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1 **Effects of desiccation practices and ploidy in cultured oysters, *Crassostrea virginica*, on**
2 ***Vibrio* spp. abundances in Portersville Bay (Alabama, USA)**
3

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Abstract

Off-bottom cultivation of oysters, *Crassostrea virginica*, is increasing in the Gulf of Mexico. The warm ambient air and water temperatures found in the Gulf of Mexico, coupled with the target market for off-bottom cultivated oysters for live raw consumption, raise concerns about the potential infections by human health pathogens, *Vibrio parahaemolyticus* and *V. vulnificus*. Regular practices associated with off-bottom cultivation, such as desiccation, expose oysters to ambient air to eliminate bio-fouling and are also known to increase these *Vibrio* spp. levels in oysters. Along with cultivation methods being introduced in the Gulf of Mexico, the use of triploid oysters is becoming increasingly popular. Triploid oysters are used a majority of the time in off-bottom cultivation due to their sterility, which results in rapid growth and high summer meat quality. Research also suggests that the lack of gonad tissue may correlate with lower *Vibrio* spp. levels in oysters. In this study, triploid and diploid oysters were cultured in Australian long line systems and subjected to two typical desiccation practices, air dried and freshwater dipped/air dried, and then evaluated for *V. parahaemolyticus* and *V. vulnificus* abundances over time. Three two-week long studies determined that *Vibrio* spp. levels in oysters that underwent either desiccation treatment returned to levels similar to those of submersed oysters by day three, referred to as returning to background levels. However, the *Vibrio* spp. levels in the treated oysters remained not significantly different from the elevated levels seen immediately following the desiccation treatment until seven days after re-submersion. There was no significant difference in *Vibrio* spp. levels between triploid and diploid oysters, nor a difference in the time of re-submersion needed to return levels to background. These results suggest that oysters that have been desiccated should be re-submersed for at least seven days

prior to harvest to mitigate any human health risk contributed by desiccation practices, regardless of oyster ploidy.

Key words: *Vibrio parahaemolyticus, Vibrio vulnificus, Crassostrea virginica,* triploid, Gulf of Mexico, aquaculture

Highlights

- This study suggests an absence of correlation between ploidy and *Vibrio* spp. levels in cultured oysters that undergo routine aquaculture desiccation during summer months in Portersville Bay, Alabama.
- This study suggests that ploidy has no effect on the amount of time needed for *Vibrio* spp. levels return to background levels after re-submersion during summer months in Portersville Bay, Alabama.
- This study identifies the length of time necessary to reduce the increased risk of *Vibrio* spp. infection from consumption of cultured oysters, *C. virginica*, that are associated with exposure to ambient air desiccation during summer months in Portersville Bay Alabama.

1. Introduction

Shellfish aquaculture in the United States generates $323 million annually, with $45 million resulting from the production of the Eastern Oyster, *Crassostrea virginica* (FAO, 2016). In 2013, the global production of *C. virginica* was 107,917 tons of oyster in shell. Aquaculture methods such as off-bottom and cage culture are widely used in the northeastern and mid-Atlantic United States with much success. In a combined effort, Auburn University, Mississippi-Alabama Sea Grant Consortium, and Louisiana State University are working with private growers to expand oyster aquaculture to the Gulf of Mexico (NOAA, 2015). In the Gulf of

74 Mexico, oyster farmers have traditionally used on-bottom cultivation of oysters on leases, laying
75 shell down as substrate for wild spat to settle and grow (Walton, 2013). With concerns about the
76 number of wild spat decreasing, oyster farmers have begun to adopt methodologies already
77 established in the Northeast and in other parts of the world (NOAA, 2015). Some of these culture
78 methods include suspended baskets, floating baskets, oyster cages, and bags suspended by legs
79 (Walton, 2013). Mississippi has begun the permitting process to allow these methodologies to be
80 used in designated areas (pers. obs.), while Florida, Louisiana, and Alabama have established
81 private growers using these off-bottom methodologies (Northern Economics, 2014). The
82 increased use of off-bottom cultivation, while beneficial to the Gulf of Mexico economy, is a
83 concern due to warm temperatures which are correlated with higher levels of *Vibrio*
84 *parahaemolyticus* and *V. vulnificus* (Johnson et al., 2010; Pfeffer et al., 2003). The warm
85 ambient air and water temperatures accompanied by regular practices associated with
86 aquaculture such as desiccation, which remove biofouling, and submersion of oysters into
87 freshwater, to remove *Polydora* spp., can lead to higher levels of *Vibrio* spp. in cultured oysters
88 (Grodeska et al., In Press; Kinsey et al., 2015).
89 *Vibrio parahaemolyticus* and *V. vulnificus* are human pathogenic bacteria commonly
90 associated with food borne illnesses, with most food borne infections (93%) coming from the
91 consumption of raw oysters (Oliver, 2013). Consumption of raw shellfish which contain high
92 abundances of these *Vibrio* spp. can cause rapid septicemia, acute gastroenteritis, and even death
93 in immune compromised individuals (Daniels et al., 2000; Jones and Oliver, 2009; Levine and
94 Griffin, 1993; Oliver, 2013). Individuals who are high risk to contract a fatal *V.*
95 *parahaemolyticus* infection, which is rare, include but are not limited to those with pre-existing
96 conditions, such as alcoholism, liver disease, renal disease, vascular disease, and/or diabetes

97 (Daniels et al., 2000). Pre-existing conditions that increase the risk of *V. vulnificus* infections,
98 which occur in ~35-50% of cases, include liver disease, such as cirrhosis or hepatitis, and open
99 wounds (Oliver, 2005; Oliver, 2006).

100 In addition to adapting grow-out methodology, researchers have experimented with and
101 modified the oysters themselves. Diploid oysters (two chromosomes) invest much of their energy
102 into developing gonadal tissue rather than growth. Triploid oysters (three sets of chromosomes)
103 are unable to reproduce, so expend more energy towards rapid growth (Nell, 2002). The use of
104 triploids also allows an extended summer market during the months that spawning diploid
105 oysters have "milky" meat that is undesirable to the consumer (Walton, 2013). De Decker et al.
106 (2011), revealed a positive correlation between *Vibrio* spp. abundances and gonadal tissue,
107 indicating that triploid oysters may harbor lower levels of certain *Vibrio* spp.. The De Decker
108 study was performed with the Pacific Oyster, *Crassostrea gigas*, and with *V. splendidus* and *V.*
109 *aestuarianus,* both of which can cause mortality in oysters, but have not been documented to
110 cause disease in humans (De Decker et al., 2011). While De Decker et al. (2011) investigated
111 *Vibrio* spp. that are not of particular interest to human health officials, their findings lead to
112 questions regarding certain *Vibrio* spp. that do have an impact on human health. Currently, a
113 majority of oyster farmers use single set triploid oysters which are acquired from hatcheries and
114 are frequently destined for the live, raw market (pers. obs.). If triploid oysters do, in fact, harbor
115 fewer *Vibrio* spp. this could be an additional benefit for the use of triploid oysters that could
116 potentially affect the calculation of risks.

