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Research Note

Effect of Gear Type on *Vibrio* spp. Levels in Farm-Raised Oysters (*Crassostrea virginica*) after Routine Handling and Resubmersion

VICTORIA L. PRUENTE,[1,2]* WILLIAM C. WALTON,[1] AND JESSICA L. JONES[2]

[1]*Auburn University Shellfish Laboratory, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, 150 Agassiz Street, Dauphin Island, Alabama 36528 (ORCID: https://orcid.org/0000-0002-0168-7823 [V.L.P.]) and* [2]*U.S. Food and Drug Administration, Division of Seafood Science and Technology, Gulf Coast Seafood Laboratory, 1 Iberville Drive, Dauphin Island, Alabama 36528, USA*

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ABSTRACT

During routine handling, cultured oysters are removed from the water and exposed to elevated temperatures, causing growth of *Vibrio vulnificus* and *Vibrio parahaemolyticus* within them. Farmers can resubmerse oysters in the water, allowing elevated *Vibrio* spp. levels to return to ambient levels within the oysters. Previous resubmersion research is limited to one aquaculture gear type during studies performed from June to September. This study aims to expand knowledge about the recovery times needed for elevated *Vibrio* levels in handled oysters from two common gear types (the adjustable longline system and the OysterGro system) during early and midsummer periods. Oysters held in both gear types were subjected to being tumbled and refrigerated or desiccated and then resubmersed into water in May and July 2018 and 2019. *Vibrio* spp. levels were measured before and after the treatments, and 3, 7, and 14 days after resubmersion, and were compared with levels in submersed oysters. All samples were tested for *V. vulnificus,* total *V. parahaemolyticus,* and pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+). Water temperatures in May were significantly lower (~5°C; $P \leq 0.009$) than in July, corresponding to lower *V. vulnificus* levels (–0.67 log MPN/g) and higher *tdh*+/*trh*+ levels (+0.56 to 0.63 log MPN/g) in control oysters. The average *Vibrio* spp. levels in control oysters from each trial did not differ between the gear types ($P \geq 0.08$). Elevated *V. vulnificus* levels recovered to ambient levels after 7 days in May and 3 days in July, regardless of gear or handling. For *V. parahaemolyticus,* the desiccated oysters required 14 days to recover in May and 7 days in July, whereas the tumbled and refrigerated oysters required 14 days or more in both months. This study had limited replication in each month, but the data suggest that the resubmersion times differ between the gear types, treatment types, and months. Future studies with more replications are needed to determine whether these trends continue.

HIGHLIGHTS

- Routine handling increased *Vibrio* spp. levels in oysters while they were out of the water.
- *Vibrio* spp. levels in untreated oysters were similar between the two gear types.
- Elevated *V. vulnificus* levels recovered after 7 days in May and 3 days in July.
- Recovery times for elevated *V. parahaemolyticus* levels were longer in May than July.
- Recovery times were longer for tumbled and refrigerated than desiccated oysters.

Key words: Desiccation; Refrigeration; Resubmersion; Tumbling; *Vibrio parahaemolyticus*; *Vibrio vulnificus*

In off-bottom oyster aquaculture, two common systems used are the adjustable longline system (ALS) and a floating cage system, such as the OysterGro system (OG) *(31)*. Both gear types hold oysters in the water column, allowing greater protection from predators than on-bottom oysters, while providing farmers ease of access for routine handling *(30, 31)*. Farmers routinely remove the oysters from the water to desiccate (air dry), tumble through a mechanical grader, and sort by size to produce a deep-cupped oyster free of biofouling organisms *(15, 24)*. Whereas routine handling produces a high-quality oyster, it can increase the levels of *Vibrio* spp. within the oysters while they are out of the water, creating a potential public health risk *(15, 22, 28)*. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are pathogenic bacteria commonly contracted from consuming raw or undercooked shellfish, with 85% of cases occurring between May and October *(12, 14, 17, 19)*. The increased public health risk that may result from routine handling can be mitigated by resubmersing the oysters after handling to allow the elevated *Vibrio* spp. levels to recover to ambient levels *(15, 16, 22)*.

Previous resubmersion research is limited to ALS gear and summer months (July to September) *(15, 22, 28),* leaving the question of how gear type and time of year could affect *Vibrio* spp. recovery times. The ALS and OG

* Author for correspondence. Tel: 817-919-8387; E-mail: vlp0006@auburn.edu.

