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Efficacy of *Vibrio parahaemolyticus* depuration in oysters (*Crassostrea gigas*)

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ABSTRACT

This study investigated the influences of seawater to oyster ratio on depuration for decontaminating *V. parahaemolyticus* in raw oysters with a goal of identifying the proper ratio of oyster to seawater capable of improving the efficacy of the depuration process. The water to oyster ratios tested in this study ranged from 1.0 to 2.5 L of artificial seawater (ASW) per oyster (40 oysters in 40, 60, 80 and 100 L ASW). The depuration efficacy for purging *V. parahaemolyticus* from oysters was highest when we applied a 2:1, followed by 1.5:1, 2.5:1, and 1:1 L of ASW/oyster. Further studies of depuration with 2:1 L of ASW/oyster found that the concentration of *V. parahaemolyticus* in oysters decreased in a nonlinear manner. The depuration curve was fitted to a one phase decay model with a coefficient of determination (R^2) of 0.933. The time for a 3 log reduction was 1.75 days with a 95% confidence interval from 1.65 to 1.85 days, which meets the FDA's requirement of larger than a 3.0 log (MPN/g) reduction as a post-harvest process for *V. parahaemolyticus* control. After 4 days levels in all trials were < 100 MPN/g meeting performance standards established by Japan and Canada. Furthermore, the time for a 3.52 log reduction was 3.17 days with a 95% confidence interval from 2.92 to 3.54 days but it took 5 days to reduce levels to < 30 MPN/g, which satisfies FDA's requirement as a post-harvest control process (> 3.52 log MPN/g reduction) for the purpose of making safety added labeling claims for *V. parahaemolyticus*.

1. Introduction

Vibrio parahaemolyticus naturally inhabits coastal waters (Kaysner and DePaola, 2001) and causes human gastroenteritis associated with seafood consumption (Hara-Kudo et al., 2003; Su and Liu, 2007). The most common symptoms associated with *V. parahaemolyticus* infections are acute gastroenteritis with diarrhea, headache, vomiting, nausea, abdominal cramps, low fever and occasionally bloody diarrhea (CDC, 2016a,b). People with compromised immune systems, especially those with chronic liver disease, have higher risks for septicemia or serious illness from *V. parahaemolyticus* (CDC, 2016a,b).

Numerous outbreaks of *V. parahaemolyticus* infections linked to consumption of raw oysters have been documented in U.S. coastal regions (DePaola et al., 2000; McLaughlin et al., 2005; CDC, 1998, 1999; 2006, 2016). The United States Centers for Disease Control and Prevention (CDC) estimates that *V. parahaemolyticus* causes 45,000 gastrointestinal illnesses each year in the United States (US); mostly from the consumption of raw oysters (Scallan et al., 2011).

The US produces more than 15.6 million kilograms of oysters each year (NOAA, 2017). Most of them are stored in refrigeration temperatures and sold live for raw consumption (Ma and Su, 2011). The threat of *Vibrio* infection following consumption of raw oysters is a major concern for public health and causes substantial economic losses to the shellfish industry. In order to reduce the risk of *V. parahaemolyticus* illness associated with raw oyster consumption, the United States Food and Drug Administration (FDA) initiated a *V. parahaemolyticus* risk assessment to predict exposure and risk of *V. parahaemolyticus* illness from consumption of raw oysters. Stricter post-harvest time-temperature measures were adopted in 2007 by the Interstate Shellfish Sanitation Conference as part of the *Vibrio parahaemolyticus* Control Plan for raw oysters handling after harvest (FDA, 2017). Post-harvest processing (PHP) is another mandated option to reduce the level of *V. parahaemolyticus* in oysters for safer consumption. Several post-harvest processes, such as low-temperature pasteurization (Andrews et al., 2000), low-dose irradiation (Andrews et al., 2003), flash-freezing followed by frozen storage (Liu et al., 2009), and high pressure processing (Ma and Su, 2011), have been developed for inactivating *V. parahaemolyticus* in raw oysters. However, most of them require either a

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significant amount of initial investment or operation costs and are lethal to oysters except the low-dose irradiation process. Cost-effective post-harvest processing for reducing *V. parahaemolyticus* in raw oysters without significant adverse effects remains to be developed.

Control authorities in other countries such as Canada and Japan have taken a different approach for *V. parahaemolyticus* risk management by mandating performance standards. Illness rates in Japan plummeted after authorities adopted a series of control measures including sanitary processing measures, use of ice and establishing a tolerance level of < 100 V. *parahaemolyticus*/g for all seafood products (Hara-Kudo et al., 2012). After a large oyster associated *V. parahaemolyticus* outbreak in Canada in 2014, authorities also implemented a number of control measures including setting a 100 MPN/g performance standards (FAO/WHO, 2016).

