 3.68×10^6 CFU g^{-1} for *V. vulnificus* (Figure 3). *Ulva* spp. had the highest count for *V. vulnificus* (3.8×10^7 CFU g^{-1}), and *Fucus* spp. had the highest count for *V. parahaemolyticus* (3.3×10^6 CFU g^{-1}).

We hypothesized that *V. vulnificus* and *V. parahaemolyticus* would vary by life strategy because microbial communities may have more time to accumulate on older tissues (Goecke et al., 2010). Instead, we found that *V. vulnificus* and *V. parahaemolyticus* abundance did not vary by life strategy (Supplementary Table S3). However, there was a clear abundance of opportunistic macroalgae (e.g., *Ulva*) present at all sites (n = 210) compared to a lack of slower-growing genera (e.g., *Fucus*, n = 23), so there may be associations not captured in this study.

We hypothesized that macroalgae morphology, which is reflective of the host's ecological niche and surface area to volume ratio, would be a significant predictor of *V. parahaemolyticus* and *V. vulnificus* abundance. We anticipated this because bacteria generally

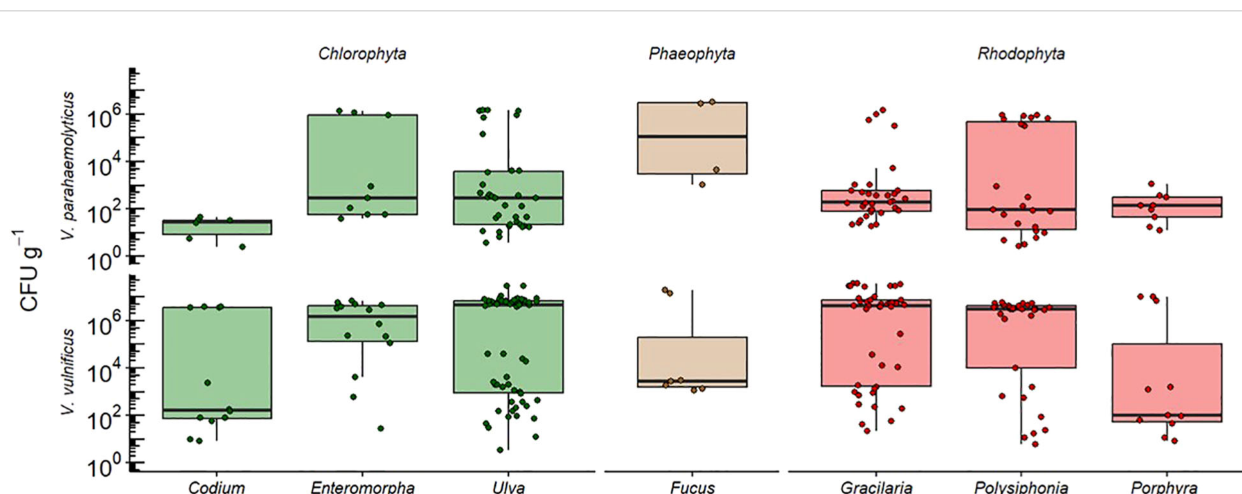


FIGURE 3
Counts of *V. vulnificus* and *V. parahaemolyticus* colony-forming units (CFUs) isolated from macroalgae samples during the study period. CFU counts were determined following the US FDA-BAM protocol, with presumptive identifications based on CHROMagar^{TM} color and morphology and species confirmation through qPCR targeting the *toxR* (for *V. vulnificus*) and *tlh* (for *V. parahaemolyticus*) genes. While not every individual colony was tested, genetic confirmation was conducted on representative colonies from each sampling site and date. Whiskers represent ± SE and the median is represented by the solid black line in the box plot. There were no significant differences in bacterial abundance between macroalgae genera.

follow the well-established ecological paradigm, where increasing habitat structural complexity increases biodiversity and richness in the system (Lemay et al., 2021). We found that *V. vulnificus* abundances were significantly higher (p = 0.0203) on the finely branched macroalgae with complex morphology and higher surface-area-to-volume ratio compared to other morphologies. *V. parahaemolyticus* abundance, however, did not vary significantly by morphology type (Supplementary Table S3).

