Flow rate of depuration system has minimal impact on *Vibrio parahaemolyticus* decontamination in Pacific oysters (*Crassostrea gigas*)

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**Abstract**

*Vibrio parahaemolyticus* infections in the United States have been linked to consumption of raw oysters. Depuration has the potential to reduce contamination in live oysters after harvest. This study investigated the impact of depuration flow rate to reduce *V. parahaemolyticus* in raw oysters. Pacific oysters (*n* = 35 per trial) were inoculated with a cocktail of *V. parahaemolyticus* (10290, 10292, 10293, BE 98-2029, and 027-1c1) in freshly prepared artificial seawater (70 L). The inoculated oysters were depurated with flow rates of 15, 20, 25, and 35 L/min at 12.5 °C for up to 5 days and *V. parahaemolyticus* contamination was determined using a three-tube most probable number (MPN) method. *V. parahaemolyticus* reductions were as flow rate moderately increased from 15 L/min (2.39 log MPN/g reduction in 5 days) to 35 L/min (3.39 log MPN/g reduction). These results suggest that depuration efficacy can be enhanced by increasing depuration flow rate to 35 L/min.

**Practical applications**

*Vibrio parahaemolyticus* can contaminate raw shellfish, including oysters, during their production and lead to outbreaks of foodborne illness. Depuration, a post-harvest process, may be used by the shellfish industry to reduce the persistence of *V. parahaemolyticus*. Previous studies have demonstrated that the depuration process can reduce *V. parahaemolyticus* in oysters; however, further optimization of the process is necessary to achieve US Food Drug Administration's targeted reduction goal (>3.52 log MPN/g). This study evaluated the impact of depuration flow rate on the reduction of *V. parahaemolyticus* in Pacific oysters. Increasing flow rates (15–35 L/min) during depuration enhanced the clearance of *V. parahaemolyticus* in these oysters; however, these conditions were unable to consistently achieve the target of >3.52 log MPN/g reduction. This study provides a reference for the industry on the variability of *V. parahaemolyticus* in individual oysters and demonstrates that practical modifications (i.e., flow rate) can be implemented in depuration systems to maximize bacterial clearance.

**KEYWORDS**

depuration, flow rate, Pacific oyster, shellfish, *vibrio parahaemolyticus*

**INTRODUCTION**

*Vibrio parahaemolyticus* is frequently isolated from raw seafood, particularly oysters, and is the species most frequently associated with foodborne Vibrio-associated gastroenteritis in the United States (Bubb, 1975; DePaola, Hopkins, Peeler, Wentz, & McPhearson, 1990). Symptoms of *V. parahaemolyticus* infection (headache, abdominal pain, nausea, diarrhea, vomiting) usually occur within 24 hr of consumption, but symptom onset may range from 4 to 96 hr (Centers for Disease Control Prevention, 2013). Most *V. parahaemolyticus* infections in the...
United States are associated with shellfish harvested from either the Gulf of Mexico or the Pacific Ocean (Cook et al., 2002). The largest outbreak of *V. parahaemolyticus* in the United States occurred in 1998 with 416 persons in 13 states suffering gastroenteritis after eating oysters harvested from Galveston Bay, Texas (Daniels et al., 2000). A 2013 *V. parahaemolyticus* outbreak involving 104 individuals along the U.S. Atlantic Coast was traced to shellfish consumption (Newton et al., 2014).

Previous research has demonstrated the ability of *V. parahaemolyticus* to grow (up to 3 log cfu/g increase in 24 hr) in oysters during warm months (Gooch, DePaola, Kaysner, & Marshall, 2001). To reduce the likelihood of infections of *Vibrio* spp. associated with raw shellfish consumption, the U.S. National Shellfish Sanitation Program (NSSP) limits the maximum time from harvest to refrigeration (≤10°C). Maximum allowable cooling time differs by region and is based on average maximum air temperature (US Food Drug Administration, 2015). While this approach will minimize the growth of *V. parahaemolyticus* in harvested oysters, it does not eliminate the risk of illness. Post-harvest processes, including thermal processing, cold storage, irradiation, and high pressure, have been demonstrated to reduce *V. parahaemolyticus* in oysters; however, these processes have a negative impact on oyster viability and product quality (Dionisio, Gomes, & Oetterer, 2009; Su, 2012). US Food Drug Administration (FDA) defines effective post-harvest processes as those that reduce levels of *Vibrio* spp. from an initial most probable number (MPN) level of 100,000/g to <30/g (3.52 log MPN/g reduction; US Food Drug Administration, 2015).