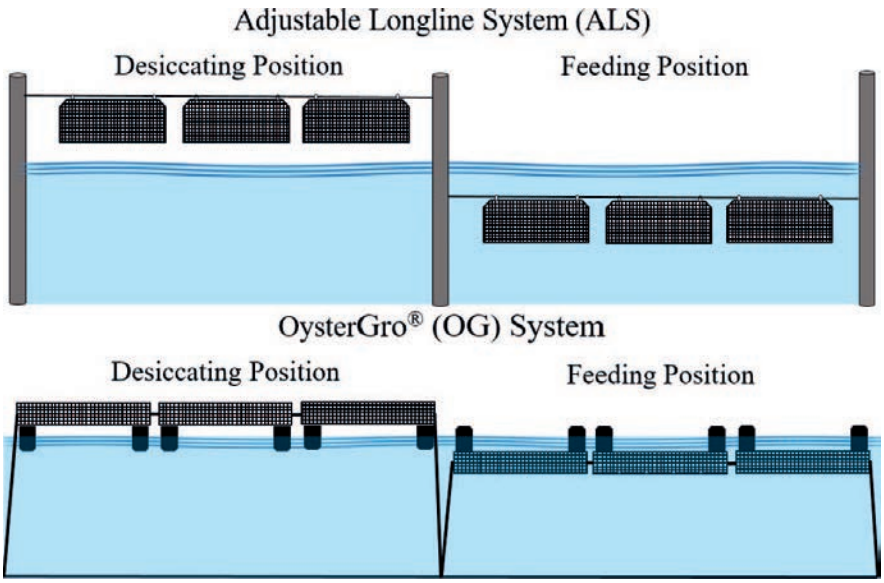

FIGURE 1. *Diagram of the adjustable longline system (ALS) and OysterGro (OG) gear types. The ALS system consists of a series of wooden pilings and PVC poles with lines tensioned between, and mesh baskets of oysters hanging from the line. The line can be raised up and down and secured in clips at various heights to allow for desiccation. The OG system consists of floating cages buoyed by air-filled pontoons at the surface of the water, filled with mesh bags containing oysters. Cages can be flipped up to expose oysters for desiccation.*

systems suspend, or float, oysters in the water column at different depths (Fig. 1), but it is unclear whether this affects the *Vibrio* spp. levels. Walton et al. *(31)* found no difference between the ambient *Vibrio* spp. levels in oysters raised in these two culture systems, but they did not test the *Vibrio* spp. levels after handling and subsequent resubmersion. Oysters in the ALS system are suspended on a rigid structure and can be hung at any height in the water column, whereas oysters in the OG system are in floating cages at the surface of the water and may be subjected to more wave action than the ALS oysters. Wave action has been shown to negatively impact the filtration rate of oysters in OG cages *(4, 23),* which could reduce the oysters' ability to efficiently purge the elevated levels of *Vibrio* spp. in that gear type.

Previous research was performed between July and September *(15, 22, 28),* and no studies were conducted during the beginning of the increased risk period (May). Cooler water temperatures are known to reduce the filtration rate of oysters and could likely reduce the purging efficiency of *Vibrio* spp. during this time *(4, 5, 13)*. Despite the cooler water temperatures, pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+) levels in oysters can be higher during late April to early May than June to September *(10, 11, 18, 32)*. Pathogenic *V. parahaemolyticus* strains required longer resubmersion periods after routine handling than *V. vulnificus* and total *V. parahaemolyticus* during June to September studies *(22, 28)*. Collectively, these factors indicate that oysters may need a longer resubmersion period for recovery of elevated levels of all *Vibrio* spp., especially pathogenic *V. parahaemolyticus,* at the beginning of the high-risk season.

To expand the existing knowledge of handling effects on *Vibrio* spp. in oysters, this study examined the effects of two gear types on the levels of *V. vulnificus,* total *V. parahaemolyticus,* and pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+) in cultured oysters before and after previously tested handling treatments *(15, 28),* and over time after resubmersion in May and July. Farm-raised oysters maintained in the ALS and OG systems were subjected to two handling treatments: desiccated overnight or tumbled and refrigerated overnight. Whereas refrigeration is a standard postharvest method to prevent vibrio proliferation *(7, 14),* we tested the potential for preharvest refrigeration during routine handling to evaluate its effect on the recovery times after resubmersion. *Vibrio* spp. levels were measured over time to determine when levels in treated oysters recovered to ambient levels in submersed (control) oysters. Results from this study will further inform public health officials and oyster farmers and aid in making informed decisions on appropriate resubmersion times for handled oysters.

MATERIALS AND METHODS

Field site and environmental monitoring. This study was performed at Auburn University's research farm site in the Grand Bay, AL. Single-set diploid oysters (*Crassostrea virginica*) were cultured in two off-bottom gear types: in BST bags suspended ~1 ft (0.3 m) below the surface on the adjustable longline system (ALS; BST Oyster Supplies, Cowell, Australia), and in the floating OysterGro cage system (OG; OysterGro, Saint-Édouard-de-Kent, New Brunswick, Canada). Oysters were stocked at standard stocking densities for each gear type: 100 to 120 oysters per bag for ALS and 150 to 200 oysters per bag for OG. Oysters remained submersed at the farm site for a minimum of 2 weeks before the start of each trial. Water temperature and salinity were recorded using a HOBO Saltwater Conductivity Data Logger (Onset Computer Corporation, Bourne, MA). During the treatment period, when oysters were out of the water, air temperature was collected from the Dauphin Island weather station at www.mymobilebay.com. Additionally, Smart Button data loggers (ACR Systems Inc., Surrey, British Columbia, Canada) were placed inside two oysters subjected to each treatment to record internal temperature.