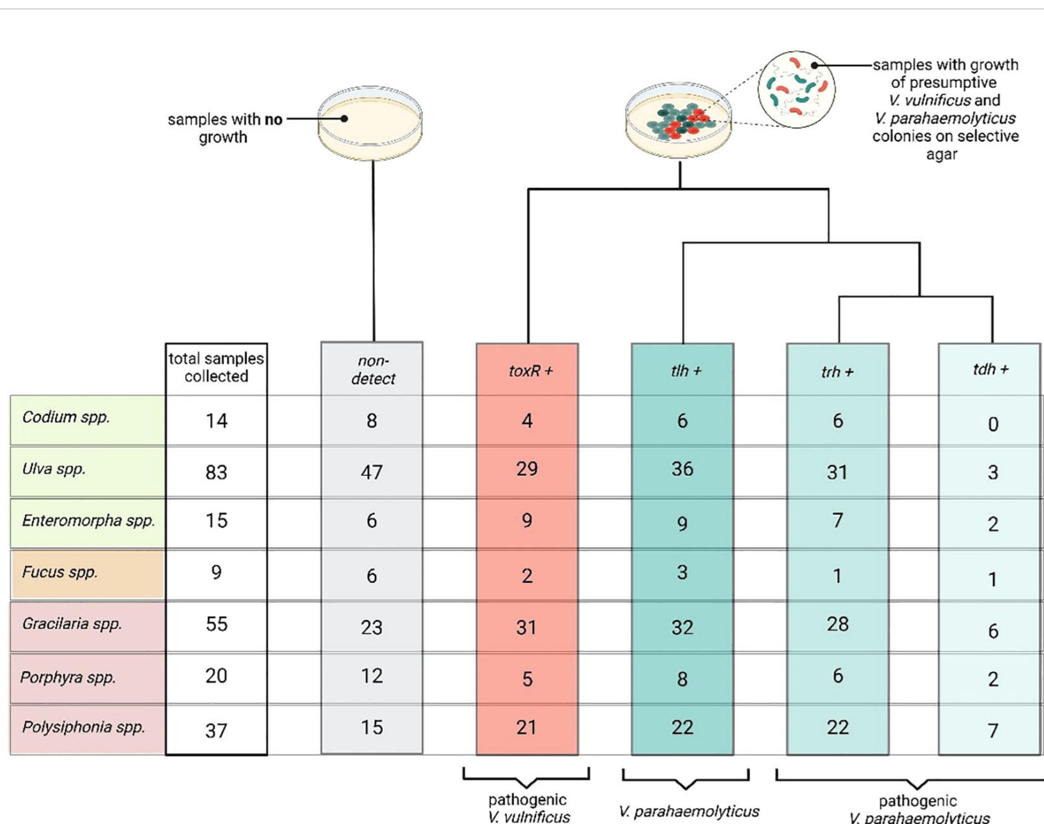
3.2 Pathogenic gene presence

Overall, 233 macroalgae samples across seven genera were taken throughout the study period. Out of the 233 samples, 117 had no growth of presumptive *Vibrio* spp. bacteria on CHROMagar plates, while 116 samples tested positive for the *V. parahaemolyticus* species identifier gene (*tlh*) and 101 tested positive for the *V. vulnificus* identifier gene (*toxR*, Figure 4). These two genes are not mutually exclusive, and both species of *Vibrio* may be present in the sample and grow on the CHROMagar plate. The genera *Ulva*, *Gracilaria*, and *Polysiphonia* have higher numbers of samples that tested positive for the *toxR* (*V. vulnificus*) and *tlh* (*V. parahaemolyticus*) genes, and these were also the genera with the most total samples collected. *V. vulnificus* is considered pathogenic with the detection of the *toxR* alone, so further analysis was not necessary. However, to determine the pathogenicity of *V. parahaemolyticus*, we quantified pathogenic gene presence (*tdh* and/or *trh*). Any sample that tested positive for the presence of the *V. parahaemolyticus* species identifier gene *tlh* was then further tested for the presence of the two pathogenic genes *trh* and *tdh*. Of the *tlh*-positive samples, 101 tested positive for *trh* and 21 tested positive for *tdh* (Figure 4). These genes are also not mutually exclusive and do often co-occur. In this study, positive *tdh* detections always co-occurred in samples that were also positive for *trh*.

