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The effect of off-bottom versus on-bottom oyster culture on total and pathogenic *Vibrio* spp. abundances in oyster tissue, water and sediment samples

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KEYWORDS

Crassostrea virginica, Aquaculture, *Vibrio parahaemolyticus*, *Vibrio vulnificus*

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HIGHLIGHTS

- Higher concentrations of total and pathogenic *V. parahaemolyticus* were isolated from oysters cultured loose on-bottom and dredged for harvest compared to those cultured off-bottom during the summer to fall in a subtidal location.
- Higher concentrations of *Vibrio* spp. were isolated from oysters that were exposed to ambient air temperatures during periods of low tide in an intertidal location.
- Based on Spearman's rank, temperature and salinity are positively correlated with *Vibrio* spp. concentrations in oysters, water and sediment
- High turbidity is associated with decreases in *Vibrio* spp. concentrations from oysters, water and sediment

ABSTRACT

Varying culture methods are commonly used for eastern oyster, *Crassostrea virginica*, aquaculture in the Northeast United States. *Vibrio vulnificus* and *V. parahaemolyticus*, two human pathogenic bacteria species, accumulate in this edible, filter feeding shellfish. This study examined the use of two methods in an intertidal area (oysters cultured in trays and in bags on sediment) and two methods in a subtidal area (oysters cultured in trays and loose on the sediment) in Massachusetts over the growing season in 2015. Abundance of total *V. vulnificus* along with total and pathogenic (*tdh*$^+$/*trh*$^+$) *V. parahaemolyticus* were determined in oysters, sediment and water using real-time PCR. Temperature, salinity, turbidity and chlorophyll were continually measured every 15 minutes at each location.

There were significantly higher abundances of total and pathogenic *V. parahaemolyticus* in on-bottom cultured oysters, while significantly higher abundances of *V. vulnificus* were identified in oysters from off-bottom culture in a subtidal location in Duxbury Bay, MA. In an intertidal location, Wellfleet Bay, MA, significantly higher abundances of total and *tdh*$^+$ *V. parahaemolyticus* were found in off-bottom oysters, but significantly higher abundances of *V. vulnificus* and *trh*$^+$ *V. parahaemolyticus* were found in on-bottom oysters.

Spearman's correlation indicated that temperature is positively associated with concentrations of *Vibrio* spp. in oysters, water and sediment, but positive correlations between salinity and *Vibrio* spp. was also observed. Conversely, turbidity had a negative effect on *Vibrio*

spp. concentrations in all sample types. There was no observed relationship inferred between chlorophyll and *Vibrio* spp. abundances in oysters, water or sediment.

1. INTRODUCTION

As of 2018, bivalve molluscs were responsible for 17.7 million tonnes of aquaculture production worldwide (Food and Agriculture Organization of the United Nations 2020), with *Crassostrea* spp. oysters responsible for 29.5% of that production. In the United States, oysters account for the highest volume of marine shellfish aquaculture production (National Marine Fisheries Services 2018). While production in the Gulf of Mexico is responsible for over half of U.S. production, oyster aquaculture along the Atlantic coast is also growing, especially in the Northeast. With the increase in oyster aquaculture, assessments involving production practices are important for improving growth and quality of the product. Two major oyster aquaculture practices include on-bottom culture, in which oysters are grown in bags or directly on the sediment, and off-bottom culture, where bags or trays are raised above the sediment. Off-bottom oyster practices utilize trays, racks or cages to hold oysters higher in the water column to increase exposure to food, promote growth, and increase avoidance of predators (Walton et al. 2013a). These methods are used to culture oysters in both intertidal and subtidal areas. In intertidal culture, oysters are exposed to variable periods of time out of the water. These diverse growing and handling practices help expand the range of locations usable for oyster production, but it is important to understand how they may affect threats to human health as a result of harmful bacteria, such as *Vibrio* spp., found within the food product's tissues.

Vibrio parahaemolyticus is a gram-negative bacterium that is ubiquitous in the marine environment. This species and its relative, *V. vulnificus*, are most common in coastal environments and are in higher concentrations in spring and summer due to increased water temperatures (Baker-Austin et al. 2012). Oysters accumulate bacteria such as *V. parahaemolyticus* and *V. vulnificus* in their tissues as a result of their filter-feeding processes. Oysters are not detrimentally affected by *V. parahaemolyticus* accumulations, but when a high concentration of pathogenic *V. parahaemolyticus* is consumed by eating uncooked oysters, humans may suffer from nausea, diarrhea, vomiting, headaches and in some cases septicemia (Nishibuchi and DePaola 2005). Two genes have been associated with the more infectious strains of *V. parahaemolyticus*: thermostable direct hemolysin (*tdh*) and thermostable direct hemolysin-

related hemolysin (*trh*). All strains of *V. vulnificus* are infectious, and consumption of this species results in gastroenteritis symptoms, while infection of open wounds exposed to contaminated seawater can result in septicemia (Strom and Paranjpye 2000).

Northern, colder regions tend to have less *V. parahaemolyticus* and *V. vulnificus* in the seawater and by consequence have lower incidence of vibriosis than southern areas such as the Gulf of Mexico (DePaola et al. 1990; Johnson et al. 2010). However, infections due to consumption of oysters does occur in the Northeast (Newton et al. 2014). As the average temperature of northern coastal waters increases due to climate change, *Vibrio* spp. may proliferate in these waters, resulting in increased accumulation in filter feeders (Baker-Austin et al. 2012). Initial levels of *Vibrio* spp. within oyster tissues are directly related to human health risk as a result of consuming raw or undercooked shellfish. As a result, the FDA requires each state that harvests eastern oyster to have *V. parahaemolyticus* and *V. vulnificus* control plans through the National Shellfish Sanitation Program (National Shellfish Sanitation Program 2019). These plans include post-harvest handling guidelines and a frequently updated since their creation in 2007. Mitigating the effect of oyster post-harvest handling and potential harmful culture practices on the pathogenic bacteria levels in the food product is important for lowering the risk they pose.

As in other states, oyster cultivation and harvest practices vary across Massachusetts, primarily due to differences in geographic and tidal characteristics. For instance, in Duxbury Bay the majority of grow out is conducted in shallow subtidal waters, where oysters are rarely exposed to air during growout, and then only for short periods of time. In contrast, much of the oyster farming in Wellfleet Bay occurs in intertidal zones where exposure times can be as high as four hours per tidal cycle. Oysters that are exposed during the tidal cycle have been associated with acute temperature changes that have led to increased levels of *Vibrio* spp. (Jones et al. 2016; Nordstrom et al. 2004; Walton et al. 2013b). Location in the water column and amount of time oysters are either closed, or actively filtering seawater, may be important to the abundance of *Vibrio* spp. present in the oyster tissues. Additionally, the effects of these culture variables on the occurrence of pathogenic versus non-pathogenic *Vibrio* spp. is unknown.