Treatments and sample collection. A total of four trials were performed (Table 1): May 2018, July 2018, May 2019, and July 2019. During the trials, three treatments were tested for each gear type (ALS and OG), with six replicate bags for each of the six combinations: a submersed control, a tumbled and refrigerated treatment (TR), and a desiccated treatment. The control oysters remained submersed throughout each trial in each gear type. Bags

TABLE 1. *Environmental data collected during the trials*[a]

Trial	Air temp (°C)[b]	Water temp (°C)[c]	Salinity (PSU)[c]
I (29 Apr.–14 May 2018)	21.4 (18.0–24.9) A	25.7 (24.1–27.6) A	16.2 (7.8–18.8) A
II (8 July–23 July 2018)	27.0 (23.6–28.2) B	30.7 (28.2–33.5) B	17.0 (6.7–23.4) A
III (28 Apr.–13 May 2019)	21.6 (20.1–23.6) A	25.4 (23.6–26.9) A	6.7 (4.5–7.7) B
IV (7 July–22 July 2019)	30.2 (27.3–33.6) C	30.3 (29.3–31.6) B	12.4 (10.6–13.0) C

[a] Means in the same column with different letters are significantly different ($P < 0.05$).
[b] Average air temperature during the treatment period, with range in parentheses, collected from mymobilebay.com from the Dauphin Island station.
[c] Average daily means, with daily ranges in parentheses. PSU, practical salinity units.

of oysters from the two handling treatments were removed from the water and transported to the Auburn University Shellfish Laboratory (~1 h), where handling treatments were applied. TR oysters were tumbled separately by bag, with oysters from each bag passing through the mechanical grader once (Chesapeake Bay Oyster Company, Wake, VA) and then were returned to their bags and placed into a walk-in cooler (0 to 4°C) for 18 ± 2 h. Desiccated oysters remained in their bags exposed to ambient outdoor temperatures for ~24 h. After 24 ± 2 h, handled oysters were resubmersed at the farm site. Triplicate samples (15 oysters per sample) were collected from the control oysters before the treatments were applied (pretreatment); then, triplicate samples were collected from each of the six gear–treatment combinations after handling treatments were applied but prior to resubmersion (posttreatment) and at 3, 7, and 14 days after resubmersion. Oyster samples were collected at the farm site and transported with blue ice to the U.S. Food and Drug Administration Gulf Coast Seafood Laboratory for analysis.

MPN and real-time PCR. Samples were processed using the three-tube most-probable-number (MPN) method *(3, 21, 25)*. In brief, oysters were cleaned under cold tap water with a sterile brush, aseptically shucked into a sterile blender, and blended for 90 s. Then, oyster homogenate was serially diluted 10-fold to 1:100,000 in phosphate-buffered saline, and 1 mL of each dilution was inoculated into triplicate tubes of alkaline peptone water *(3, 21)*. Three tubes containing 10 mL of alkaline peptone water were inoculated with 1 g of oyster homogenate each. MPN tubes were incubated for 18 to 24 h at 35°C and then examined for turbidity; a 1-mL aliquot from each turbid tube was heated at 95°C for 10 min, resulting in a crude DNA extract that was cooled on ice or was directly stored at –20°C until further analysis. For real-time PCR analysis, extracts were thawed and centrifuged at 12,500 × *g* for 2 min. A 2-μL aliquot of the supernatant was tested for the presence of *V. vulnificus,* total *V. parahaemolyticus* (*tlh*), and pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+) using the real-time PCR assays as previously described *(22, 25)*. Using a standard MPN table, the number of positive MPN tubes was used to determine the levels of each *Vibrio* target *(3)*.

Statistical analysis. Environmental data (water temperature, air temperature, and salinity) were used to calculate average daily means, minimums, and maximums, and general linear models were used to determine any statistical differences among trials. Prior to analysis, all *Vibrio* spp. data (reported as MPN per gram of oyster homogenate) were log transformed. In instances where *Vibrio* spp. were not detected (<0.3 MPN/g), half of the limit of detection was substituted prior to log transformation. General linear models were used to compare the *Vibrio* spp. levels in control oysters between the two gear types for each individual trial. This model was also used to compare the *Vibrio* spp. levels in all control oysters among the May and July trials. Based on these results, the May trials were analyzed separately from the July trials to explore the differences between the months. All *Vibrio* spp. data are reported as log MPN per gram ± 95% confidence interval.