3.3 Environmental characteristics and pathogenic *Vibrio* abundance

We hypothesized that both *V. parahaemolyticus* and *V. vulnificus* abundances would be linked to environmental parameters because previous research has established these links, particularly with salinity, temperature, and, to varying degrees, nutrient concentrations (Thompson and Polz, 2006; Baker-Austin et al., 2010; Takemura et al., 2014; Paranjpye et al., 2015; Sha et al., 2022; Kalvaitienė et al., 2023). The bulk of water column physiochemical parameters linked to *Vibrio* spp. abundance is inconsistent across studies and relationships dependent on taxonomic, geographic, and temporal resolution (Takemura et al., 2014). Our ZINB model identified NH_4^+, NO_x, and salinity as the most significant predictors of *V. parahaemolyticus* abundance (CFU g^{-1}), with the lowest AIC values supporting their inclusion in the model (Table 2). In contrast, *V. vulnificus* abundances were best predicted by TSS, DIP, and salinity, based on AIC model selection (Table 2). These predictor variables are the environmental parameters collected at each sampling date, which were used to assess their association with *Vibrio* spp. abundance. Despite these being significant predictors, the overall explanatory power of the model remained limited (Nagelkerke's R^2 = 0.20 for *V. parahaemolyticus* and 0.05 for *V. vulnificus*), indicating that other unmeasured factors may also be influencing *Vibrio* dynamics in these samples.

The final models were chosen based on AIC values, indicating the model's quality of model fit and complexity, where a lower AIC



	total samples collected	non-detect	toxR +	tlh +	trh +	tdh +
Codium spp.	14	8	4	6	6	0
Ulva spp.	83	47	29	36	31	3
Enteromorpha spp.	15	6	9	9	7	2
Fucus spp.	9	6	2	3	1	1
Gracilaria spp.	55	23	31	32	28	6
Porphyra spp.	20	12	5	8	6	2
Polysiphonia spp.	37	15	21	22	22	7

FIGURE 4
Summary of macroalgae samples collected, plated, and tested for *Vibrio* species and pathogenic genes. The first column represents the total number of macroalgae samples collected for each genus, including both positive and negative detections. The second column (gray, non-detected) indicates samples that did not grow colonies with the characteristic *Vibrio* morphology on CHROMagar™ and were therefore not tested further. The third column (red, *toxR*+) represents samples that contained colonies confirmed as *V. vulnificus* via qPCR detection of the *toxR* gene, which is species-specific and associated with pathogenicity. The fourth column (blue, *tlh*+) shows samples with *V. parahaemolyticus*, confirmed through detection of the *tlh* gene. The next columns display samples that tested positive for the pathogenic *trh* (blue, *trh*+) and *tdh* (blue, *tdh*+) genes, both of which indicate virulent *V. parahaemolyticus*. *tdh* was only detected in samples that were also positive for *trh*, suggesting the presence of highly virulent serotypes. Created in BioRender. Geisser, A. (2025) https://BioRender.com/0fwtfx1.

value indicates a model with a better trade-off between goodness-of-fit and the number of parameters. Nagelkerke's R^2 was calculated to assess the explanatory power of the model as it is scaled to resemble the R^2 from linear regression more closely, allowing for a clearer interpretation. For stepwise AIC values for each model, please see Supplementary Tables S4, S5.

4 Discussion

Here, we show that *V. parahaemolyticus* and *V. vulnificus* are present on a variety of common temperate macroalgae. The

TABLE 2 ZINB models were created with the predictor variables determined to be statistically significant to *Vibrio* spp. abundance.