117 Most studies of *C. virginica*, *V. parahaemolyticus*, and *V. vulnificus* have focused on dry
118 storage and post-harvest methodologies that increase *Vibrio* spp, to determine the length of time
119 it takes for *Vibrio* spp. levels to multiply after harvest. That information helps inform public

120 health officials on the amount of time oysters can be held without refrigeration before they pose
121 an increased risk to illness. In contrast, this study focused on reducing *Vibrio* spp. abundances
122 prior to harvest and to ensure that routine aquaculture practices do not increase the risk of illness
123 to consumers. The objective was to determine whether *V. parahaemolyticus* and *V. vulnificus*
124 abundances differ between diploid and triploid oysters over time when subjected to common
125 aquaculture desiccation practices including 27-hour ambient air dry and 3-hour freshwater dip
126 prior to 24-hour ambient air dry, followed by re-submersion, and any interaction between ploidy
127 and these desiccation practices. Measuring these *Vibrio* spp. abundances across time may help
128 determine the length of time needed to mitigate effects of such desiccation practices. A
129 significant relationship between either ploidy, desiccation practices, days since re-submersion,
130 and/or any interactions effect on *Vibrio* spp. could help provide information to the oyster farming
131 industry and influence public health decisions.

132 **2. Materials and Methods**

133 **2.1. Sampling Location and Conditions**

134 The fieldwork was conducted at Auburn University's research field site in Portersville
135 Bay, Coden, Alabama (Mississippi Sound), a shallow (1-2 m) firm mud bottom site with a small
136 tide (0.5-1.0 m). At this site, mid-summer salinities typically range from 15 to 25 PSU and water
137 temperatures range from 25-30 °C (Walton, 2013). These field conditions were expected to be
138 favorable to proliferation of pathogenic *Vibrio* spp. (WHO-FAO, 2005). Environmental data
139 during study were retrieved from mymobilebay.com using the Cedar Point station. These data
140 included daily mean and daily minimum and maximum of salinity, water temperature, wind
141 speed, precipitation across trials, and mean air temperature for the period of desiccation.

142 **2.2. Submersion and Treatment of Oysters**

143 During this study, data were collected during three two-week long replicate trials in 2015.
144 The trials started July 15th and ended September 1st to ensure favorable conditions for *Vibrio* spp.
145 growth. Sampling over time was done to determine the length of time needed for *Vibrio* spp.
146 abundances in oysters of both desiccation treatments and ploidy to return to levels not
147 significantly different from those of submersed oysters, which will hereafter be referred to as
148 returning to submersed levels.

149 Oysters were stocked in replicate baskets (BST Oyster Supplies, Australia), each with
150 100-120 diploid oysters, and another group of baskets were each stocked with 100-120 triploid
151 oysters from the same half-sibling brood. The triploid oyster brood was verified using flow
152 cytometry (Allen, 1983). All baskets were submersed in one batch on an Australian Adjustable
153 Long-Line culture system (ALS) at the study site alternating ploidies by bay, and maintained at a
154 depth un-exposed to air during even extreme low tides for a minimum of 14 days prior to
155 sampling (and typically greater than 45 days for each subsequent trial). This extended
156 submersion period prior to any sampling allowed oysters to reach ambient *Vibrio* spp. levels
157 (Grodeska et al., 2017; Walton et al., 2013a).

158 In any single trial of the three, six randomly selected baskets per ploidy were subjected to
159 a 3-hour freshwater dip, and then allowed to air dry for 24 hours (hereafter freshwater dipped).
160 Another three to six randomly selected baskets per ploidy were subjected to 27 hours of
161 desiccation at ambient air temperatures (hereafter air dried). Six baskets per ploidy were left in
162 the water, and designated as the control (hereafter submersed). One sample of 12-15 oysters was
163 taken out of each of three randomly chosen baskets for each ploidy prior to any treatment and
164 were represented as $T_{pre\text{-}treatment}$. This was used to determine what the initial *Vibrio* spp.
165 abundances were prior to treatment for each ploidy. Additionally, immediately prior to re-

166 submersion, $T_{post\text{-}treatment}$, three samples of 12-15 oysters were randomly selected from three
167 baskets per treatment for each ploidy to determine the effect of 'desiccation treatment' (including
168 submersed) on *Vibrio* spp. abundances. During each trial, a sample of 12-15 oysters was
169 randomly collected from three baskets per treatment for each ploidy at the following time points:
170 1, 2, 3, 7, 10, and 14 days after re-submersion (hereafter referred to as 'T_x' where the sub-script x
171 designates the number of days of re-submersion). All samples were packed in coolers with ice
172 packs buffered by burlap sacks to prevent direct contact with the oysters.

173 Due to the amount of time that processing required and a parallel project, diploid samples
174 were shipped to either Auburn University Aquatic Microbiology Lab (AU AML) or delivered to
175 the FDA Gulf Coast Seafood Laboratory (FDA GCSL). Prior to initiation of the study, multiple
176 samples were split between GCSL and Auburn labs to ensure no statistically significant
177 difference between results generated at the two laboratories. During the split sample analysis and
178 this study, oysters destined for the FDA GCSL were held in a cooler overnight to mimic shipping
179 conditions required to deliver oysters to AU AML. AU AML processed diploid samples from
180 $T_{post\text{-}treatment}$ and T_7, T_{10}, and T_{14} and FDA GCSL processed diploid samples from $T_{pre\text{-}treatment}$ and
181 T_1, T_2, and T_3. All triploid samples, along with all of trial III oysters, were processed at the FDA
182 GCSL.