Oysters are filter-feeding animals, which are capable of filtering large volumes (13 L/h) of seawater in relatively short periods of time (6 hr; Loosanoff, 1958). Filter-feeding also enables oysters to release contaminants like bacteria (Chae, Cheney, & Su, 2009; Sunnotel et al., 2007), viruses (Sobsey, Davis, & Rullman, 1987), and marine toxins (Cunningham & Tripp, 1973) into the bulk water. To take advantage of this biological process, depuration, a controlled process of holding oysters in a recirculating, sterilized seawater system, has been proposed as an option to reduce *V. parahaemolyticus* contamination of raw oysters (Bloginowski & Stewart, 1983). Salmonella, *Escherichia coli*, and *Pseudomonas* spp. have been shown to be easily reduced in oysters depurated at ambient temperature; however, *Vibrio* spp. persist in the oysters for longer periods of time (Son & Fleet, 1980; Vasconcelos & Lee, 1972). Depuration at reduced temperatures (7–15°C, 5 days) has been shown to reduce *V. parahaemolyticus* in oysters (Phuvasate, Chen, & Su, 2012); however, further optimization is necessary to achieve the FDA target of a 3.52 log cfu/g reduction.

Flow rates for depuration systems must be operated at minimal 107 L per minute per cubic meter of shellfish to achieve oxygen levels necessary maintaining oyster viability following harvest (US Food Drug Administration, 2015). Previous research has demonstrated that increasing the flow rate of seawater has a positive effect on oyster filtering rate (Wilson-Ormond, Powell, & Ray, 1997). Lewis (2010) demonstrated that increasing the flow rate (68 L/min) of the depuration system led to increased reductions (up to 4.56 log cfu/g) of *Vibrio vulnificus* in Eastern oysters. The objective of this study was to determine the influence of depuration flow rate on the reduction of *V. parahaemolyticus* in the Pacific oysters.

## 2 MATERIALS AND METHODS

### 2.1 *V. parahaemolyticus* culture preparation

Five clinical strains of *V. parahaemolyticus* 10290 (serotype O4 : K12, tdh+, trh+), 10292 (serotype O6 : K18, tdh+, trh+), 10293 (serotype O1 : K56, tdh+, trh+), BE 98-2029 (serotype O3 : K6, tdh+, trh–), 1C1-O27 (serotype O5 : K15, tdh+, trh–) were previously obtained from the FDA Pacific Regional Laboratory Northwest (Bothell, WA). Strains were individually grown in 10 mL tryptic soy broth (Difco; Becton Dickinson, Sparks, MD) supplemented with 1.5% NaCl (TSB-Salt) at 35–37°C for 16–18 hr. Each enriched culture was streaked onto a thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Difco; Becton Dickinson) plate and incubated at 37°C for 18–24 hr. One single colony from each TCBS plate was transferred into 10 mL TSB-Salt and incubated at 35–37°C for 4 hr. Five enriched cultures of *V. parahaemolyticus* were then pooled into a 50-mL sterile centrifuge tube and harvested by centrifugation at 3,000 × g at 5°C for 15 min. Pelleted cells were resuspended in 2% salt solution to produce a culture suspension of 8–9 log cfu/mL.

### 2.2 Oyster inoculation with *V. parahaemolyticus*

Freshly harvested raw Pacific oysters (small size; ≥30 g/oyster) were collected from Washington oyster farm (Chetlo Harbor Shellfish, Ilwaco, WA) and transported on ice to the Seafood Laboratory (Astoria, OR). Artificial seawater (ASW) was prepared by dissolving Instant Ocean salt (Aquarium systems, Inc., Mentor, OH) in deionized water to achieve a salinity of 30 ppt (parts per thousand). Oysters were washed with tap water to remove mud and then placed in a high-density polyethylene (HDPE) tank (45 × 30 × 30 cm; Nalgene, Rochester, NY) containing 20 L ASW. Oysters were held under ambient conditions (≥20°C) with air continuously pumped into the tank overnight to keep oxygen level favorable to maintain biological activities of oysters. For inoculation, oysters (*n* = 35) were then submersed in ASW (20 L) containing the *V. parahaemolyticus* cocktail at a cell density of ≥5 log cfu/mL and held overnight as described above (Su, Yang, & Hase, 2010).

### 2.3 Depuration treatment

Oysters were depurated in a laboratory-scale system composed of a 15 W Gamma UV sterilizer (Current-USA, Inc., Vista, CA), a water chiller (Delta star; Aqua Logic, Inc., San Diego, CA), temperature controller (set at 12.5°C; Ranco ETC, Delphos, OH), and a recirculating pump (Pan World Co., Ltd., Jyousou-shi, Japan). Depuration process was conducted with 35 oysters in 70 L of ASW (oyster : water = 1:2) using flow rates of 15, 20, 25, or 35 L/min for a maximum of 5 days.