	Model parameters	AIC	Nagelkerke's R^2
V. parahaemolyticus	Salinity + NH_4 + NO_x	645	0.20
V. vulnificus	TSS + DIP + salinity	995	0.05

abundances of *V. parahaemolyticus* and *V. vulnificus* documented in this work are on par or higher than those reported in previous studies investigating macroalgae and pathogenic *Vibrio* spp. For example (Gonzalez et al., 2014), report maximum abundances of 1.2 × 10^4 CFU g^{-1} of *V. parahaemolyticus* and 3.2 × 10^3 CFU g^{-1} of *V. vulnificus* on *Gracilaria* spp. during the growing season in Virginia, in the mid-Atlantic region of the United States. Additionally, (Barberi et al., 2020). found a maximum of 8.0 × 10^2 CFU g^{-1} of *V. parahaemolyticus* on *Saccharina latissima* during its growing season in the Gulf of Maine in the Northern Atlantic United States. Another study from Japan on *Porphyra* spp. and *Undaria* spp. reported 86 MPN g^{-1} of *V. vulnificus* and 110 MPN g^{-1} of *V. parahaemolyticus* (Mahmud et al., 2007; Mahmud et al., 2008). (MPN and CFU are considered equivalent units and thus directly comparable, with MPN being a statistics-based measurement and CFU being a direct counting measurement. MPN can have a lower limit of detection, but CFU can provide finer-scale information). Our initial hypothesis that both *Vibrio* spp. would vary by macroalgae morphology type was not supported for *V. parahaemolyticus* and very weakly supported for *V. vulnificus*. These data suggest that while *V. vulnificus* may prefer to colonize

different morphological types of macroalgae, morphology is not the main driver of pathogenic *Vibrio* spp. abundance, at least in this study.

Another key metric used widely to describe *Vibrio* bacteria and their role in the environment is their pathogenicity levels and strain types. While the presence of virulence genes like *tdh* and *trh* is crucial for determining the pathogenic potential of a *V. parahaemolyticus* strain, these genes do not determine the serotype directly. Instead, they indicate that the strain may be more virulent or have a higher potential to cause illness. The serotype is established through serotyping methods that assess the O and K antigens, whereas the virulence genes provide additional information about the strain's pathogenic characteristics (Iida et al., 1997; Nair et al., 2007). Samples that are positive for both *V. parahaemolyticus* pathogenic genes, however, may indicate the presence of the O3:K6 serotype, which has been linked to the *V. parahaemolyticus* pandemic clonal complex (VpCC) (Martinez-Urtaza et al., 2005; Gonzalez-Escalona et al., 2011; Martinez-Urtaza et al., 2016). Environmental isolates with the *tdh* and *trh* genes have rarely been detected in the past, with only 0%–6% testing positive for both genes found in coastal regions of the U.S., Europe, and Asia between 1995 and 2000 (Nishibuchi et al., 1992; DePaola et al., 2000). The potential spread of the predominant pandemic clone, O3:K6, highlights the evolving nature of *V. parahaemolyticus* virulence (Bej et al., 1999; Okura et al., 2003; Parvathi et al., 2006). The historical rarity of environmental isolates containing both virulence genes indicates that these strains are now potentially becoming more prevalent. This trend could reflect changes in environmental conditions, local human activities, climate change, or likely a combination of all these changes, necessitating further research into the factors driving these emerging genetic patterns. Similarly, *V. vulnificus* serotypes are based on O antigens; however, no single serotype is consistently linked to virulence the way O3:K6 is in *V. parahaemolyticus* (Bisharat et al., 1999). Instead, *V. vulnificus* is classified into three biotypes based on pathogenicity: biotype 1, responsible for most human infections; biotype 2, primarily affecting eels but occasionally infecting humans; and biotype 3, which has been linked to wound infections specifically in Israel (Tison et al., 1982; Høi et al., 1998; Warner and Oliver, 2008). Key genetic markers, such as the *vcg* (virulence-correlated gene) and *rrn* (ribosomal RNA operon) type, differentiate clinical and environmental strains, with the *vcgC* genotype and type B rRNA more commonly associated with human infections (Wright et al., 1981; Gulig et al., 2005; Rosche et al., 2005). As warming ocean temperatures expand its geographical range, *V. vulnificus* infections are increasingly reported in higher latitudes, emphasizing the need for ongoing environmental surveillance and molecular epidemiology to assess future risks (Johnson et al., 2010). The model results investigating environmental drivers of *Vibrio* abundance mostly align with general previous reported trends. For example, *V. parahaemolyticus* grows at higher salinities, with optimum growth found in salinity approximately 25‰, which is similar to the mean salinities from the sites in this study, ranging from 25‰ to 29‰ (Martinez-Urtaza et al., 2008). Nutrient concentrations, particularly nitrogen, may influence *Vibrio* spp. for varying reasons. The bacteria may simply be responding to an influx of nitrogen stimulating growth of host organisms, as well as using the nutrients for their own growth and metabolism (Blackwell and Oliver, 2008).