183

184 **2.3. Sample Analysis**

185 Samples were processed following standard protocols according to Bacteriological
186 Analytical Manual (BAM). Upon opening the cooler, 12-15 oysters from each experimental
187 group were cleaned, shucked, and homogenized in a sterile food blender. The samples were then
188 processed for *V. parahaemolyticus* and *V. vulnificus* abundances by direct plating; samples were

189 plated on T1N3 to isolate *V. parahaemolyticus* and VVA to isolate *V. vulnificus.* After colonies
190 were lifted and lysed to filters, the remainder of processing was completed at the FDA GCSL
191 using alkaline phosphatase-labeled oligonucleotide probe colony hybridization for confirmation
192 (McCarthy et al., 1999; Wright et al., 1993). Probe-positive colonies were counted and reported
193 in CFU/g.
194

195 **2.4. Statistical Analysis**
196

197 Environmental data were collected from mymobilebay.com using the Cedar Point site.
198 Environmental parameters that were analyzed included water temperature, salinity, wind speed,
199 precipitation, and air temperature collected during the 27 hours oysters were subjected to
200 desiccation treatments; these data were used to perform one-way ANOVA. Air temperature daily
201 mean along with the minimum and maximum values were calculated. The remaining data were
202 collected for the entire duration of each trial and the daily mean along with daily mean minimum
203 and maximums were calculated. The daily means were used to perform one-way ANOVA to
204 compare between trials, except precipitation. The daily mean maximum for precipitation was
205 used to perform one-way ANOVA. Using the data that were previously described for each one-
206 way ANOVA, a main effects model was performed to determine environmental effects on *Vibrio*
207 spp..

208 All *Vibrio* spp. data were log transformed and triplicate samples averaged so that
209 replication was at the trial level. A two-way ANOVA was performed to determine if there was a
210 difference in Vibrio spp. abundances at the trial level. To assess whether the two treatments
211 (freshwater dipped and air dried) successfully elevated *Vibrio* spp. abundances, $T_{post\text{-}treatment}$ levels
212 compared to un-manipulated $T_{pre\text{-}treatment}$ levels, a two-way ANOVA was performed to compare

213 effects of treatment and ploidy on *Vibrio* spp. abundances with a post-hoc multiple comparisons

214 t-test.

215 A three-way ANOVA was performed to compare the effects of ploidy, treatment, and days since

216 re-submersion on *Vibrio* spp. abundances. A student's t-test was used for all post-hoc

217 comparisons where a significant effect was found. All ANOVAs and post-hoc comparisons were

218 considered significant at $p < 0.05$. All statistical calculations were performed using the JMP

219 statistical program (SAS Institute Inc., Cary, NC).

220

221 **3. Results**

222 **3.1. Environmental Parameters**

223 Although significant differences of environmental parameters (Table 1) were detected

224 between trials, there was no apparent effect ($p > 0.30$) on *Vibrio* spp. abundance within the

225 oysters.

226

227 **3.2. Initial Effect of Desiccation Treatment**

228

229 No significant difference of *Vibrio* spp. abundance in oysters was detected among trials

230 ($p < 0.05$), so all trials were combined for further analysis. At the onset of the trials, there was no

231 effect of ploidy ($p = 0.20$), nor an interaction between treatment and ploidy ($p = 1.00$) on *V.*

232 *parahaemolyticus* abundances (Table 2); therefore, further analysis was completed with

233 combining data from diploids and triploids. The treatments had a highly significant ($p < 0.01$)

234 effect on *V. parahaemolyticus* abundances (Table 2). Among the different treatments, the

235 abundances in the oysters from two manipulated treatments [air dried (4.6 log MPN/g) and

236 freshwater dipped (4.5 log MPN/g)] were significantly higher ($p < 0.01$) than abundances in

237 either the $T_{pre\text{-}treatment}$ oysters (3.0 log MPN/g) or the submersed oysters (3.2 log MPN/g), but did

238 not differ ($p = 0.83$) from each other (Table 3, Fig. 1). Additionally, the abundances in oysters
239 from the submersed treatment did not differ ($p = 0.37$) from those in the pre-treatment oysters
240 (Table 3, Fig. 1).

241 Similarly, for *V. vulnificus*, there was no effect of ploidy ($p = 0.52$) nor an interaction (p
242 = 0.84) between treatment and ploidy (Table 4). There was an effect of treatment ($p = <0.01$).
243 Among the different treatments, abundances in oysters from the two manipulated treatments [air
244 dried (4.9 log MPN/g) and freshwater dipped (4.9 log MPN/g)] were significantly higher ($p <$
245 0.01) than abundances in either the $T_{pre\text{-}treatment}$ oysters (3.8 log MPN/g) or the submersed oysters
246 (3.9 log MPN/g), but the levels did not differ ($p = 0.99$) from each other (Table 5, Fig. 2).
247 Additionally, abundances in oysters from the submersed treatment did not differ ($p = 0.89$) from
248 the pre-treatment oysters (Fig. 2).

249 **3.3. Effect of Ploidy over Re-submersion Time**

250 There was no effect of ploidy on either *V. parahaemolyticus* ($p = 0.06$) or *V. vulnificus* (p
251 = 0.28), despite triploids tending to have lower *V. parahaemolyticus* and *V. vulnificus*
252 abundances than diploid oysters (Fig. 3). Additionally, no significant interaction ($p \geq 0.38$) of
253 ploidy with treatment or time (Tables 6 and 7, respectively) was identified. Due to the lack of
254 effect of ploidy, data from both ploidies were combined for further analysis. While no statistical
255 analysis was performed comparing the means and standard error of Vibrio levels within each
256 trial shows similar trends (Table 8 and 9).

257 **3.4. Effects of Time and Desiccation Treatment Interactions**

258 When ploidy data were combined, there were significant interactions ($p \leq 0.04$) between
259 time and treatment (Tables 6 and 7, respectively) for both *V. parahaemolyticus* and *V. vulnificus*.
260 *V. parahaemolyticus* abundances reached those similar to submersed (4.0 log CFU/g) at T_3 for