### 2.4 Microbiological analysis

Concentrations of *V. parahaemolyticus* in oysters were determined using the three-tube MPN method (Kaysner & DePaola, 2004). Oysters (*n* = 5–6/time point) were randomly selected for analysis at each time point (i.e., days 0, 1, 3, 5). Individual oysters were transferred from the depuration tank, shocked with a sterile shucking knife, and the oyster meat was aseptically transferred to a sterile blender jar.
Oyster meat was homogenized with an equal volume of sterile alkaline phosphate buffer saline (PBS; pH 7.4) for 1 min at high speed by a laboratory blender (Waring Laboratory, Torrington, CT). Twenty grams of homogenized oyster sample (1:2 dilution) was mixed with 80 mL of PBS to prepare 1:10 dilution sample suspension. Additional 10-fold dilutions of the sample suspension were prepared using PBS. Sample dilutions were individually inoculated into three tubes of alkaline peptone water (APW; pH 8.5; Difco; Becton Dickinson). APW tubes were incubated at 37 °C for 16–18 hr. A 3-mm loopful from the top 1 cm of each turbid APW tube was streaked onto individual thiosulfate-citrate-bile salt-sucrose agar (TCBS) plates and incubated at 35–37 °C for 18–24 hr. Round, green or bluish colonies with 2–3 mm diameter on a TCBS plate after incubation was considered positive for V. parahaemolyticus.

### 2.5 Statistical analysis

Microbiological population levels were log-transformed and statistically analyzed using mixed model analysis of variance (ANOVA), and multiple-comparison of Student’s t-test using JMP (SAS Institute, Inc., Cary, NC). Significant differences between reductions of V. parahaemolyticus at various times and flow rates during depuration were established at p < .05. Linear fit was used to describe the rate of reduction over time.

### 3 RESULTS AND DISCUSSION

Survivors of V. parahaemolyticus over time at 15 L/min is reported in Figure 1. Depuration at 15 L/min resulted in reduction of the average V. parahaemolyticus population from 4.93 to 2.54 log MPN/g in 5 days. There was a large variability in the results during the first 3 days of depuration, with day 3 exhibiting the widest range from 2.46 to 4.38 log MPN/g (standard error = 0.33). This range is likely due to differences in filtering rates and activity of individual oysters. Linear estimates of survivors indicated a reduction rate of 0.41 log MPN/g/day of V. parahaemolyticus in oysters using the 15 L/min flow rate ($R^2 = .67$).

Calculated log-reductions and rates of reduction for V. parahaemolyticus at four different flow rates are shown in Table 1. The 15 L/min flow rate resulted in the lowest reduction of V. parahaemolyticus (2.39 log MPN/g). Increasing the depuration flow rate to 20 or 25 L/min for 5 days enhanced the reduction of V. parahaemolyticus to 2.68 and 2.80 log MPN/g, respectively (Table 1). Further increase in flow rate to 35 L/min resulted in an average reduction of 3.39 log MPN/g of V. parahaemolyticus in oysters after 5 days. As the flow rate increased from 15 to 35 L/min, the rate of V. parahaemolyticus reduction significantly increased from 0.42 to 0.64 log reduction/day (p < .05). However, none of these depuration treatments achieved FDA’s targeted 3.52 (MPN/g) log reduction of V. parahaemolyticus for post-harvest shellfish processing (US Food Drug Administration, 2015). This agrees with our previous study which reported that depuration at 12.5 °C in 25 L/min did not achieve >3.52 log MPN/g reduction (3.33 log MPN/g; Phuvasate et al., 2012). Multiple depuration studies have demonstrated significant oyster-to-oyster variability, which makes validation of the efficacy of a biologically driven process especially challenging.

Based on mixed model ANOVA, the difference of mean survivors of V. parahaemolyticus is most significantly impacted by duration of

![FIGURE 1 Survivors (log MPN/g) of V. parahaemolyticus in laboratory-contaminated individual oysters during depuration with flow rate of 15 L/min for up to 5 days. Markers indicate V. parahaemolyticus levels in individual oysters ($n = 5$ per time point)](image)
TABLE 1  Reduction of V. parahaemolyticus (log MPN/g) after 5 days of depuration and rate of reduction (log MPN/g/day) of V. parahaemolyticus in oysters using various depuration flow rates at 12.5 °C

<table>
<thead>
<tr>
<th>Flow rate (L/min)</th>
<th>Reduction on day 5 (log MPN/g)</th>
<th>Rate of reduction (log MPN/g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (n = 1)</td>
<td>2.39 ± 0.12A</td>
<td>0.42</td>
</tr>
<tr>
<td>20 (n = 1)</td>
<td>2.68 ± 0.11A</td>
<td>0.48</td>
</tr>
<tr>
<td>25 (n = 4)</td>
<td>2.80 ± 0.13A</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>35 (n = 3)</td>
<td>3.39 ± 0.12B</td>
<td>0.64 ± 0.05</td>
</tr>
</tbody>
</table>

Note. *n* Means the number of replicates for each flow rate. Tests with flow rate of 15 and 20 L/min lack replication of tests. MPN = most probable number.

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