This study aimed to quantify *Vibrio* spp. concentrations in oyster tissues and correlate those with culture method and *Vibrio* spp. concentrations in water and sediment samples in two important culture locations in Massachusetts, US. The goal was to determine if tidal cycle or

culture methods plays a role in total *V. parahaemolyticus* and *V. vulnificus*, as well as pathogenic *V. parahaemolyticus* (*tdh*$^+$/ *trh*$^+$) accumulation in oysters.

2. METHODS

2.1 Treatments and Sample Collection

Samples of adult, submarket sized eastern oysters were collected from two oyster aquaculture locations in Cape Cod Bay, Massachusetts, US. At each location, off-bottom and on-bottom culture methods were utilized. Duxbury Bay was chosen as the subtidal location, where direct on-bottom oysters were dredged for sampling, and the off-bottom cultures were hand collected from trays containing oysters that were raised ~0.5 m above the sediment. The second location, Wellfleet Bay, is an intertidal location where oysters experienced periods of exposure during tidal cycles. There, oysters were collected by hand from direct on-bottom culture bags or from off-bottom trays containing oysters that were raised ~0.5m above the sediment. Collections were made during low tide.

At the time of oyster collection, water and sediment cores were collected in triplicate at the culture sites of each of the four groups. Water samples were collected at each culture site using sterile 1 L glass bottles. Sediment cores were taken at the sediment surface directly next to each culture site. All samples were kept on ice in coolers during transport. Each 1 L of water was vacuum filtered through 0.8µm, 0.45 µm, then 0.22 µm mixed cellulose filter membranes (Wilkem Scientific Ltd). Filters were then stored at -80°C until DNA extraction. Sediment samples were immediately stored at -80°C upon arrival at the lab.

Environmental parameters were collected from buoys equipped with Yellow Springs Instruments Inc. (YSI) sondes as part of Cape Cod Cooperative Extension's water quality monitoring program. Temperature (°C), salinity (ppt), turbidity (NTU), and chlorophyll (µg/L) measurements were recorded every fifteen minutes in the bays of Wellfleet and Duxbury over the course of the study in 2015. Due to sensor malfunction, salinity data in Duxbury was not reported throughout the entire sampling period. Because changes in the *Vibrio* spp. content of an oyster or the environment are likely affected by changes in environmental parameters over several days, three-day averages were calculated for the period prior to each collection date. For each parameter, this provides a better understanding of the bacteria's response to recent environmental conditions.

155

156 *2.2 DNA extraction and real-time PCR*

157 Sampling occurred every two weeks from April 2015 through October 2015. At each
158 sampling point, one set of ten oysters was collected from each of the four experimental groups.
159 Oysters were cleaned, sterilely shucked and all tissues and associated fluids were blended at high
160 speed for five minutes to create a homogeneous mixture. From the resulting homogenate, 10g
161 were added to 90ml APW, in triplicate, and incubated for 20 hours at 150 rpm and at 35°C.
162 Then, 1 mL of culture was added to a 1.5 mL microcentifuge tube, and DNA was extracted
163 following manufacturer's instructions using the MoBio Power Food Kit with an alternate lysis
164 step that included sample incubation at 65°C for 10 min. DNA extraction from the filters was
165 carried out using the MoBio PowerWater Kit with the same alternate lysis step as the oyster
166 homogenate samples. At the time of extraction, sediment samples were defrosted and 1g of
167 sediment was taken from the top layer of the sediment core at the location of the water/sediment
168 interface. DNA extractions were carried out using the MoBio PowerSoil Kit following
169 manufacturer's instructions. Again, an alternate lysis method was included following the
170 addition of Solution PS1 where samples were incubated at 65°C for 10 min.

171 Real-time PCR amplification on all oyster, water and sediment samples was carried out
172 following methods outlined by Scro et al. (2019). Briefly, DNA was tested for total *V.*
173 *parahaemolyticus* and *V. vulnificus* in a duplex qPCR assay. Then all extracts deemed positive
174 for *V. parahaemolyticus* were screened using a triplex qPCR for pathogenic genes tdh^+ and trh^+.
175 Plasmids were used for standard curve generation for all gene targets. Previous work suggested
176 that the qPCR method used here was not specific for trh^+ *V. parahaemolyticus* and that false
177 amplification would occur in the presence of *V. alginolyticus* (Scro et al. 2019). While the gene
178 from *V. parahaemolyticus* is 98% similar to *trh* that has been found in strains of *V. alginolyticus*,
179 work was completed in the original study that designed the primers to ensure zero cross-
180 contamination (Gonzalez-Escalona et al 2006; Nordstrom et al. 2007). Therefore, trh^+ *V.*
181 *parahaemolyticus* samples are included in this analysis.

182

183 *2.3 Statistical Analysis*

184 *Vibrio* spp. concentrations, including virulence genes, were enumerated by copy number
185 from qPCR assays. Concentrations were log transformed for data visualization and statistical

analysis. Despite transformation, concentrations did not achieve normal distribution and non-parametric analyses were conducted. Statistical significance ($p < 0.05$) of observed differences in concentrations for each target by location and treatment within each location was evaluated by non-parametric Wilcoxon rank-sum tests. The same methods were repeated for *Vibrio* spp. concentrations in water and sediment samples. Correlations between *Vibrio* spp. concentrations, total and pathogenic, and environmental parameters were assessed using Spearman's rank correlation coefficient (r_s). Spearman's rank was also used to correlate all target concentrations in oysters with those of the surrounding water and sediment. Based on Spearman's rank correlation, correlations were either strong ($r_s > 0.75$), moderate ($r_s = 0.5 – 0.75$) or weak ($r_s < 0.5$). Wilcoxon tests were also used to compare water quality parameters between the two locations. Statistical analysis of all data gathered was completed through R studio (version 3.6.2).

3. RESULTS

3.1 Environmental parameters

Water quality parameters varied significantly between the two locations. Over the course of the study, water temperatures in the subtidal location, Duxbury Bay, 7.85 – 22.64°C, were significantly lower ($p < 0.0001$) than in the intertidal location, Wellfleet Bay, 10.59 – 27.05 °C. Average daily water temperature reached its maximum during late August in Wellfleet Bay and in early September in Duxbury Bay (Fig. 1A). Salinity was significantly greater ($p = 0.0003$) in Duxbury, with a range of 27.29 to 32.62 ppt, compared to 27.55 to 31.29 ppt in Wellfleet (Fig. 1B). Turbidity measurements at both locations reflect the cyclical pattern of turbidity, with higher turbidity before low tide and during spring tides. Both Duxbury and Wellfleet experience higher relative turbidity during the spring season and late fall (Fig. 1C). The intertidal flats of Wellfleet experience extreme tidal cycles causing a larger range of turbidity (0.84 – 57.71) than Duxbury (0.97 – 21.97 NTU). Overall, Wellfleet Bay's turbidity was significantly higher than Duxbury ($p = 0.0003$). Chlorophyll abundance was significantly greater ($p < 0.0001$) in Wellfleet, with a range of 2.57 to 19.37 µg/L. In Duxbury, chlorophyll ranged from 1.82 to 14.81 µg/L (Fig. 1D).