The data from the May 2018 and 2019 trials (trials I and III) were pooled and analyzed using a linear mixed-effects model to determine the effects of gear, treatment, and days since resubmersion, and the interaction between the two fixed effect variables. A random effect of trial was included to account for any between-trial variation. The data from July 2018 and 2019 trials (trials II and IV) were analyzed similarly. Initially, pretreatment *Vibrio* spp. levels were compared with the posttreatment levels in the handled oysters to determine how handling affected the *Vibrio* spp. levels. Then, if a significant interaction between treatment and days since resubmersion was detected, individual *t* tests were performed for each time point to compare the treatment levels with the control levels. Each of the *Vibrio* spp. was considered "recovered" when the treatment levels were not significantly higher than the submersed control levels for each gear type ($\alpha = 0.05$). All data analyses were performed in R Studio using the

TABLE 2. Vibrio *spp. levels in submersed control oysters by trial*[a]

Trial	*V. vulnificus*	Total *V. parahaemolyticus*	Pathogenic *V. parahaemolyticus*	
			tdh+	*trh*+
I	3.18 (±0.71) A	3.51 (±0.60) A	0.43 (±0.63) A	0.36 (±0.71) A
II	4.36 (±0.48) B	3.33 (±0.52) AB	−0.42 (±0.41) B	−0.48 (±0.36) BC
III	3.88 (±0.42) C	3.07 (±0.44) C	−0.01 (±0.62) C	−0.24 (±0.53) C
IV	4.04 (±0.44) C	3.20 (±0.38) BC	−0.41 (±0.58) B	−0.52 (±0.34) B

[a] Average *Vibrio* spp. levels ($n = 15$) in submersed control oysters during each trial (±95% CI), reported as log MPN per gram. Means in the same column with different letters are significantly different.

TABLE 3. Vibrio *spp. levels before and after handling treatments, but prior to resubmersion*[a]

Vibrio spp.	Days							
	ALS pre	ALS post	ALS TR	ALS des	OG pre	OG post	OG TR	OG des
May trials (2018–2019)								
V. vulnificus	3.15 ± 0.60	3.35 ± 0.90	2.83 ± 0.26	**5.11 ± 0.72**	3.55 ± 0.49	2.90 ± 0.20	2.94 ± 0.57	**5.04 ± 1.05**
Total *V. parahaemolyticus*	3.19 ± 0.32	3.43 ± 0.50	2.94 ± 0.46	**4.95 ± 0.38**	3.29 ± 0.43	2.86 ± 0.35	3.05 ± 0.42	**4.83 ± 0.53**
Pathogenic *V. parahaemolyticus* (*tdh*+)	0.49 ± 0.67	0.38 ± 0.43	0.61 ± 0.63	**2.24 ± 0.49**	0.69 ± 0.67	0.29 ± 0.66	0.13 ± 0.44	**2.22 ± 0.45**
Pathogenic *V. parahaemolyticus* (*trh*+)	0.19 ± 0.77	0.13 ± 0.59	0.48 ± 0.43	**2.04 ± 0.58**	0.25 ± 0.86	0.21 ± 0.66	0.19 ± 0.28	**2.30 ± 0.47**
July trials (2018–2019)								
V. vulnificus	4.14 ± 0.32	**4.71 ± 0.51**	4.33 ± 0.46	**6.04 ± 0.38**	3.94 ± 0.44	4.27 ± 0.36	4.00 ± 0.34	**5.97 ± 0.22**
Total *V. parahaemolyticus*	2.96 ± 0.27	3.30 ± 0.68	2.94 ± 0.15	**5.74 ± 0.40**	3.22 ± 0.55	3.18 ± 0.46	3.48 ± 0.28	**5.90 ± 0.30**
Pathogenic *V. parahaemolyticus* (*tdh*+)	−0.63 ± 0.44	−0.34 ± 0.44	−0.62 ± 0.30	**1.90 ± 0.38**	−0.69 ± 0.29	−0.47 ± 0.40	−0.58 ± 0.26	**1.94 ± 0.33**
Pathogenic *V. parahaemolyticus* (*trh*+)	−0.76 ± 0.14	**−0.34 ± 0.39**	−0.66 ± 0.17	**1.96 ± 0.27**	−0.63 ± 0.30	−0.48 ± 0.46	−0.59 ± 0.37	**2.19 ± 0.40**

[a] *Vibrio* spp. levels in pre- and posttreatment oysters, reported as log MPN per gram ± SD ($n = 6$). Numbers in bold represent posttreatment levels that were significantly higher than pretreatment levels, as determined by *t* tests. ALS, adjustable longline system; pre, pretreatment; post, posttreatment; TR, tumbled and refrigerated; des, desiccated; OG, OysterGro system.

nlme package *(27, 29)*. All figures were created in SigmaPlot (Systat Software, San Jose, CA).