261 oysters from air dried (4.3 log CFU/g) and freshwater dipped treatments (4.5 log CFU/g) (Fig.4).
262 Specifically, at $T_{post\text{-}treatment}$, T_1, and T_2, abundances in oysters of the two desiccation treatments
263 (air dried, freshwater dipped), which ranged from 4.0 log CFU/g (T_2 air dried) to 4.6 log CFU
264 ($T_{post\text{-}treatment}$ air dried, T_1 freshwater dipped), were significantly higher ($p \leq 0.01$) than the
265 abundances in oysters of the submersed treatment, which ranged from 3.2 log CFU/g (T_0, T_1) to
266 3.4 log CFU/g (T_2), but from T_3 onward there were no significant differences among all three
267 treatments within any given number of days submersed. In addition, there were not any
268 significant differences ($p \geq 0.05$) of *V. parahaemolyticus* abundances between the two
269 desiccation treatments within any given number of days. (Fig. 4).
270 Importantly, the abundances of *V. parahaemolyticus* in submersed oysters differed
271 significantly among days (Fig. 4); for example, the levels at T_3 (4.0 log CFU/g) were
272 significantly higher than at $T_{post\text{-}treatment}$, T_1, T_2, T_{10} and T_{14} which ranged from lowest at T_{10} (3.1
273 log CFU/g) to the highest at T_2 and T_{14} (3.4 log CFU/g). Additionally, at T_3 the abundances in
274 oysters from desiccated samples returned to those in submersed oysters [air dried oysters (4.6 log
275 CFU/g), freshwater dipped oysters (4.5 log CFU/g)], but did not decrease from initially elevated
276 levels ($T_{post\text{-}treatment}$) until T_7 [air dried oysters (3.7 log CFU/g), freshwater dipped oysters (3.6 log
277 CFU/g)].
278 For *V. vulnificus*, abundances in oysters that underwent desiccation treatments reached
279 those similar ($p = 0.14$) to submersed oysters (3.7 log CFU/g) at T_2 in air dried (4.1 CFU/g) and
280 freshwater dipped (4.2 CFU/g) treatment samples (Fig. 5). Specifically, at days $T_{post\text{-}treatment}$ and
281 T_1, the abundances in oysters from the two treatments (air dried, freshwater dipped) were
282 significantly higher ($p \leq 0.01$) than the levels in submersed oysters. By T_3 and for the remainder
283 of the study, there were no significant differences ($p \geq 0.05$) in *V. vulnificus* abundances in

284 oysters among the three treatments. In addition, there were no significant differences ($p \geq 0.05$)
285 between the abundances in the two treatments at any given number of days submersed.

286 The abundances of *V. vulnificus* in submersed oysters did not differ significantly ($p \geq$
287 0.05) among days. Despite this lack of variation in abundances of *V. vulnificus* in submersed
288 oysters, there was variation was observed between treatments among days. At $T_{2,}$ abundances in
289 oysters from both treatments were significantly lower than elevated $T_{post\text{-}treatment}$ levels [air dried
290 oysters (4.9 log CFU/g), freshwater dipped oysters (4.9 log CFU/g)]. There was an increase in *V.*
291 *vulnificus* levels at $T_{3,}$ abundances in oysters from freshwater dipped treatments returned to $T_{post\text{-}}$
292 $_{treatment}$ elevated levels (4.5 log CFU/g), while abundances in air dried treatment oysters remained
293 significantly lower. The abundances in oysters from freshwater dipped treatments again
294 decreased from $T_{post\text{-}treatment}$ levels at T_7 (3.8 log CFU/g) and did not return to elevated levels for
295 the remainder of the study.

296 **4. Discussion**

297 Based on these data, ploidy of oysters does not appear to provide a significant increase or
298 reduction of risks associated with *V. vulnificus* and *V. parahaemolyticus*. Notably, however, for
299 *V. parahaemolyticus*, triploids tended to have lower abundances than diploid oysters; this was
300 also seen in Walton et al. (2013b). With no significance, the effect of ploidy on *Vibrio* spp.
301 abundances remains intriguing but appears to be overwhelmed by other factors.

302 Environmental parameters during this study, such as water temperatures greater than
303 15˚C (Gooch et al., 2002; Murphy and Oliver, 1992) and salinity between 5 and 25 PSU (Bryan
304 et al., 1999; Hoi et al., 1998; Kaspar and Tamplin, 1993) were conducive to *Vibrio* spp. growth.
305 This was evident in the effectiveness of both routine desiccation practices, air dried and
306 freshwater dipped, to significantly increase *Vibrio* spp. abundances compared to levels in

307 continually submersed and $T_{pre\text{-}treatment}$ oysters. The increase of *Vibrio* spp. abundances by 1 to 1.5
308 logs demonstrates a greater human health risk associated with oysters subjected to routine
309 aquaculture practices, and indicates a rationale for special requirements being associated with
310 desiccation and re-submersion practices. When comparing between treatments, neither the air
311 dried nor freshwater dipped treatment had an increased risk or benefit, in regards to affecting
312 *Vibrio* spp. levels. At each time point, for both *Vibrio* spp. abundances no significant differences
313 were found between the two manipulated desiccation treatments.
314 Furthermore, there was a clear pattern of desiccated treatments returning to submersed
315 levels within three days. However, we note that there was significant variation in the abundances
316 in submersed oysters, which suggests that the ambient abundances of *Vibrio* spp. changed and, in
317 some cases, increased. In those cases, a lack of difference between desiccated treatments and the
318 submersed treatment did not indicate that the desiccated oysters were reducing *Vibrio spp.*
319 abundances, but rather that they were simply converging. Public health recommendations are
320 generally based on exposing oysters to practices that may elevate abundances and determining
321 the length of time until those abundances return to submersed levels; however, it is important to
322 note that in most cases *Vibrio* spp. abundances in oysters that underwent desiccation treatments
323 did not decrease from initial elevated levels until after returning to submersed levels, except for
324 air dried *V. vulnificus* abundances. It is imperative that *Vibrio* spp. abundances in oysters are
325 given time to decrease from elevated levels because this, in theory, removes the effects of
326 desiccation practices. While abundances returned to submersed levels by day three, elevated
327 levels do not significantly decrease from the initial elevated levels ($T_{post\text{-}treatment}$) until day seven,
328 except for *V. vulnificus* abundances in air dried oysters which decreased by two days post re-
329 submersion. Seven days allows time for abundances to return to submersed levels and decrease

330 from those initially elevated levels. This supports a recommendation of seven days of re-
331 submersion prior to harvesting and is consistent with the recommendation resulting from the
332 different analytical and statistical methodologies used in Grodeska et al. (2017).
333 Notably, this study did not sample between three days and seven days. The combined
334 results of *Vibrio* spp. abundances returning to ambient between two to three days and levels
335 significantly decreasing at or before day seven suggests that oysters subjected to routine
336 desiccation practices may need fewer than seven days to remove the increased associated risk.
337 Further investigation of the effects of desiccation practices, especially days four, five, and six,
338 may result in a recommendation of less than seven days of re-submersion prior to harvest.