3.2 Vibrio *spp. concentrations in a subtidal culture location*

217 *Vibrio* spp. concentrations in oyster tissues from Duxbury varied significantly between
218 the two growing methods for all targets (Fig. 2). The first detection of *V. parahaemolyticus*
219 populations occurred in May, while *V. vulnificus* was not detected until mid-June. Both *V.*
220 *parahaemolyticus* and *V. vulnificus* reached peak concentrations in oyster tissues in late July.
221 Based on the Wilcoxon rank-sum analysis, oysters grown in direct on-bottom cultures had
222 significantly higher levels of total *V. parahaemolyticus* at five different time points, while
223 oysters grown off-bottom in trays had significantly higher concentrations of *V. parahaemolyticus*
224 only once, in June (Fig. 2A). In contrast, *V. vulnificus* concentrations were significantly higher in
225 off-bottom oysters more frequently than in oysters grown on the sediment (Fig. 2B). Pathogenic
226 targets reached peak concentrations in oyster tissues in late September for *tdh*$^+$ *V.*
227 *parahaemolyticus* (Fig. 2C) and late July for *trh*$^+$ *V. parahaemolyticus* samples (Fig. 2D). Both
228 genes were found in significantly higher concentrations in oyster tissues in those grown on-
229 bottom than those grown off-bottom at three different time points over the course of the study.

230 *Vibrio* spp. abundances data from water samples were inconsistent and were lower than
231 levels detected in oyster homogenate (Fig. 4). Pathogenic *V. parahaemolyticus* were detected
232 infrequently in water and sediment samples; and these were not included in further analyses.
233 Spikes in *V. parahaemolyticus* concentrations in water samples were observed at both on and
234 off-bottom sample areas in September in Duxbury (Fig. 4A). At two time points, total *V.*
235 *parahaemolyticus* concentrations were significantly higher in water surrounding on-bottom
236 oysters versus off-bottom oysters grown in trays. Concentrations of *V. vulnificus* in water
237 surrounding on-bottom oysters remained low (< 0.5 log copies) throughout the sampling period.
238 At the off-bottom culture area, three spikes in *V. vulnificus* concentrations were observed in
239 May, July and September (Fig. 4B). These spikes were significantly higher based on Wilcoxon
240 rank-sum tests.

241 *Vibrio* spp. abundances in sediment core samples were lower than those detected in
242 oysters. Pathogenic *V. parahaemolyticus* was rarely detected in sediment samples, and thus not
243 included in further analyses. Sediment core samples from on-bottom culture sites had
244 significantly higher concentrations of *V. parahaemolyticus* in August through mid-September
245 (Fig. 5B). Peak *V. parahaemolyticus* was detected in late July from sediment in the off-bottom
246 culture location and in early August in the on-bottom culture location. For both sample sites, *V.*

vulnificus levels peaked in early September, but overall were below 1.5 log copies with no significant differences detected (Fig. 5B).

A moderate, positive significant correlation was identified between total *V. parahaemolyticus* and *trh*$^+$ *V. parahaemolyticus* in oyster tissues (Table 1). Positive correlations were also observed for levels of *Vibrio* spp. in environmental samples and oyster tissues. Both water and sediment total *V. parahaemolyticus* had significant correlations with *tdh*$^+$ and *trh*$^+$ *V. parahaemolyticus* in oysters. Likewise, *V. vulnificus* concentrations in the water samples were moderately and positively correlated with *V. vulnificus* concentrations in oyster tissues. Temperature was strongly correlated with *V. vulnificus* levels in oyster tissue and *V. parahaemolyticus* levels isolated from sediment samples. There was also moderate positive correlation between temperature and *Vibrio* spp. levels in water samples and both *tdh*$^+$ and *trh*$^+$ *V. parahaemolyticus* in oyster tissues. Salinity was positively correlated with *Vibrio* spp., showing a strong relationship with oyster *V. vulnificus* concentrations, and a moderate relationship with oyster pathogenic *V. parahaemolyticus* concentrations and total water *V. parahaemolyticus* concentrations. Turbidity was negatively correlated with all *Vibrio* spp. concentrations except total *V. parahaemolyticus* in oyster tissues. These correlations were moderate based on r_s. Similarly, turbidity was negatively correlated with total *V. vulnificus* and *V. parahaemolyticus* levels in water and total *V. parahaemolyticus* levels in sediments. Chlorophyll levels resulted in weak negative correlations with total *V. parahaemolyticus* in water and total *V. vulnificus* in sediment.

3.3 Vibrio *spp. concentrations in an intertidal culture location*

Vibrio parahaemolyticus were found in significantly higher concentrations in oysters from off-bottom culture than on-bottom culture at all but one time point (Fig. 3A). In early June, oysters from on-bottom culture had significantly higher concentrations of *V. parahaemolyticus* than off-bottom oysters. Unlike *V. parahaemolyticus*, *V. vulnificus* levels were higher in on-bottom cultured oysters for a majority of the study (Fig. 3B). This was significant in both September collections. Off-bottom cultured oysters had significantly higher concentrations of *tdh*$^+$ *V. parahaemolyticus* at eight of the thirteen collection points, and *trh*$^+$ *V. parahaemolyticus*

were significantly higher in late May (Fig. 3C, D). In contrast, on-bottom oysters had significantly higher levels of *trh*$^+$ *V. parahaemolyticus* in July, August and September (Fig. 3D).

Water samples from the off-bottom culture location had significantly higher concentrations of *V. parahaemolyticus* from the end of June through the end of September, with peak concentrations detected in August (Fig. 4C). There was one time point, in May, where *V. parahaemolyticus* was found in significantly higher concentrations in water from on-bottom culture location. Concentrations of *V. vulnificus* were more variable in water over the sampling period, with peaks detected in August for the off-bottom culture location and late September for the on-bottom culture location (Fig. 5D). Water from the off-bottom culture locations had significantly higher *V. vulnificus* concentrations in late June and early July. The opposite was observed in early June water samples, where levels were significantly higher in the on-bottom culture location.

In Wellfleet, *Vibrio* spp. concentrations isolated from sediment cores were generally lower than those isolated from oyster tissues and water sample. Sediment samples from off-bottom culture frequently had higher levels of *V. parahaemolyticus* and were significantly higher than sediment samples from on-bottom culture in both August collections (Fig. 5C). Concentrations of *V. vulnificus* in sediments were inconsistent and generally low with no pattern observed in either location (Fig. 5D).