RESULTS

Environmental data and *Vibrio* spp. variation. May trials had significantly lower (~5°C) air and water temperatures than July trials (Table 1; $P \leq 0.01$). Trial IV had a significantly higher mean ambient air temperature at 30.2°C than any other trial. Water temperatures did not significantly differ ($P \geq 0.51$) within the May or July trials. There were significant differences between the average daily salinities among the trials, with higher salinities during 2018 (trials I and II) than 2019 (trials III and IV). *Vibrio* spp. levels in control oysters of each gear type did not significantly differ (Table 2; $P \geq 0.08$), except for one instance. On average, total *V. vulnificus* levels in the OG control oysters in trial I were 0.52 ± 0.51 log MPN/g higher than the levels in the ALS control oysters ($P = 0.04$).

Treatment effects on *Vibrio* spp. before resubmersion. After the treatments were applied in May, but prior to resubmersion, the changes in *Vibrio* spp. levels depended on the treatment type (Table 3). The *Vibrio* spp. levels in the TR oysters (regardless of gear type) either decreased from pretreatment control levels by as much as 0.56 ± 0.75 log MPN/g (*tdh*+) or increased by as much as 0.28 ± 0.80 log MPN/g (*trh*+). However, the changes in *Vibrio* spp. levels in the TR oysters were not significant ($P \geq 0.13$). *Vibrio* spp. levels in desiccated oysters (regardless of gear type) significantly increased ($P < 0.001$) from pretreatment levels, ranging from an increase of 1.49 ± 0.86 log MPN/g for *V. vulnificus* to an increase of 2.05 ± 0.80 log MPN/g for pathogenic *V. parahaemolyticus* (*trh*+).

Similarly, after the treatments were applied in July, the *Vibrio* spp. levels in the TR oysters decreased as much as 0.02 ± 0.56 log MPN/g below pretreatment levels (total *V. parahaemolyticus*) or increased up to 0.26 ± 54 log MPN/g above pretreatment levels (total *V. parahaemolyticus*). However, these changes in TR oysters were not significant ($P \geq 0.34$). In the desiccated oysters, the *Vibrio* spp. levels significantly increased ($P < 0.001$) from pretreatment levels, by 2.03 ± 0.46 log MPN/g for *V. vulnificus* up to 2.82 ± 0.51 log MPN/g for pathogenic *V. parahaemolyticus* (*trh*+). Additionally, the posttreatment *V. vulnificus* levels in the ALS control oysters in July were significantly ($P = 0.03$) greater than the pretreatment levels, by 0.57 ± 0.50 log MPN/g, but this effect was not observed in the OG control oysters ($P = 0.18$; Table 3).

***Vibrio* spp. recovery times after resubmersion.** In May and July, for all *Vibrio* spp. tested, there were significant interactions between treatment type and time since resubmersion (Supplemental Table S1). Therefore, individual analyses were performed at each sampling time point to determine when levels recovered to control levels by gear type. After 7 days of resubmersion in May, *V. vulnificus* levels in the treated oysters were not significantly higher than the control oysters of each gear type ($P \geq 0.11$;