339 **5. Conclusion**

340 This study was conducted using routine aquaculture practices, in an approved aquaculture
341 location in the Gulf of Mexico to determine if, under these experimental conditions, diploid or
342 triploid oysters would contain significantly different *Vibrio* spp. abundances during time of re-
343 submersion. We have concluded that, while there is a tendency for triploids to have lower
344 abundances of *V. parahaemolyticus* than diploids, triploid oysters do not have significantly
345 different *Vibrio* spp. abundances compared to diploid oysters. When oysters underwent
346 desiccation treatments (air dried or freshwater dipped), there was no apparent effect between
347 those two treatments on *Vibrio* spp. abundances. Overall, it is important to note that, while
348 *Vibrio* spp. abundances may have returned to submersed levels by day three, there is a possibility
349 they are still affected by desiccation treatments until seven days after re-submersion (our next
350 sampling period). Allowing *Vibrio* spp. levels in oysters to return to submersed levels as well as
351 decrease from initially elevated levels removes the increased risk associated with routine
352 desiccation practices. For these reasons this study supports a recommendation of seven days of
353 re-submersion after routine desiccation practices prior to harvest in either diploid or triploid
354 oysters.

355

356

357

358 Table 1: Mean environmental data (salinity, water temperature, wind speed, and precipitation)
359 with mean daily minimum and maximums over each trial and averaged air temperature for date
360 of treatment (desiccation) with averaged minimum and maximums. Superscript letters denote
361 significant differences.

Trials	Environmental data				
	Water Temp (°C)	Salinity (PSU)	Wind Speed (knots)	Precipitation (cm)	Air Temp (°C)
I	31.0 ^{A} (30.1-32.0)	19.9 ^{B} (16.3-24.2)	9.6 ^{A} (1.8-19.8)	0.003 ^{A}	29.8 ^{A} (28.8-30.7)
II	30.2 ^{B} (29.3-31.1)	23.7 ^{A} (21.1-26.8)	10.4 ^{A} (3.3-19.9)	0.009 ^{A}	28.8 ^{B} (26.6-30.0)
III	29.6 ^{B} (28.7-30.6)	20.9 ^{B} (18.4-24.0)	7.4 ^{B} (1.0-14.0)	0.0006 ^{A}	28.6 ^{C} (26.7-29.7)

362
363 Table 2: ANOVA table for $T_{pre\text{-}treatment}$ and $T_{post\text{-}treatment}$ *V. parahaemolyticus* abundances;
364 submersed, air dried, and freshwater dipped. Lines in bold represent significant differences
365 (alpha = 0.05).

Source	DF	Sum of Squares	F Ratio	Prob> F
Treatment	**3**	**12.76**	**27.33**	**<0.01**
Ploidy	1	0.28	1.80	0.20
Treatment*Ploidy	3	0.01	0.02	1.00

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367 Table 3: Post-hoc t-test comparison of *V. parahaemolyticus* levels for $T_{pre\text{-}treatment}$ (Pre) and $T_{post\text{-}treatment}$;
368 submersed, air dried (Air), and freshwater dipped (Freshwater). Lines in bold represent
369 significant differences (alpha = 0.05).

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Air	**Pre**	**1.58**	**0.23**	**1.10**	**2.06**	**<0.01**
Freshwater	**Pre**	**1.53**	**0.23**	**1.05**	**2.01**	**<0.01**
Air	**Submersed**	**1.37**	**0.23**	**0.89**	**1.85**	**<0.01**
Freshwater	**Submersed**	**1.32**	**0.23**	**0.84**	**1.80**	**<0.01**
Submersed	Pre	0.21	0.23	-0.27	0.69	0.37
Air	Freshwater	0.05	0.23	-0.43	0.53	0.83

370 Table 4: ANOVA table for $T_{pre\text{-}treatment}$ and $T_{post\text{-}treatment}$ *V. vulnificus* levels; submersed, air dried,
371 and freshwater dipped. Lines in bold represent significant differences (alpha = 0.05).

Source	DF	Sum of Squares	F Ratio	Prob> F
Treatment	**3**	**6.16**	**6.62**	**<0.01**
Ploidy	1	0.13	0.43	0.52
Treatment*Ploidy	3	0.26	0.27	0.84

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373 Table 5: Post-hoc t-test comparison of *V. vulnificus* levels for $T_{pre\text{-}treatment}$ (Pre) and $T_{post\text{-}treatment}$;
374 submersed, air dried (Air), and freshwater dipped (Freshwater). Lines in bold represent
375 significant differences (alpha = 0.05).

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value

Freshwater	**Pre**	**1.04**	**0.32**	**0.36**	**1.72**	**<0.01**
Air	**Pre**	**1.03**	**0.32**	**0.35**	**1.72**	**<0.01**
Freshwater	**Submersed**	**0.99**	**0.32**	**0.31**	**1.67**	**<0.01**
Air	**Submersed**	**0.99**	**0.32**	**0.31**	**1.67**	**<0.01**
Submersed	Pre	0.04	0.32	-0.64	0.73	0.89
Freshwater	Air	0.004	0.32	-0.68	0.69	0.99

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377 Table 6: ANOVA table of the test of effects of time, treatment, and ploidy on *Vibrio*
378 *parahaemolyticus* abundances in oysters. Lines in bold represent significant differences (alpha =
379 0.05).

Source	DF	Sum of Squares	F Ratio	Prob> F
Model	**41**	**38.24**	**4.99**	**<0.01**
Error	84	15.69		
C. Total	125	53.93		
Time	**6**	**17.82**	**15.90**	**<0.01**
Treatment	**2**	**7.13**	**19.09**	**<0.01**
Ploidy	1	0.66	3.54	0.06
Time*Treatment	**12**	**10.59**	**4.72**	**<0.01**
Time*Ploidy	6	1.21	1.08	0.38
Treatment*Ploidy	2	0.18	0.49	0.62
Time*Treatment*Ploidy	12	0.65	0.29	0.99

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389 Table 7: ANOVA table of the test of effects of time, treatment, and ploidy on *Vibrio vulnificus*
390 abundances in oysters. Lines in bold represent significant differences (alpha = 0.05).