Based on Spearman's rank correlation coefficient there was a moderate positive relationship between total and pathogenic, *tdh*$^+$ and *trh*$^+$, *V. parahaemolyticus* (Table 2). Positive correlations were also found for *Vibrio* spp. levels in environmental samples and oyster tissues. Specifically, *V. parahaemolyticus* levels in water were moderately correlated with *tdh*$^+$ concentrations in oysters, while *V. vulnificus* levels in water were weakly correlated with *V. vulnificus* concentrations in oysters. There were strong and moderate positive correlations between temperature and oyster *trh*$^+$ *V. parahaemolyticus* and total *V. vulnificus* levels, respectively. Temperature also had a moderate positive correlation with *V. parahaemolyticus* water concentrations and a weak positive correlation with *V. parahaemolyticus* sediment concentrations. Turbidity was negatively, but not significantly correlated with oyster *Vibrio* spp. concentrations. A moderate negative relationship was observed between turbidity and *trh*$^+$ *V. parahaemolyticus*. A weak negative correlation was noted between turbidity and environmental

306 *V. parahaemolyticus* in sediment samples. Lastly, chlorophyll was positively correlated with
307 total *V. vulnificus* isolated from water.

308

309 **4. DISCUSSION**

310 Off-bottom and on-bottom culture practices are common in oyster aquaculture not only in
311 the Northeast United States, but also in other Atlantic coastal waters of the U.S. During this
312 study, detectable levels of *Vibrio* spp. were found in all aquacultured oysters from two locations
313 on the southeastern shores of Massachusetts from May through October 2015. Following well
314 established seasonal trends, peaks in total and pathogenic *V. parahaemolyticus* and total *V.*
315 *vulnificus* from oyster tissues were observed in both Duxbury and Wellfleet Bays during the
316 warmer summer months of July and August. Generally, total *V. vulnificus* concentrations were
317 lower than total *V. parahaemolyticus* concentrations. Previous work from Massachusetts also
318 found higher concentrations of *V. parahaemolyticus* over *V. vulnificus* in oyster tissues (Scro et
319 al. 2019).

320 For all culture types in both locations, *trh*$^+$ *V. parahaemolyticus* was more abundant than
321 *tdh*$^+$ *V. parahaemolyticus*, with the exception of off-bottom oysters in Wellfleet Bay, where the
322 two pathogenic genes had similar abundances. Several studies have shown great variability in the
323 *tdh*$^+$ and *trh*$^+$ genes measured in water column and oyster tissue samples from Atlantic coastal
324 waters including the Gulf of Mexico (Cox and Gomez-Chiarri 2012; Watkins and Cabelli 1985;
325 West et al. 2013; Zimmerman et al. 2007). However, only one previous study has shown that *trh*$^+$
326 *V. parahaemolyticus* strains can be more abundant than *tdh*$^+$ strains as observed in one location
327 in Massachusetts (Scro et al. 2019). Much of the variability of pathogenic *V. parahaemolyticus* is
328 still unknown in Massachusetts waters due to a lack of studies.

329 The data revealed more correlations between environmental parameters and *Vibrio* spp.
330 levels in oysters, water and sediment samples from Duxbury Bay than Wellfleet Bay. Wellfleet
331 Bay culture sites were also in close proximity to the mouth of the Herring River, where cooler
332 watershed could have played a role in the bacterial proliferation. The most likely reason for the
333 lack of relationships in Wellfleet samples is the intertidal nature of the location, which causes
334 major changes in water conditions throughout each day, creating an environment that may inhibit

335 *Vibrio* spp. growth on surfaces. As a result, variability in *Vibrio* spp. targets in Wellfleet are not
336 correlated with the environmental factors monitored.
337 Total *V. parahaemolyticus* concentrations in oysters did not show a correlation with any
338 of the environmental parameters observed during this study. The same was observed for total *V.*
339 *parahaemolyticus* concentrations in sediment and water samples. Conversely, and potentially
340 importantly, total *V. vulnificus* and pathogenic *V. parahaemolyticus* concentrations in oysters
341 were positively correlated with temperature for both locations. These correlations suggest that
342 pathogenic *V. parahaemolyticus* may respond differently than total *V. parahaemolyticus* to
343 temperature within oyster tissues. Previous studies have shown that ratios between pathogenic
344 and total *V. parahaemolyticus* can vary depending on temperatures (DePaola et al. 2003; Johnson
345 et al. 2010). This relationship between temperature and *Vibrio* spp. is well established (Baker-
346 Austin 2012; DePaola et al. 2003; Johnson et al. 2010; Kaneko and Calwell 1973; Motes et al.
347 1998; Zimmerman et al. 2007). With the rise of water temperatures due to climate change,
348 predicting and mitigating risks of pathogenic *Vibrio* spp. outbreaks continues to be an important
349 public health issue.
350 Despite the lack of correlation with *V. parahaemolyticus* in our study, salinity has been
351 reported to contribute to *Vibrio* spp. abundance (Blackwell and Oliver 2007; Zimmerman et al.
352 2007). However, the optimum salinity for *V. parahaemolyticus* growth has been found to be 22–
353 24 ppt, whereas *V. vulnificus* grows best at lower salinities (0 to 10 ppt) with growth still
354 occurring at 20 to 25 ppt (Blodgett 2010; Johnson et al. 2010; Randa et al. 2004). In both
355 locations, salinity never dropped below 27 ppt. There was no observed correlation between
356 salinity and *Vibrio* spp. in Wellfleet. However, salinity was found to be correlated with *V.*
357 *vulnificus* concentrations in Duxbury, suggesting that there may still be a positive correlation
358 between *Vibrio* spp. and salinity above the optimal salinity range (Johnson et al. 2012; Nigro et
359 al. 2011; Reyes-Velázquez et al. 2010; Sobrinho et al. 2010).
360 Wellfleet Bay showed higher and more variable levels of turbidity than Duxbury Bay.
361 Other studies have cited a positive relationship of *Vibrio* spp. in oysters with turbidity due to the
362 filtering of more suspended material and high nutrient levels of turbid waters (Blackwell &
363 Oliver 2008; Johnson et al. 2010; Johnson et al. 2012; Parveen et al. 2008; Zimmerman et al
364 2007). However, a negative correlation was noted between increased turbidity and *Vibrio* spp.
365 concentrations in Wellfleet oyster tissues. Water pumping rates can decline by more than 57% in