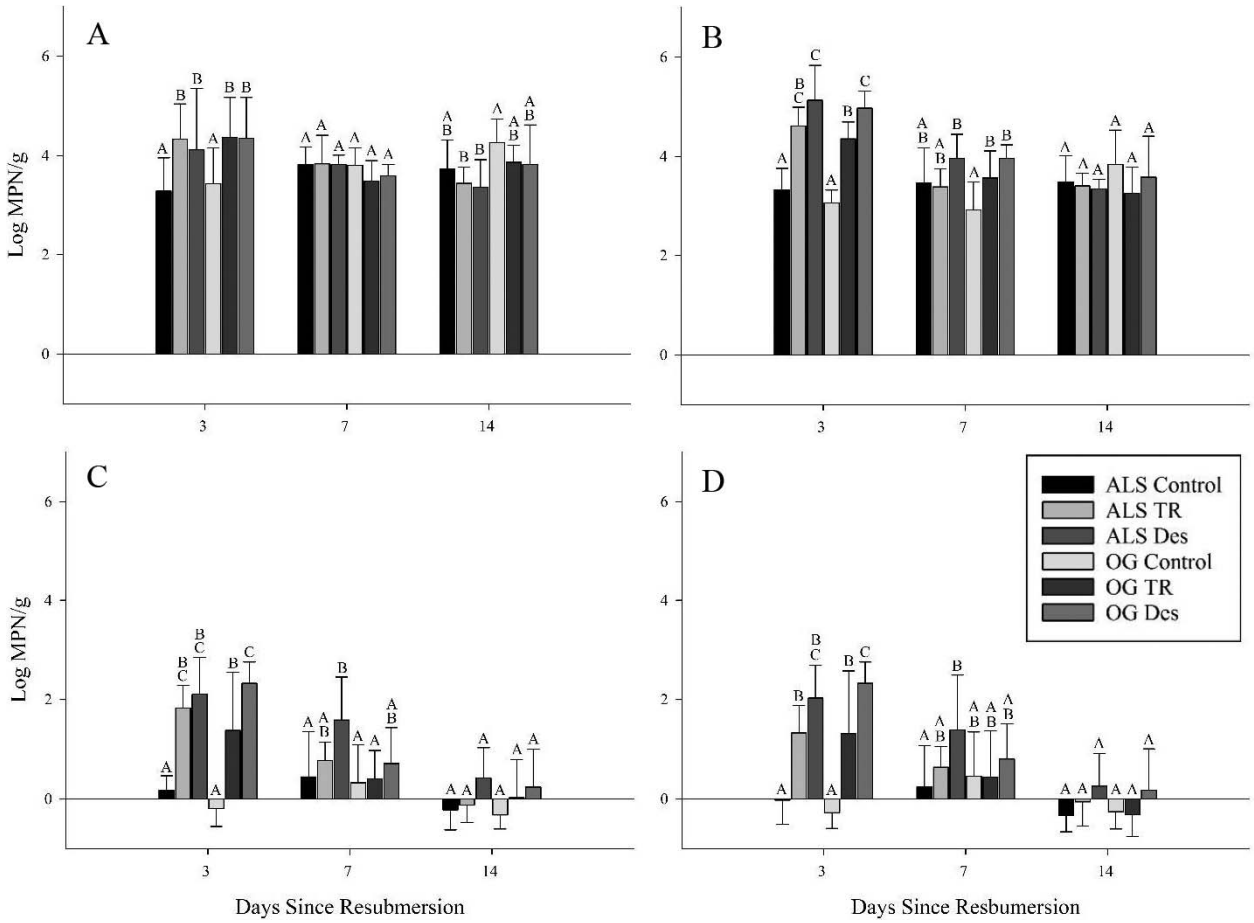

FIGURE 2. *Mean log-transformed* Vibrio *levels for* V. vulnificus *(A), total* V. parahaemolyticus *(B),* V. parahaemolyticus *(*tdh*+) (C), and* V. parahaemolyticus *(*trh*+) (D) during May resubmersion trials. ALS, adjustable longline system gear; OG, OysterGro gear; Control, submersed control; TR, tumbled, refrigerated; Des, desiccated.* x *Axis shows the days since resubmersion. Bars represent standard deviations, and letters represent significant differences in* Vibrio *spp. levels (*n = *6).*

Fig. 2A). In contrast, all *Vibrio* spp. levels in treated oysters were not significantly higher than control levels after 3 days of resubmersion in July ($P \geq 0.05$; Fig. 3A). For *V. vulnificus,* the recovery times did not differ between the gear types within the month, but they tended to be longer in May than in July (Table 4).

In May, regardless of treatment, the elevated total *V. parahaemolyticus* levels in the ALS oysters recovered to control levels after 7 days ($P \geq 0.08$), whereas the levels in the OG oysters recovered after 14 days ($P \geq 0.06$; Fig. 2B). In July, the levels in the desiccated oysters of both gear types recovered after 7 days of resubmersion ($P \geq 0.12$; Fig. 3B), whereas the OG TR oysters required 14 days to recover ($P = 0.98$). The total *V. parahaemolyticus* levels in the ALS TR oysters were 0.84 ± 0.50 log MPN/g higher than in the control ($P = 0.002$) after 14 days of resubmersion and did not return to ambient *V. parahaemolyticus* levels during the July study (Table 4). The *tdh*+ and *trh*+ *V. parahaemolyticus* levels in OG oysters (regardless of treatment) recovered after 7 days of resubmersion in May ($P \geq 0.39$; Fig. 2C and 2D). Conversely, there was a difference in pathogenic *V. parahaemolyticus* recovery times between the treatments in ALS: elevated levels recovered after 7 days in TR oysters ($P = 0.44$) and after 14 days in desiccated oysters ($P = 0.06$). During the July trials, the pathogenic *V. parahaemolyticus* levels in oysters of both gear types recovered after 7 days of resubmersion ($P \geq 0.14$), except for the OG TR oysters, which required 14 days for the *tdh*+ levels to return to ambient ($P = 0.51$; Fig. 3C and 3D).

DISCUSSION

Farm-raised oysters were placed in two common gear type systems (ALS and OG) and subjected to a common handling treatment (desiccation) and a potential new handling treatment (TR). These routine handling practices were followed by a 2-week resubmersion period to allow the oysters to purge elevated levels of *Vibrio* spp. back to ambient levels. Data from four trials were used to determine the recovery times for *Vibrio* spp. in oysters of four handling–gear type combinations during early May and July. Although water temperatures were lower in the May trials, the *Vibrio* spp. in the submersed control oysters were not always lower because control oysters had lower total *V. vulnificus* levels and higher pathogenic *V. parahaemolyticus* (*tdh*+/*trh*+) levels in May than in July, similar to previous findings *(10, 11, 18, 32)*. There was only one instance in which the *Vibrio* spp. levels in oysters were different