Our model identified nitrogen and TSS as factors associated with *Vibrio* spp. abundance, albeit with a weak correlation. While these relationships were not strong, they align with previous research suggesting that *Vibrio* spp. may respond to nutrient availability and suspended particles in multiple ways (Blackwell and Oliver, 2008). Elevated nitrogen levels may indirectly promote *Vibrio* spp. abundance by stimulating the growth of macroalgal hosts, thereby providing more surface area and organic matter for bacterial colonization (Thompson and Polz, 2006; Wang et al., 2020). Additionally, nitrogen could directly support *Vibrio* metabolism and growth (Thompson and Polz, 2006). Similarly, the presence of TSS may provide microhabitats for *Vibrio* attachment, shielding them from environmental fluctuations and predation (Venkateswaran et al., 1990; Main et al., 2015; Liang et al., 2019). However, the relatively weak association observed in this study suggests that while TSS may play a role, it is not the primary driver of *Vibrio* spp. abundance in these macroalgal communities. The weak correlations observed could also reflect the complexity of *Vibrio* spp. dynamics, where multiple interacting environmental factors influence bacterial abundance. Future research incorporating finer-scale analyses of nutrient cycling, organic matter availability, and particulate-associated *Vibrio* communities may help clarify these relationships. Previous studies have also reported that the concentration of suspended particles in the water column is an important predictor of pathogenic *Vibrio* spp. abundance (Venkateswaran et al., 1990; Fries et al., 2008). *Vibrio* spp. attach to particles in the water column that provide refuse from grazing, higher nutrient concentrations, and buffers from environmental fluctuations (Eiler et al., 2006). While *V. vulnificus* can be found across a wide range of salinities (4‰ to 37‰), it functions optimally at salinities between 10‰ and 25‰, which is consistent with the salinities in this study (Noorian et al., 2023). Although our model identified key environmental predictors of *V. vulnificus* abundance, the overall model performance was weak, indicating that additional unmeasured factors may be influencing *V. vulnificus* dynamics.

We were surprised that temperature was not a significant predictor of pathogenic *Vibrio* spp. in this study, as rising water temperature is linked to higher metabolism and overall increased fitness (Karvonen et al., 2010). However, our study was conducted between May and October, during which temperatures ranged from 16.3°C to 27.4°C, which is well within the known survival and growth range for *Vibrio* spp. It is possible that within this relatively constrained temperature window, other environmental variables, such as nutrient availability, particle attachment, or competition with other microbes, exerted a stronger influence on *V. vulnificus* and *V. parahaemolyticus* abundance. Additionally, while temperature is a well-established driver of *Vibrio* seasonality, its role in explaining fine-scale variation in abundance within a single season may be less

pronounced. As coastal waters continue to warm, including in Narragansett Bay, the seasonal window of *Vibrio* prevalence may extend earlier into the spring and persist later into the fall, which could have implications for future monitoring and risk assessments.