366 highly turbid waters, which could explain the negative correlations observed in this study
367 (Loosanoff 1948; Loosanoff and Tommers 1948). A spike in turbidity was recorded in Wellfleet
368 during the month of June. This increase in particulate matter could explain why there was
369 significantly higher *V. parahaemolyticus* levels observed in on-bottom cultured oysters versus
370 off-bottom.
371 Chlorophyll levels varied in both locations, with Wellfleet slightly higher than Duxbury;
372 however, chlorophyll abundance was not found to be positively associated with *Vibrio* spp.
373 concentrations in oysters. Two negative relationships were observed in Duxbury between
374 chlorophyll and water *V. parahaemolyticus* and sediment *V. vulnificus* concentrations. Previous
375 studies have found positive correlations between chlorophyll and *Vibrio* spp. concentrations,
376 much like the relationship observed in Wellfleet water *V. vulnificus* levels (Johnson et al. 2010;
377 Phillips et al. 2007; Randa et al. 2004; Watkins and Cabelli 1985). The positive correlation
378 between Wellfleet *V. vulnificus* concentrations isolated from water with chlorophyll levels may
379 be due to the coincidental correlation between temperature and chlorophyll (Thompson et al.
380 2004). Furthermore, the correlations observed in Duxbury water *V. parahaemolyticus* and
381 sediment *V. vulnificus* levels were weak and given the inconsistent and low level of *Vibrio* spp.
382 in water and sediment samples, they are not likely representative of the true relationship with
383 chlorophyll. Cooler temperatures, combined with the less algal growth, could also indicate why
384 the expected positive correlation between chlorophyll and *Vibrio* spp. levels was not observed in
385 Duxbury Bay.
386 There were observed differences in *Vibrio* spp. depending on culture method within each
387 location. Oysters grown using direct on-bottom culture in Duxbury had higher concentrations of
388 total and pathogenic (*tdh*$^+$/*trh*$^+$) *V. parahaemolyticus* than those grown off-bottom in trays.
389 Populations of *V. parahaemolyticus* and *V. vulnificus* are higher and more frequently detected in
390 sediment versus water samples (Johnson et al. 2012). *Vibrios* are surface loving organisms, so it
391 is likely that the populations are sustained by organic matter from both sediment and
392 phytoplankton in the water column (Johnson et al. 2010; Rehnstam-Holm et al. 2010). The
393 higher *V. parahaemolyticus* concentrations in the water and sediment samples surrounding the
394 on-bottom cultured oysters likely lead to the elevated levels in oyster tissues. In addition, off-
395 bottom culture from subtidal growing locations has been shown to decrease *Vibrio* sp.
396 concentrations by an average of 13% (Cole et al. 2015). Contrary to *V. parahaemolyticus*

findings, *V. vulnificus* concentrations in oyster tissues and water column samples were found to be significantly higher in the off-bottom culture site. Similar to pathogenic *V. parahaemolyticus*, total *V. vulnificus* was positively correlated between oyster and water samples. It should also be noted that oysters from on-bottom culture in Duxbury were dredged for harvest instead of hand collected. Dredging has been previously described as a stressor for oysters: it degrades water quality, increases sedimentation, releases anoxic sediment, and can lead to the depletion of oxygen surrounding oysters following the resuspension of nutrients that assist phytoplankton production (Lenihan and Peterson 1998; Wilbur and Clarke 2010). It has been previously shown that a mechanical stressor, like dredging, can cause increased rates of mortality and *Vibrio* sp. loads as a result (Lacoste et al. 2001). The added stress on the oyster can explain the increased total and pathogenic *V. parahaemolyticus* concentrations from on-bottom oysters.

Oysters grown using on-bottom culture in Wellfleet had lower levels of total and *tdh*$^+$ *V. parahaemolyticus*, while levels of total *V. vulnificus* and *trh*$^+$ *V. parahaemolyticus* were relatively similar for the two culture practices. Exposure of the oysters during the tidal cycle leads to elevated oyster temperatures and resultant spikes in *V. parahaemolyticus* levels, with larger increases observed with respect to pathogenic strains (Ben-Horin et al. 2021; Jones et al. 2016). Sampling at or shortly after low tide, as in this study, would then result in higher levels of *Vibrio* spp. in the oysters (Grodeska et al. 2017; Jones et al. 2016). However, levels can return to those found in submerged oysters of subtidal and intertidal locations with the incoming tide (Ben-Horin et al. 2021). Many aquaculturists use desiccation practices such as exposing oyster to air during tidal cycles to reduce fouling organism infestation (Fitridge et al. 2012). While this common practice does reduce biofouling, internal oyster temperatures and air temperatures are high, leading to *Vibrio* spp. proliferation and levels that can be four to eight times higher than those of oysters prior to exposure (Grodeska et al. 2017; Jones et al. 2016; Nordstrom et al. 2004).

Elevated concentrations of *Vibrio* spp. can be depurated and returned to ambient levels by means of re-submersion for as little as one to four days following tidal or desiccation-based air exposure (Grodeska et al. 2017; Jones et al. 2016; Pruente et al. 2020). Re-submergence practices are required in Massachusetts for oysters removed for culling, sorting or antifouling practices, but is not currently included in *Vibrio* spp. control plans for oysters that are exposed for long periods of time in intertidal locations as indicated by this study (Massachusetts

Department of Marine Fisheries 2020; NSSP 2019). Understanding the response of *Vibrio* spp. to exposure time or position within the water column is necessary in order to effectively manage the risk of *V. parahaemolyticus* to human health and the shellfish industry.

5. CONCLUSION

Vibrio spp. abundances can vary depending on tidal exposure and culture practices used for growing oysters. Off-bottom aquaculture in subtidal areas generally results in lower concentrations of *V. parahaemolyticus* and *V. vulnificus*, but farmers should be careful in growing areas with variable tides. This data shows that intertidal areas with long periods during which the oysters are not covered by water, during which oysters stay closed, increases the abundance of *Vibrio parahaemolyticus* in the oysters. Thus, this study supports previous reports that *V. parahaemolyticus* levels rise following periods of desiccation or exposure at low tide. This study builds upon the well-established relationship between *Vibrio* spp. and temperature and salinity, but suggests that a more complicated relationship exists with turbidity and chlorophyll, requiring further exploration. Results from this study can help inform future *Vibrio* spp. control plans within Northeast aquaculture states in order to reduce initial bacterial levels in oyster tissues thereby lowering human health risk.

CRediT authorship contribution statement

Abigail K. Scro: Investigation, Formal analysis, Writing – Original Draft, Visualization
James Westphalen: Formal analysis, Writing – Original Draft
Hauke L. Kite-Powell: Conceptualization, Methodology, Writing – Review & Editing
John W. Brawley: Conceptualization, Methodology, Resources, Writing – Review & Editing
Roxanna M. Smolowitz: Conceptualization, Investigation, Methodology, Resources, Writing – Review & Editing