Source	DF	Sum of Squares	F Ratio	Prob> F
Model	41	22.67	2.14	<0.01
Error	84	21.67		
C. Total	125	44.33		

Time	**6**	**9.91**	**6.40**	**<0.01**
Treatment	**2**	**4.57**	**8.87**	**<0.01**
Ploidy	1	0.30	1.18	0.28
Time*Treatment	**12**	**6.01**	**1.94**	**0.04**
Time*Ploidy	6	0.30	0.20	0.98
Treatment*Ploidy	2	0.17	0.33	0.72
Time*Treatment*Ploidy	12	1.40	0.45	0.94

	Trials	I			II			III		
	Treatment	Submersed	Air	Freshwater	Submersed	Air	Freshwater	Submersed	Air	Freshwater
Day since resubmersion	0	3.45 (±0.10)	5.14 (±0.39)	5.11 (±0.14)	4.38 (±0.13)	4.71 (±0.33)	5.79 (±0.13)	2.01 (±0.24)	3.56 (±0.68)	4.44 (±0.10)
	1	3.48 (±0.15)	4.84 (±0.35)	3.04 (±0.29)	1.79 (±0.46)	4.82 (±0.68)	5.57 (±0.09)	2.96 (±0.36)	3.42 (±1.08)	2.88 (±1.30)
	2	3.77 (±0.73)	2.83 (±1.40)	3.99 (±0.37)	4.75 (±0.11)	5.16 (±0.30)	4.67 (±0.30)	2.87 (±0.29)	3.93 (±0.16)	2.39 (±1.00)
	3	3.50 (±0.36)	4.20 (±0.44)	2.25 (±0.81)	3.11 (±0.40)	4.21 (±0.30)	3.12 (±0.90)	2.83 (±0.10)	3.02 (±0.35)	2.44 (±0.26)
	7	3.81 (±0.35)	3.94 (±0.16)	3.44 (±0.03)	3.56 (±0.30)	3.21 (±0.17)	3.77 (±0.20)	3.09 (±0.12)	2.65 (±0.18)	2.46 (±0.21)
	10	3.63 (±0.00)	3.77 (±0.20)	3.16 (±0.10)	3.66 (±0.52)	3.63 (±1.25)	3.47 (±0.17)	2.01 (±0.80)	3.86 (±0.59)	2.6 (±0.02)
	14	3.51 (±0.65)	3.41 (±0.22)	2.67 (±0.19)	2.99 (±0.22)	2.97 (±0.00)	2.48 (±0.00)	3.19 (±0.40)	2.93 (±0.16)	3.64 (±0.18)

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395 Table 8: Mean log CFU/g *V. parahaemolyticus* abundances with standard error across for all treatments and trials.

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	Trials	I			II			III		
	Treatment	Submersed	Air	Freshwater	Submersed	Air	Freshwater	Submersed	Air	Freshwater
Day since resubmersion	0	4.31 (±0.07)	5.27 (±0.48)	5.66 (±0.00)	4.44 (±0.29)	4.79 (±0.08)	6.04 (±0.00)	2.94 (±0.73)	4.41 (±0.97)	4.99 (±0.22)
	1	3.54 (±0.28)	4.88 (±0.28)	4.83 (±0.51)	3.37 (±0.30)	4.55 (±0.56)	5.09 (±0.30)	2.7 (±0.26)	2.87 (±0.93)	3.52 (±1.46)
	2	4.64 (±0.33)	2.63 (±1.11)	4.46 (±0.14)	3.88 (±0.39)	3.66 (±0.29)	4.4 (±0.32)	2.97 (±0.38)	3.19 (±0.29)	4.04 (±0.07)
	3	4.52 (±0.18)	3.77 (±0.06)	4.26 (±0.20)	3.58 (±0.06)	3.5 (±0.24)	2.58 (±0.92)	2.75 (±0.11)	2.45 (±0.09)	2.7 (±0.37)
	7	4.6 (±0.19)	3.86 (±0.11)	3.46 (±0.08)	3.59 (±0.23)	3.19 (±0.40)	2.89 (±0.16)	3.77 (±0.20)	3.06 (±0.28)	2.32 (±0.19)
	10	4.11 (±0.24)	3.52 (±0.29)	3.22 (±0.30)	3.68 (±0.36)	1.8 (±0.17)	2.12 (±0.26)	2.46 (±1.02)	3.86 (±1.06)	2.9 (±0.29)
	14	4.71 (±0.08)	4.4 (±0.13)	3.66 (±0.17)	3.46 (±0.35)	2.18 (±0.00)	1.79 (±0.00)	3.5 (±0.24)	3.07 (±0.15)	2.9 (±0.29)

398 Table 9: Mean log CFU/g *V. vulnificus* abundances with standard error across for all treatments and trials.

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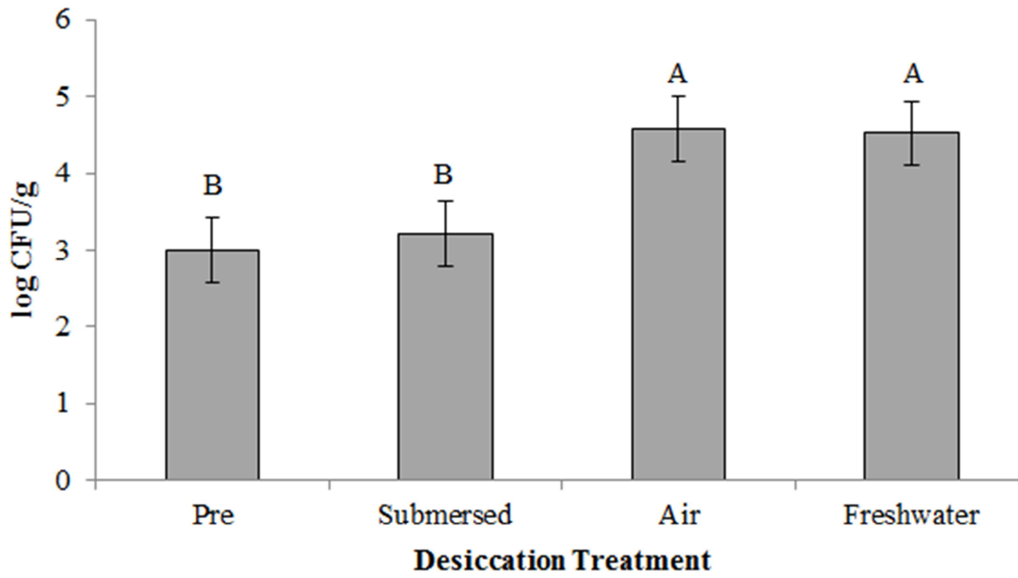

403

404 Figure 1: Mean *V. parahaemolyticus* abundances with standard error bars across all trials of
405 treatment; $T_{pre\text{-}treatment}$ (Pre) and $T_{post\text{-}treatment:}$ submersed, air dried (Air), and freshwater dipped
406 (Freshwater). Different letters indicate significant differences as determined by the post hoc
407 student t-test.