Depuration is a process for purging microbial contaminants by holding shellfish in clean seawater under controlled conditions over time (Blogoslawski and Stewart, 1983). Depuration has been used for reducing sewage-associated bacteria, such as coliforms and *E. coli* in shellfish. The efficacy of a depuration process is largely dependent on the biological activities of oysters and the nature of the microorganism. Temperature is the most critical factor affecting the pumping rate. The volume of water pumped by oysters is regarded as a predictor of biological activity of oysters (Loosanoff, 1958). Other environmental factors, including salinity and dissolved oxygen, are also important for shellfish to purge contaminants effectively during depuration. Reports demonstrate that depuration in clean water at room temperature is effective for reducing sewage-associated pathogens but not effective in reducing *V. parahaemolyticus* (Eyles and Davey, 1984). Depuration at refrigerated temperatures (5 °C for 6 days, 7–15 °C for 5 days) has been shown to reduce *V. parahaemolyticus* in Pacific oysters (*Crassostrea gigas*) by > 3.0 log (MPN/g) (Su et al., 2010; Phuvasate et al., 2012). However, none of these depuration processes could achieve 3.52 log reduction of *V. parahaemolyticus* as a post-harvest process required by the NSSP for the purpose of added safety labeling claims (FDA, 2017). Optimal ratios of depuration water to oyster have not been investigated. Thus, the objectives of this study were to determine the optimum ratio of seawater to oysters and the time required under optimum conditions to achieve > 3.52 (log MPN/g) reduction of *V. parahaemolyticus* in the Pacific oysters (*Crassostrea gigas*).

2. Materials and methods

2.1. Preparation of clinical V. parahaemolyticus strains

Five clinical *V. parahaemolyticus* strains (10290, 10292, 10293, BE98-2029, O27-1C1) obtained from the culture collection of the Food and Drug Administration Pacific Regional Laboratory Northwest (Bothell, WA) were used in the present study. Their serotypes and virulence factors were shown in Table 1.

Each strain was individually grown in 10 ml tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD, USA) supplemented with 1.5% NaCl (TSB-Salt) for 16–18 h at 37 °C. Each culture was streaked onto a thiosulfate-citrate-bile salts-sucrose (TCBS; Difco, Becton Dickinson; Sparks, MD, USA) agar plate and incubated for 18–24 h at 37 °C. A single colony from each TCBS plate was picked and incubated in TSB-Salt (10 ml) for 4 h at 37 °C. The TSB-Salt cultures were pooled into a 50-mL sterile centrifuge tube and harvested by centrifugation at 3000 × *g* (Sorvall RC-5B, Kendro Laboratory Products, Newtown, CT, USA) at 5 ± 1 °C for 15 min. Pelleted cells were collected and re-suspended in 50 ml sterile 2% NaCl to obtain a multi-strain cocktail suspension of 10^{8-9} CFU/ml.

Table 1
Characteristics of *Vibrio parahaemolyticus* (*Vp*) strains used in depuration experiments.

Vp Strains	Serotype	Virulence factors		
		tlh	*tdh*	*trh*
10290	O4:K12	+	+	+
10292	O6:K18	+	+	+
10293	O1:K56	+	+	+
BE98-2029	O3:K6	+	+	–
O27-1c1	O5:K15	+	+	–

2.2. Oyster preparation

Freshly harvested Pacific oysters (*Crassostrea gigas*) obtained from Oregon Oyster Farms (Yaquina Bay, Newport, OR, USA) were delivered to the laboratory in a cooler with ice on the day of harvest. The initial *V. parahaemolyticus* levels in oyster samples used for our experiments were non-detectable at harvest.

Oysters were selected with an average length of 8.0 ± 0.7 cm and weight of 29.7 ± 5.2 g and washed with tap water to remove mud on the shell and then placed in a 45 L rectangular high-density polyethylene (HDPE) tank (45 cm × 30 cm × 30 cm) containing aerated artificial seawater (ASW) with a salinity of 30 ppt. The ASW was prepared by dissolving Instant Ocean salt (Systems Inc., Mentor, OH, USA) in deionized water according to manufacturer's instructions. Two drops of marine microalgae concentrate (Shellfish Diet, 1800; Reed Mariculture Inc., Campbell, CA, USA) was added to the ASW by using a disposable pasteur and oysters were held at room temperature (20–22 °C) overnight to resume their biological activities.

2.3. Inoculation of oyster with V. parahaemolyticus

Forty five oysters held in ASW at room temperature (20–22 °C) overnight were transferred to an identical tank of 20 L of fresh ASW (30 ppt) containing a mixture of five *V. parahemolyticus* strains at a level of approximate 10^5 CFU/ml. Accumulation of *V. parahaemolyticus* in oysters was conducted at room temperature overnight (16–18 h) to achieve a target level of contamination in oysters of about 10^{4-5} CFU/g. Air was also pumped into the ASW to keep dissolved-oxygen (DO) levels favorable for oyster pumping and uptake of *V. parahaemolyticus*.