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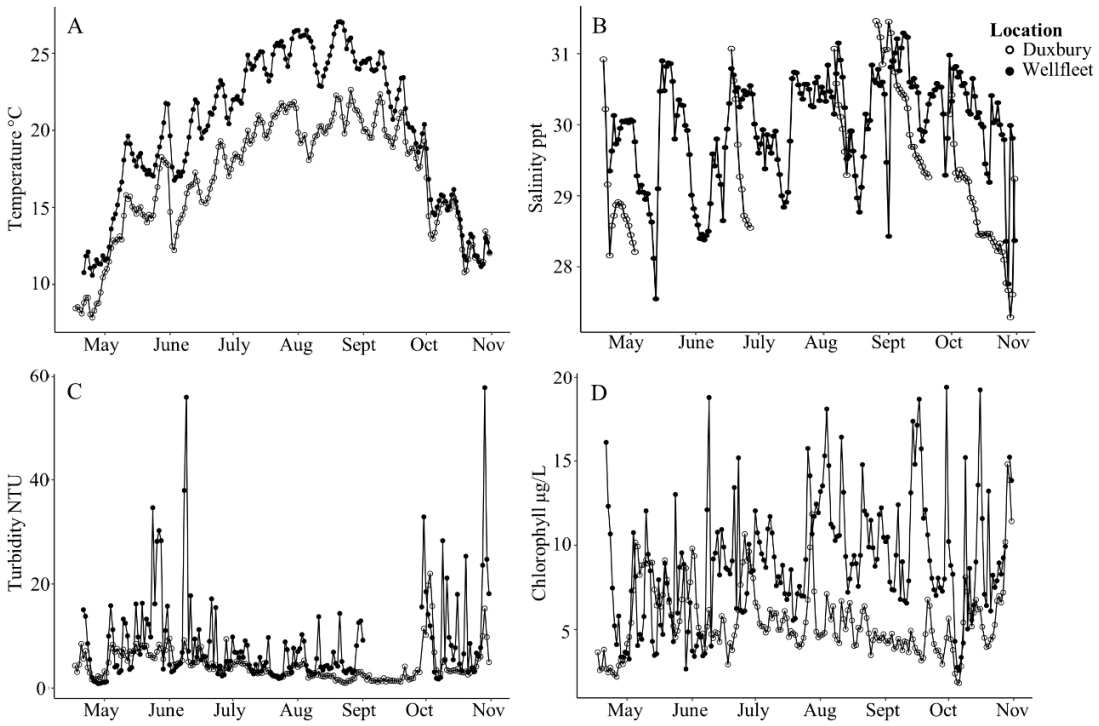

601
602 Figure 1. Average daily water quality parameters for two locations in Cape Cod Massachusetts
603 during 2015. Measurements recorded include temperature (A), salinity (B), turbidity (C), and
604 chlorophyll (D).

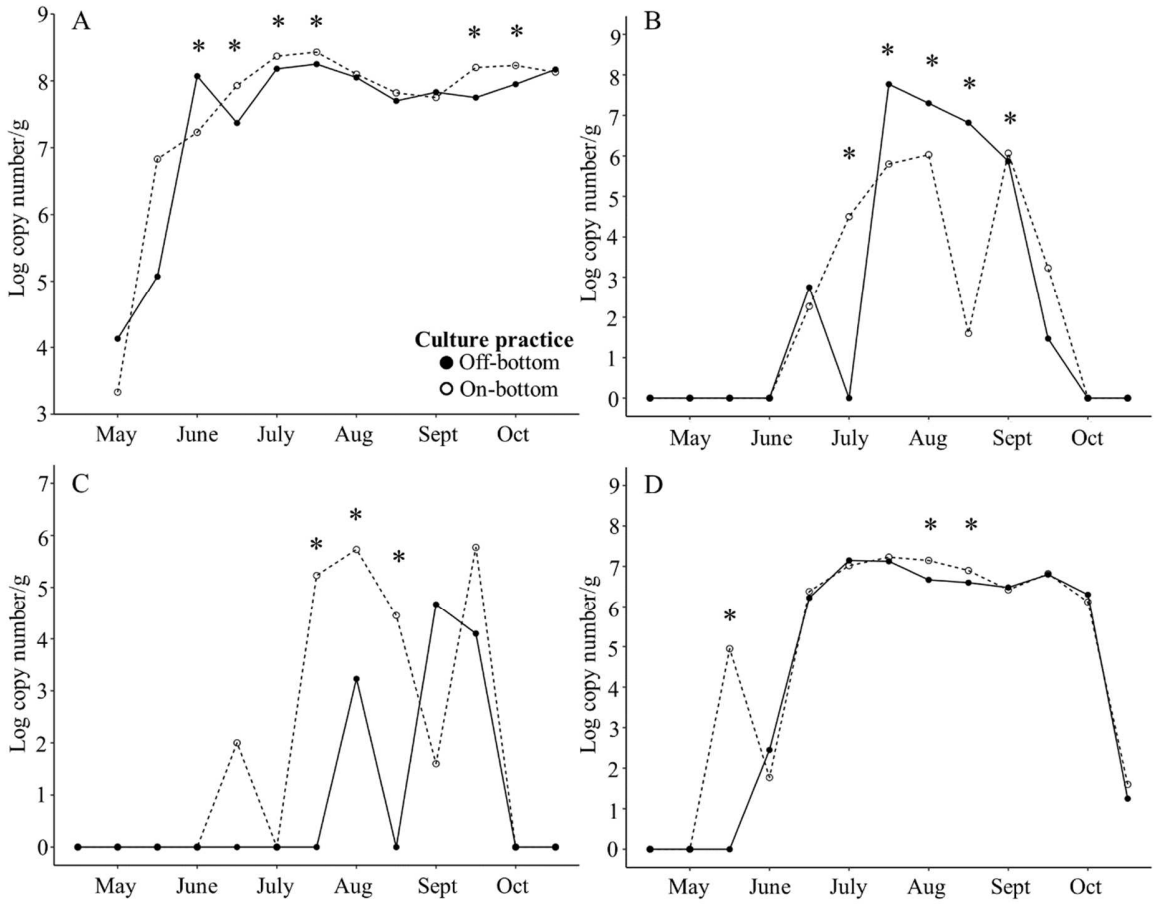

Figure 2. Mean log transformed *Vibrio* spp. concentrations in oyster tissues from Duxbury Bay, Massachusetts (a subtidal location) over a five-month period. Aquacultured oysters were collected from either 'Off-bottom' culture, where oysters were grown in trays above the sediment, or 'On-bottom' culture, where oysters were grown directly on sediment. Levels are represented as total *V. parahaemolyticus* (A), total *V. vulnificus* (B), *tdh*$^+$ *V. parahaemolyticus* (C), and *trh*$^+$ *V. parahaemolyticus* (D). Asterisks indicate time points were differences in culture practices are statistically significant ($p < 0.05$) based on Wilcoxon rank-sum test.