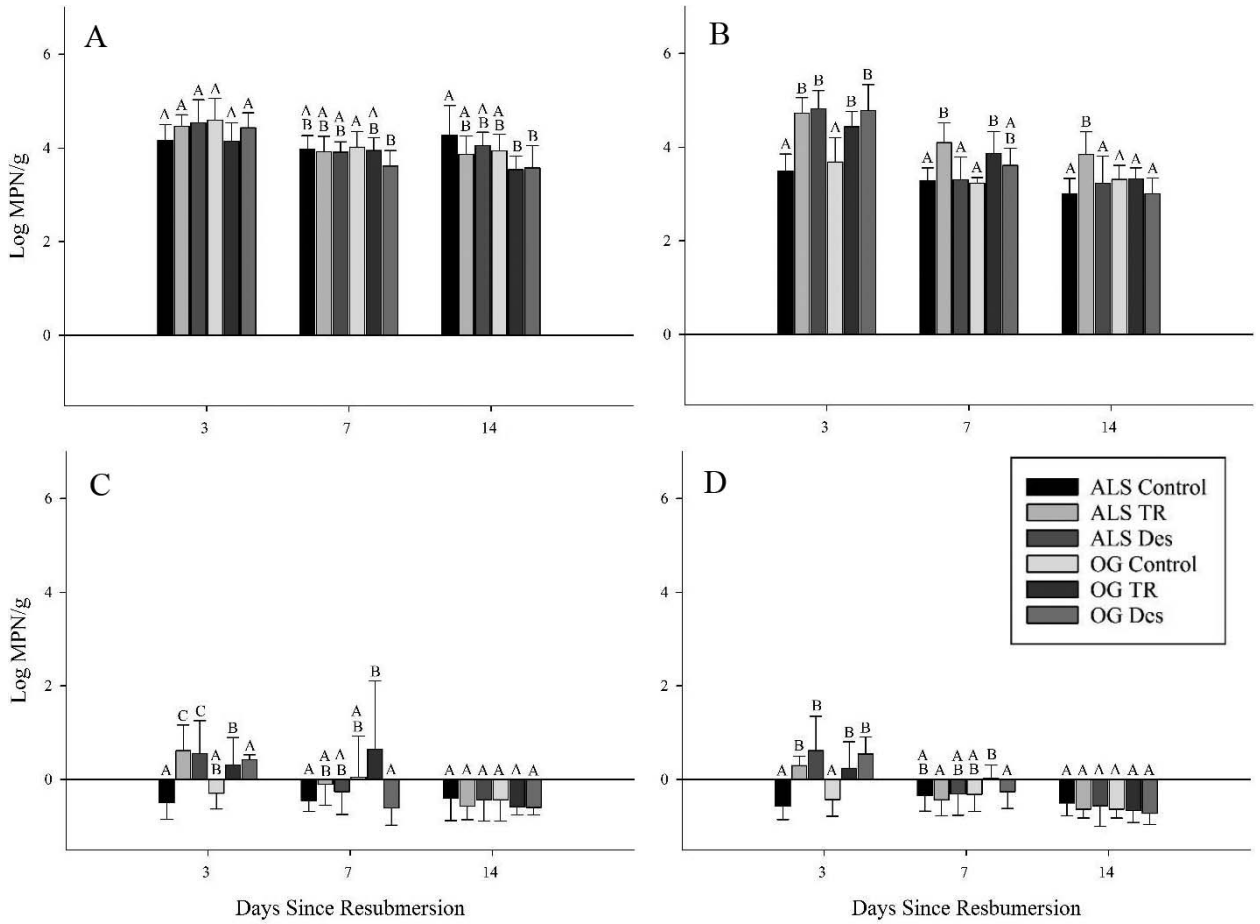

FIGURE 3. *Mean log-transformed* Vibrio *levels for* V. vulnificus *(A), total* V. parahaemolyticus *(B),* V. parahaemolyticus *(*tdh*+) (C), and* V. parahaemolyticus *(*trh*+) (D) during July resubmersion trials. ALS, adjustable longline system gear; OG, OysterGro gear; Control, submersed control; TR, tumbled, refrigerated; Des, desiccated.* x *Axis shows the days since resubmersion. Bars represent standard deviations, and letters represent significant differences in* Vibrio *spp. levels (*n = 6*).*

TABLE 4. *Number of days for* Vibrio *spp. levels to return to control levels*[a]

Vibrio spp.	Days			
	ALS TR	ALS des	OG TR	OG des
May trials (2018–2019)				
V. vulnificus	7	7	7	7
Total *V. parahaemolyticus*	7	7	14	14
Pathogenic *Vp* (*tdh*+)	7	14[b]	7	7
Pathogenic *Vp* (*trh*+)	7	14[b]	7	7
July trials (2018–2019)				
V. vulnificus	3	3	3	3
Total *V. parahaemolyticus*	>14	7	14	7
Pathogenic *Vp* (*tdh*+)	7	7	7[b]	7
Pathogenic *Vp* (*trh*+)	7	7	7	7

[a] Number of days after resubmersion when *Vibrio* spp. levels in treatment oysters were not significantly different from control oysters ($P > 0.05$), as determined by the mixed-effects model. ALS, adjustable longline system; TR, tumbled and refrigerated; des, desiccated; OG, OysterGro system.