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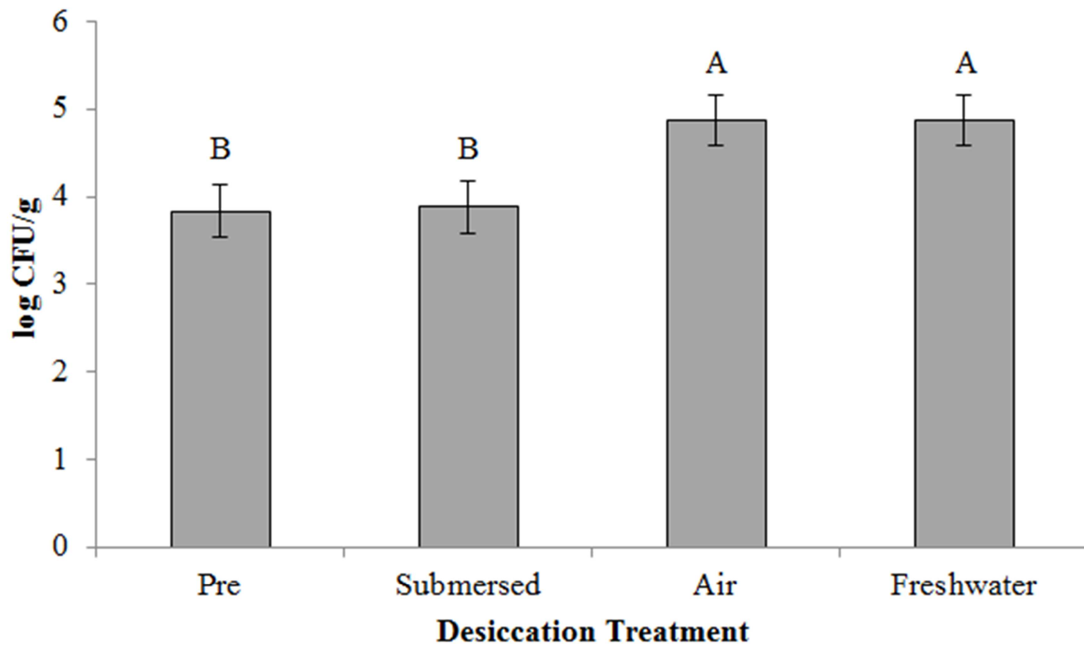

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411 Figure 2: Mean *V. vulnificus* abundances with standard error bars across all trials of $T_{pre\text{-}treatment}$
412 (Pre) and $T_{post\text{-}treatment;}$ submersed (Sub), air dried (Air), and freshwater dipped (Freshwater).
413 Different letters indicate significant differences as determined by the post hoc student t-test.

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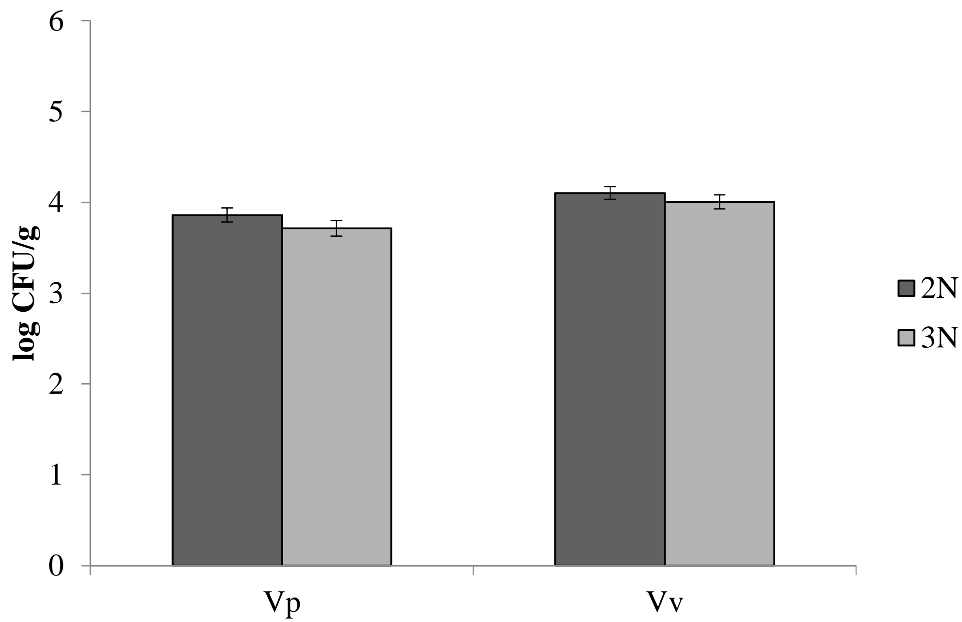

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417 Figure 3: Comparison of mean log transformed CFU/g of *V. parahaemolyticus* (Vp) and *V.*
418 *vulnificus* (Vv) by ploidy standard error bars (combined across treatments and time). The key
419 describes ploidy: diploids (2N) and triploids (3N). No significant differences were found
420 between ploidy.