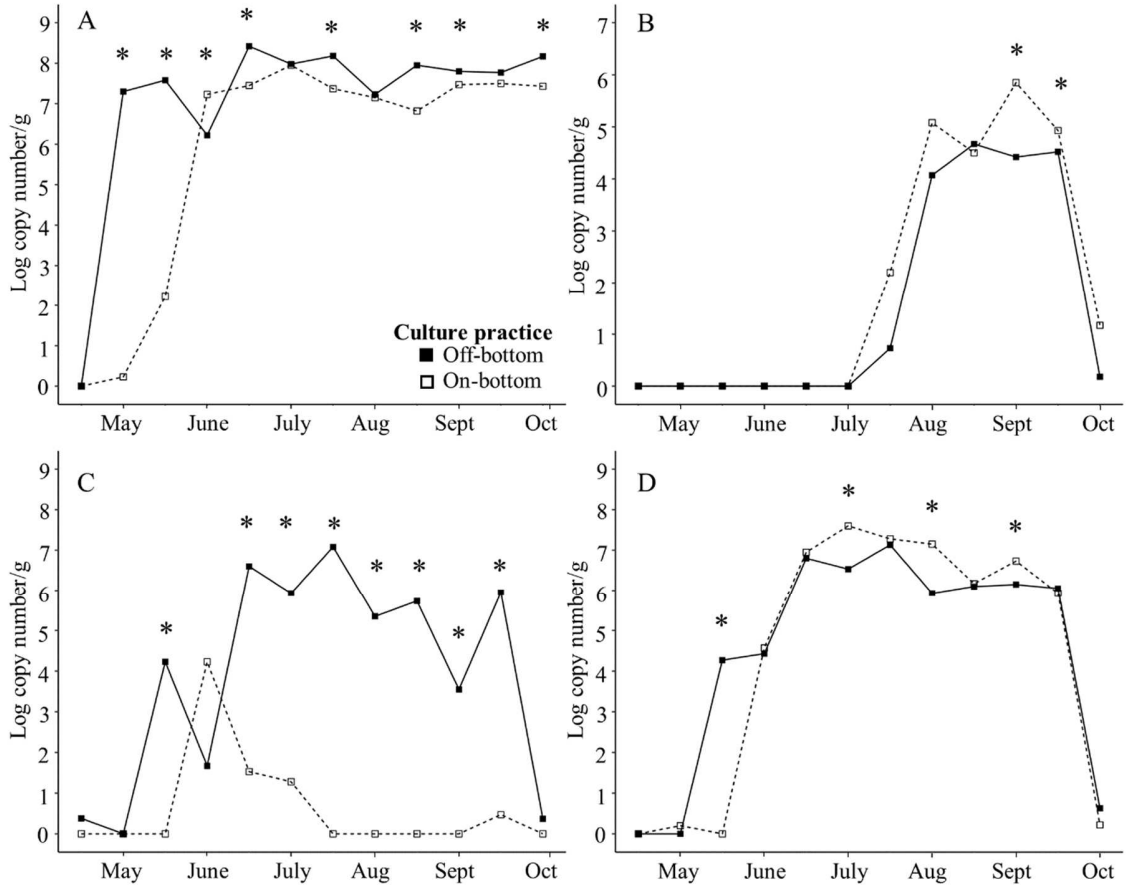

Figure 3. Mean log transformed *Vibrio* spp. concentrations in oyster tissues from Wellfleet Bay, Massachusetts (an intertidal location) over a five-month period. Aquacultured oysters were collected from either 'Off-bottom' culture, where oysters were grown in trays above the sediment, or 'On-bottom' culture, where oysters were grown in bags on sediment. Levels are represented as total *V. parahaemolyticus* (A), total *V. vulnificus* (B), *tdh*$^+$ *V. parahaemolyticus* (C), and *trh*$^+$ *V. parahaemolyticus* (D). Asterisks indicate time points were differences in culture practices are statistically significant ($p < 0.05$) based on Wilcoxon rank-sum test.

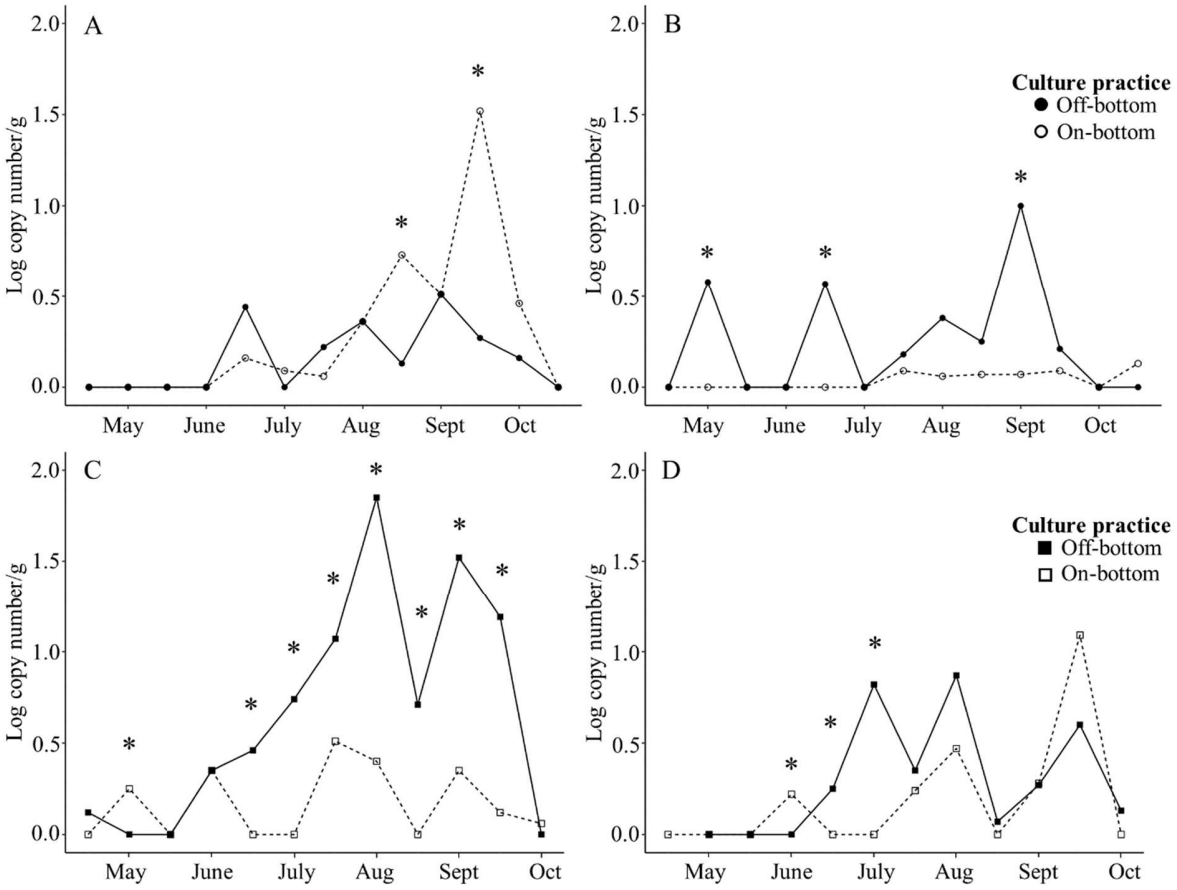

Figure 4. Mean log transformed total *Vibrio* spp. concentrations in water column samples from two Massachusetts locations over a five-month period. Water samples were collected directly next to 'Off-bottom' or 'On-bottom' aquacultured oysters. Levels are reported for Duxbury (circles) as total *V. parahaemolyticus* (A) and total *V. vulnificus* (B), and for Wellfleet Bay (squares) as total *V. parahaemolyticus* (C) and total *V. vulnificus* (D). Asterisks indicate time points were differences in culture practices are statistically significant ($p < 0.05$) based on Wilcoxon rank-sum test.