[b] Cases where statistical significance does not agree with biological relevance (i.e., *Vibrio* spp. levels in the treatment oysters were still over 0.5 log MPN/g higher than levels in control oysters).

between the gear types (total *V. vulnificus* levels in trial I), similar to previous findings where *Vibrio* spp. levels were similar between gear types *(31)*. The higher *V. vulnificus* levels in the OG oysters during trial I could have resulted from the oysters experiencing higher surface water temperatures or greater wave action than the ALS oysters. The mean air temperatures were lower in May, corresponding to lower increases of *Vibrio* spp. in oysters during desiccation in May than in July (Table 3). Regardless of these differences, the air temperatures in both months created optimal conditions for *Vibrio* spp. growth *(6, 8, 9, 14, 26)* and resulted in significant increases in *Vibrio* levels during oyster exposure. The TR oysters had insignificant initial increases in *Vibrio* spp. levels, and in some cases, the levels decreased, as previously described for refrigeration *(6, 7, 9, 14, 26)*.

The recovery times required for levels in oysters to return to ambient levels in this study varied among the *Vibrio* spp. target (Table 4). *V. vulnificus* levels in oysters of all gear and treatment combinations recovered after 7 days in May and 3 days in July. For total *V. parahaemolyticus,* the TR oysters (regardless of gear type) required 14 days or more of resubmersion in July, whereas the desiccated oysters required only 7 days. This was in contrast to previous findings from Portersville Bay, AL *(28),* where

recovery times were the same between the TR and desiccated treatments. The TR oysters in this study appeared to experience a delay in filter feeding after resubmersion, possibly due to the effect of different environmental conditions experienced at this study site, indicating the potential for spatial and temporal variability in recovery times. There was no difference in recovery time (7 days) between desiccated oysters in either gear type in July for *V. parahaemolyticus*. This same trend did not hold true in May, when the desiccated oysters of both gear types required 14 days of resubmersion for all *Vibrio* spp. to recover. The variation in recovery times between May and July indicates that the cooler month of May requires a longer resubmersion period (14 days) for *V. parahaemolyticus* than is required in June to September. This could be due to the variability in total and pathogenic *V. parahaemolyticus* levels found during early May, combined with the reduced filtration of oysters in cooler water temperatures *(5, 10, 11, 13, 18, 32)*.

The data from this study were analyzed using a mixed-effects model as previously described *(28)*; however, the question of biological relevance versus statistical significance was raised. It can be inferred from the quantitative risk assessments that a 0.5-log MPN/g increase in levels increases the risk of infection three- to sixfold for *V. vulnificus* and threefold for *V. parahaemolyticus (1, 2)*. Additionally, this threshold of 0.5 log MPN/g takes into account an average combined method error and sample-to-sample variability of 0.5 log MPN/g *(15, 16, 20, 22, 28)*. Therefore, observed differences in means above this threshold could be assumed to be "real" (not an artifact of sample or method variability), raising concerns about risk of illness. This biological relevance "threshold" and the mixed-effects model were in agreement on determination of recovery times, except for two of the 32 conditions examined in the pooled analyses: in May, the *tdh*+ and *trh*+ levels in the ALS desiccated oysters were 0.63 ($\pm$0.73) and 0.60 ($\pm$0.63) log MPN/g higher than the control levels on day 14, and the model showed these differences as not significant ($P \geq 0.06$). Using this biological threshold appears to be a more conservative approach for public health, suggesting that longer recovery times are needed for *Vibrio* spp. in oysters to return to ambient levels than determined by the model. Whereas the use of a more conservative alpha with the models is one option, a biologically relevant difference in means of 0.50 MPN/g could be used as an alternative metric for decision making.

This study subjected cultured oysters to routine handling practices that elevated *Vibrio* spp. levels within the oysters, and it determined the time required for the elevated levels to return to ambient levels after resubmersion. As a result of the limited sampling days and low level of replication, data from this study are limited in statistical power and may not be well suited for use in making regulatory decisions about resubmersion periods. Future studies should conduct additional sampling between 7 and 14 days after resubmersion to refine the recovery time. Despite these limitations, this study revealed valuable trends that have started to fill in existing knowledge gaps and, ultimately, can be used to inform future studies to further investigate resubmersion. Factors from similar studies were tested (i.e., ALS gear type, summer months, handling treatments) *(15, 16, 22, 28)* along with several new factors, including resubmersion in a different water body (Grand Bay, AL), a cooler month (May), and an additional gear type (OG). The addition of new factors revealed that geographical location and time of year may have an effect on the resubmersion time required for cultured oysters to purge *Vibrio* spp. levels after routine handling.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/JFP-20-318.s1

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