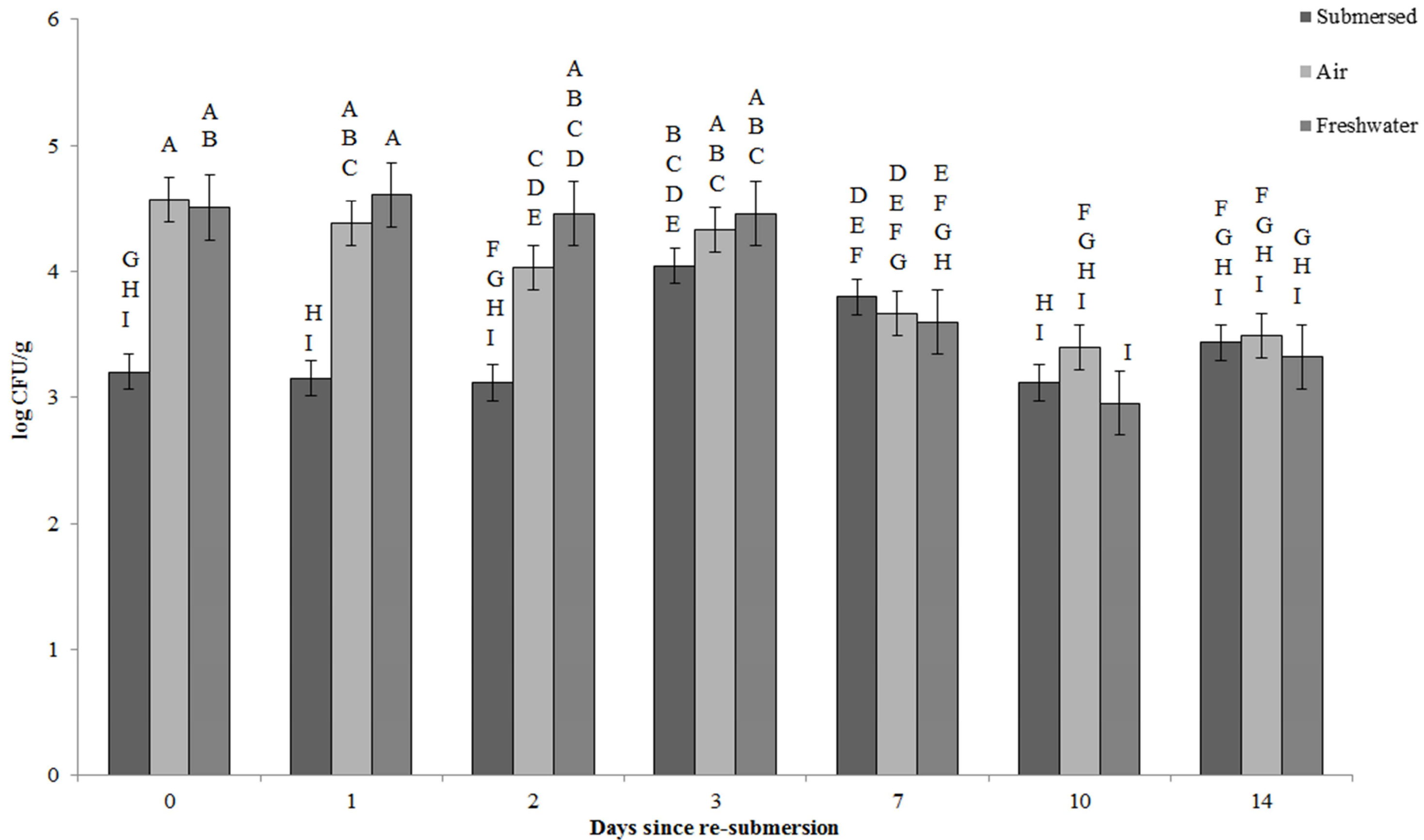

Figure 4: Mean log transformed *V. parahaemolyticus* (y-axis) with standard error bars. With a key describing desiccation treatment; submersed (Sub), 27 hour desiccation (Air), and 3 hour freshwater dip with 24 hour desiccation (Freshwater). Days since re-submersion are shown on the x-axis, where day 0 is immediately prior to re-submersion ($T_{post\text{-}treatment}$). Letters located above bars are representative of significant differences as determined by post-hoc student t-test results.

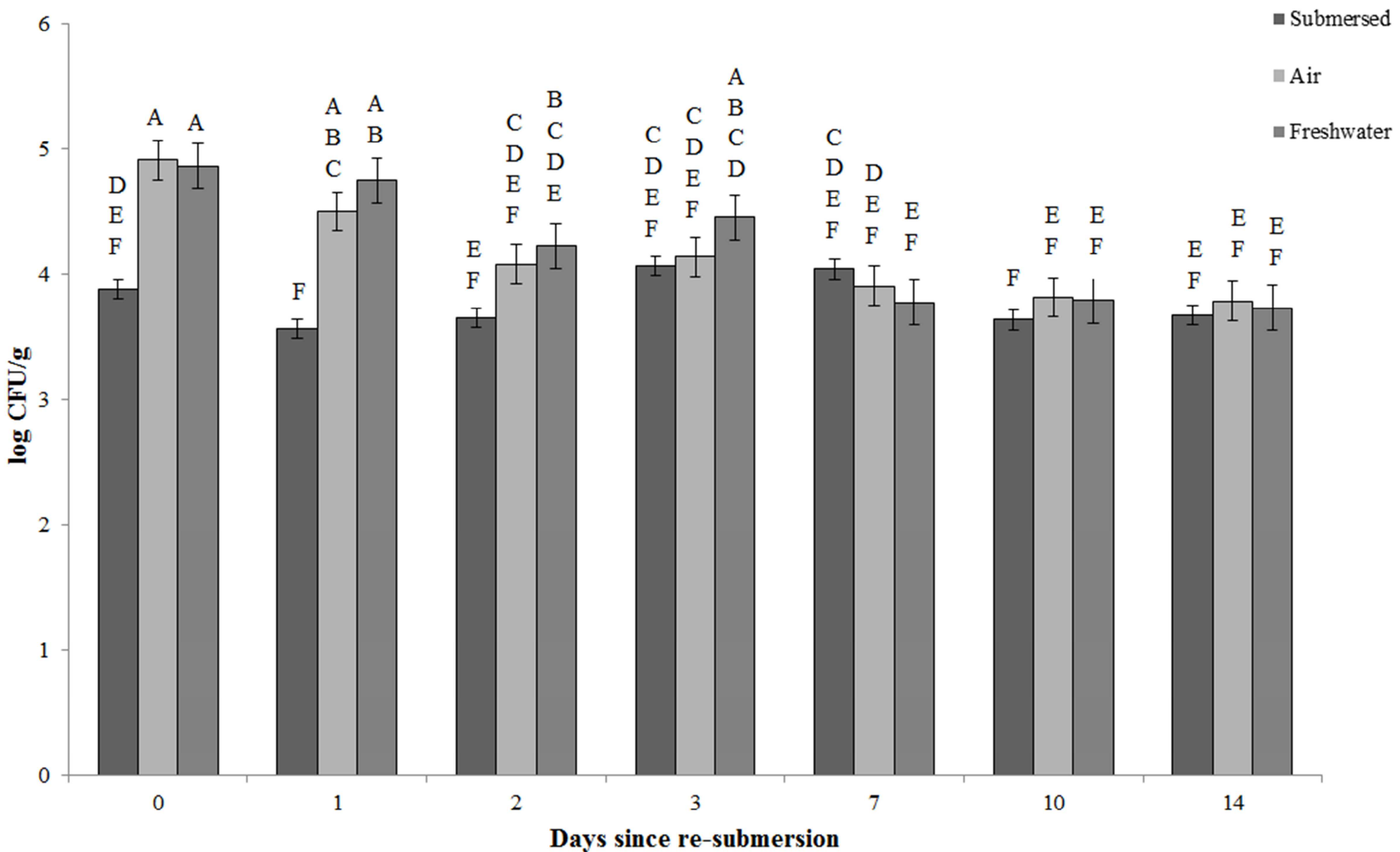

Figure 5: Mean log transformed *V. vulnificus* (y-axis) with standard error bars. With a key describing desiccation treatment; submersed (Sub), 27 hour desiccation (Air), and 3 hour freshwater dip with 24 hour desiccation (Freshwater). Days since re-submersion are shown on the x-axis, where day 0 is immediately prior to re-submersion($T_{post\text{-}treatment}$). Letters located above bars are representative of significant differences as determined by post-hoc student t-test results.

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