4.1 Why are *Vibrio* spp. on macroalgae?

Vibrio spp. are recognized as highly successful opportunists and generalists in various environments (Goecke et al., 2010; Samsing and Barnes, 2024). Unlike many marine bacteria, *Vibrio* spp. thrive even without a host association (Lovell, 2017). These bacteria are found in diverse ecosystems, from near-shore areas (Kaneko and Colwell, 1973; DePaola et al., 1994; DePaola et al., 2000; Cox and Gomez-Chiarri, 2012) to the open ocean (Martinez-Urtaza et al., 2016) and from tropical regions (Deepanjali et al., 2005; Zimmerman et al., 2007) to as far north as Alaska and Scandinavia (Høi et al., 1998; McLaughlin et al., 2005; Baker-Austin et al., 2013). Since *Vibrio* spp. do not rely on a host organism for survival, their presence on macroalgae suggests that they may benefit from this association. Pathogenic *Vibrio* spp. have been found to be more abundant on macroalgae surfaces than in the surrounding water (Mahmud et al., 2007; Mahmud et al., 2008). Additionally, higher abundances of pathogenic *Vibrio* spp. are found in sediments and particulate matter compared to their free-living presence in the water column (Parveen et al., 2008; Johnson et al., 2012; Gutierrez West et al., 2013; Vezzulli et al., 2013; Main et al., 2015). The absence of a strong relationship between pathogenic *Vibrio* spp. abundance and environmental parameters may actually provide helpful insight to understanding macroalgae as a host for *V. parahaemolyticus* and *V. vulnificus*. Similar to water column particles and sediments, the surface of macroalgae offers a micro-niche habitat conducive to bacterial survival and proliferation as it provides a buffer against environmental fluctuations (Beleneva et al., 2006; Englebert et al., 2008; Goecke et al., 2010; Saha et al., 2020). The ability of pathogenic *Vibrio* spp. to form biofilms on both biotic and abiotic surfaces facilitates their persistence in the environment, making macroalgae an excellent host (Baker-Austin et al., 2018). While beyond the scope of this study, it is possible that *Vibrio* spp. may benefit their macroalgae host by producing secondary metabolites that inhibit the colonization of other microbes, potentially enhancing the overall health of the macroalgae (Goecke et al., 2010). While we aimed to reduce potential confounding from macrofouling epibionts by excluding visibly colonized macroalgae, we recognize that microscopic epibionts may still have played a role in shaping *Vibrio* dynamics. Epibionts, including other microbes, small invertebrates, or protists, could have influenced *Vibrio* spp. through a variety of mechanisms (e.g., serving as hosts, providing additional organic matter, competing for resources, or producing antimicrobial compounds) (Egan et al., 2013; Dang and Lovell, 2016). Previous studies have shown that epiphytic microbial communities can both facilitate and inhibit *Vibrio* colonization, depending on species interactions and environmental conditions (Lemire et al., 2015; Liu et al., 2022; Liu et al., 2024). Future work incorporating metagenomic or microscopy-based approaches could help clarify the potential role of epibionts in structuring *Vibrio* populations on macroalgae.

4.2 Implications and future research

There is limited knowledge about the abundance of *V. parahaemolyticus* and *V. vulnificus* on macroalgae. This study highlights that macroalgae in this temperate estuary are a reservoir of pathogenic *V. parahaemolyticus* and *V. vulnificus*. The environmental parameters measured here were not the key drivers of *Vibrio* spp. abundance. Thus, we may not have captured a large enough range of the parameters we measured, we may be missing key environmental parameters, or importantly, environmental conditions matter less when the *Vibrio* can attach to and be protected by the macroalgae host.

Though pathogenic *Vibrio* were found on all genera of macroalgae, this does not directly translate to human disease. An infectious dose of a pathogen often combined with poor immune status or previous skin lesion is required to cause disease in the host organism. Thus, the pathology of an infectious dosage in both *V. parahaemolyticus* and *V. vulnificus* is highly variable and situationally dependent (Drake et al., 2007). However, this study does demonstrate that as macroalgae continue to proliferate and our climate warms, macroalgae may be an emerging route of human exposure to pathogenic *Vibrio* spp. worthy of study. Future research could focus on quantifying *V. parahaemolyticus* and *V. vulnificus* abundance across a wider range of systems, seasons, and environmental parameters, as well as investigating potential species-specific chemical interactions underpinning the relationship between pathogenic *Vibrio* spp. and macroalgae host.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

AG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. AS: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing – review & editing. RS: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. RF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1549732/full#supplementary-material

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