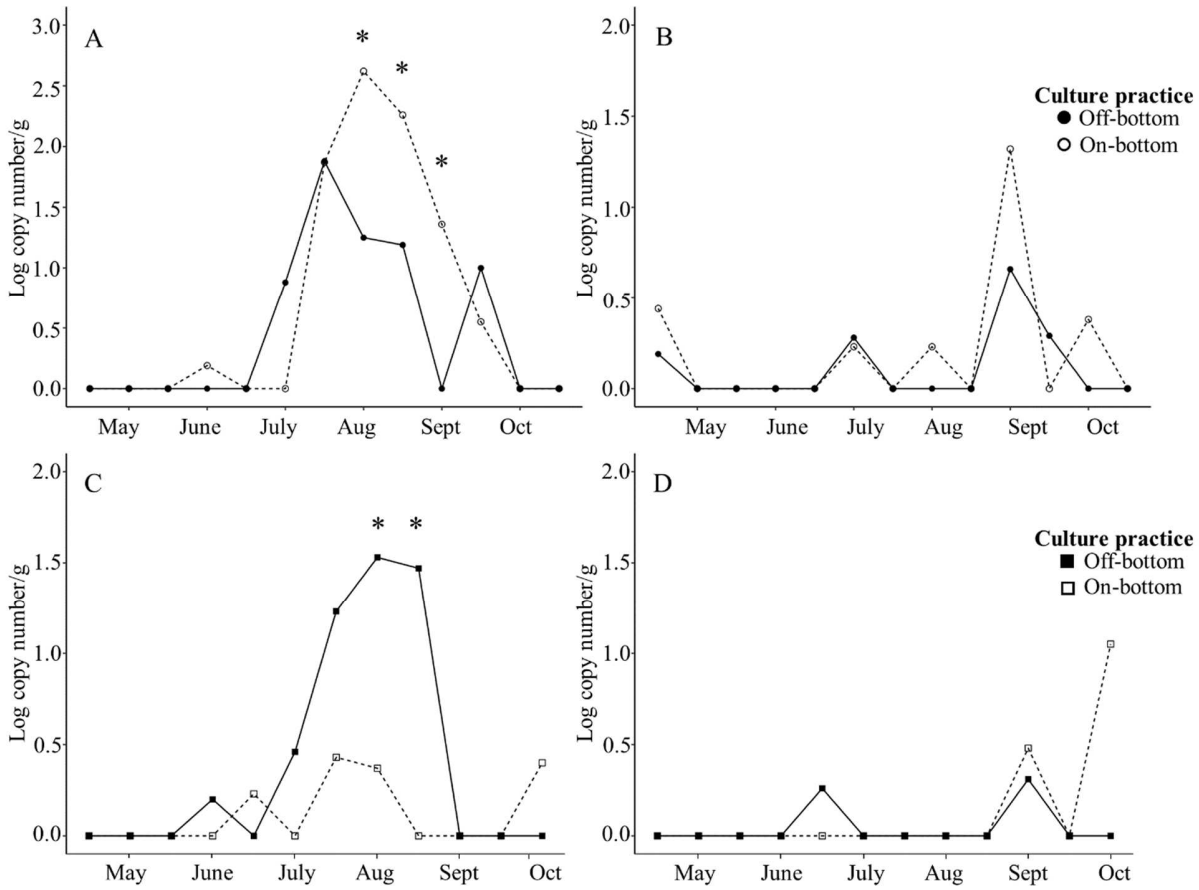

Figure 5. Mean log transformed total *Vibrio* spp. concentrations in sediment samples from two Massachusetts locations over a five-month period. Sediment samples were collected directly next to 'Off-bottom' or 'On-bottom' aquacultured oysters. Levels are reported for Duxbury Bay (circles) as total *V. parahaemolyticus* (A) and total *V. vulnificus* (B), and for Wellfleet Bay (squares) as total *V. parahaemolyticus* (C) and total *V. vulnificus* (D). Asterisks indicate time points were differences in culture practices are statistically significant ($p < 0.05$) based on Wilcoxon rank-sum test.

Table 1. Correlation between *Vibrio* spp. abundances, temperature, salinity, turbidity, and chlorophyll in Duxbury, MA over a 6-month period as determined by Spearman's rank correlation coefficient (r_s). Correlations were determined as strong ($r_s > 0.75$), moderate ($r_s = 0.5 – 0.75$) or weak ($r_s < 0.5$). Grey boxes indicate statistical significance ($p < 0.05$).

		Oyster				Water		Sediment	
		V. parahaemolyticus			*V. vulnificus*	*V. parahaemolyticus*	*V. vulnificus*	*V. parahaemolyticus*	*V. vulnificus*
		total	***tdh*⁺**	***trh*⁺**					
Oyster	***tdh*⁺**	0.280							
	***trh*⁺**	0.686							
Water	***V. parahaemolyticus***	0.329	0.675	0.587					
	V. vulnificus	0.053	0.417	0.318	0.614	0.490			
Sediment	***V. parahaemolyticus***	0.315	0.577	0.741	0.661	0.465	0.376		
	V. vulnificus	0.004	0.166	0.151	0.095	0.247	-0.049	0.042	
	Temperature	0.278	0.659	0.725	0.807	0.568	0.524	0.747	0.137
	Salinity	-0.209	0.564	0.630	0.858	0.687	0.512	0.413	0.231
	Turbidity	-0.315	-0.623	-0.572	-0.668	-0.545	-0.607	-0.591	-0.051
	Chlorophyll	-0.131	-0.239	-0.134	-0.145	-0.459	0.027	0.038	-0.443

Table 2. Correlation between *Vibrio* spp. abundances, temperature, salinity, turbidity, and chlorophyll in Wellfleet, MA over a 6-month period as determined by Spearman's rank correlation coefficient (r_s). Correlations were determined as strong ($r_s > 0.75$), moderate ($r_s = 0.5 – 0.75$) or weak ($r_s < 0.5$). Grey boxes indicate statistical significance ($p < 0.05$).

		Oyster				Water		Sediment	
		V. parahaemolyticus			*V. vulnificus*	*V. parahaemolyticus*	*V. vulnificus*	*V. parahaemolyticus*	*V. vulnificus*
		total	***tdh*⁺**	***trh*⁺**					
Oyster	***tdh*⁺**	0.632							
	***trh*⁺**	0.527							
Water	***V. parahaemolyticus***	0.310	0.574	0.394					
	V. vulnificus	0.244	0.400	0.259	0.457	0.679			
Sediment	***V. parahaemolyticus***	0.165	0.281	0.374	0.230	0.510	0.212		
	V. vulnificus	0.263	-0.061	0.105	0.254	0.191	0.039	-0.104	
	Temperature	0.333	0.268	0.765	0.701	0.581	0.395	0.481	0.085
	Salinity	0.310	0.112	0.239	0.218	0.123	0.243	0.140	0.028
	Turbidity	-0.207	-0.307	-0.568	-0.203	-0.356	-0.036	-0.488	0.423
	Chlorophyll	0.004	0.033	0.128	0.402	0.222	0.567	-0.110	0.085