2.4. Depuration process

Inoculated oysters were transferred to a laboratory recirculating depuration system equipped with a 15 W Gamma UV sterilizer (Current-USA Inc., Vista, CA, USA) and a water chiller (Delta star, Aqua Logic, Inc., San Diego, CA, USA). In each trail, different initial volumes of ASW (40, 60, 70, 80 and 100 L) were used for depurating forty oysters inoculated with *V. parahaemolyticus* at 12.5 °C for 5 days, resulting in various water-oyster ratios of 1.0, 1.5, 2.0 or 2.5 L of ASW/oyster. During each sample time, a volume of water was removed to maintain constant water/oyster ratio. The salinity of ASW for depuration was 30 ppt (pH 8.3 with no adjustment) and recirculating flow rate 1500 L/h (25 L/min) to ensure sufficient dissolved oxygen in water.

2.5. Microbiological analysis

Populations of *V. parahemolyticus* in oysters during depuration were determined by the three-tube most-probable-number (MPN) method according to the Food and Drug Administration Bacteriological Analytical Manual (Kaysner and DePaola, 2004). Six oysters were randomly picked from the depuration system every day and shucked with a sterile shucking knife. The oyster meat was homogenized with an equal volume of sterile phosphate buffer saline (PBS; pH 7.4) at high speed for 1 min using a two-speed laboratory blender (Waring Laboratory, Torrington, CT, USA) to prepare 1:2 dilution sample suspensions. Twenty grams of the 1:2 dilution sample suspension was mixed with 80 ml of PBS to make 1:10 sample dilution. Additional 10-fold

dilutions were prepared with sterile PBS. All sample dilutions were individually inoculated into 3 tubes of alkaline peptone water (APW; pH 8.5; Difco, Becton Dickinson, USA). Inoculated APW tubes were incubated at 35–37 °C overnight. A 3-mm loopful from the top 1 cm of each turbid APW tube was streaked onto individual thiosulfate-citrate-bile saltsucrose agar (TCBS) plates and incubated at 35–37 °C for 18–24 h. Formation of round, green or bluish colonies with 2–3 mm diameter on a TCBS plate after incubation was considered positive for *V. parahaemolyticus*. Total *V. parahemolyticus* levels in oysters were determined according to the 3-tube MPN table. The efficacy of the UV sterilizer in inactivating *V. parahaemolyticus* cells released from oysters into re-circulating ASW was verified daily by plating the ASW on TCBS plates followed by incubation at 37 °C for 24 h.

2.6. Depuration curve generation

Additional seven trials were conducted under the best oyster stocking density identified (water/oyster ratio of 2:1) to generate a depuration curve. The concentration (log MPN/g) of *V. parahaemolyticus* in the oysters at each sampling, Y(*t*), was adjusted for the initial concentration (Y_i) of *V. parahaemolyticus* to obtain the log change (Y_Δ) as follows:

$$Y_\Delta = Y_i - Y(t)$$

The log change for 42 samples (7 trials x 6 replicate oyster samples per sampling time) per sampling time were graphed as a function of time (0, 1, 2, 3, 4, 5 days) and were then fitted to a one phase decay model:

$$Y_\Delta = (Y_i \text{ - Plateau})*\exp(-K*t) + \text{Plateau}$$

where Plateau was the minimum concentration of *V. parahaemolyticus* obtained and K (d^{-1}) was the rate constant. The depuration curve was fitted using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA). The values for Y_i (0 log MPN/g) and Plateau (−3.70 log MPN/g) were fixed during curve fitting. The interpolation function of Prism was used to obtain the times (± 95% confidence intervals) for a 3 log or 3.52 log reduction of *V. parahaemolyticus* during depuration.

2.7. Statistical analysis

Results of microbiological tests were converted to log values and analyzed with One-Way ANOVA and Tukey-Kramer multiple-comparison Test using the R program (R foundation, Vienna, USA). Significant differences among means of each treatment over time were established at a level of $P < 0.05$.

3. Results

3.1. Effects of water to oyster ratios on V. parahaemolyticus populations in oysters during depuration

Changes of *V. parahaemolyticus* populations in oysters during five days of depuration processes are reported in Fig. 1. Among the different ratios of oyster to water tested, the smallest reduction of *V. parahaemolyticus* (2.45 log MPN/g) in Pacific oysters after 5 days of process was observed from depuration in 40 L of ASW (1:1 L of ASW/oyster). Increasing the water volume to 60 L for depuration (1.5:1 L of ASW/oyster) resulted in a greater reduction of 3.54 and 3.74 log (MPN/g) of *V. parahaemolyticus* in oysters after 4 and 5 days, respectively. Further increasing the water volume to 80 L (2:1 L of ASW/oyster) for depuration yielded 3.60 and 3.91 log (MPN/g) reductions of *V. parahaemolyticus* in oysters after 4 and 5 days of process, respectively (Fig. 1). The depuration efficacy for purging *V. parahaemolyticus* from oysters was highest when the water to oyster ratio was 2:1, followed by 1.5:1, 2.5:1 and 1:1. It was believed that depuration at 12.5 °C with a water to oyster ratio of either 1.5:1 or 2:1 could deliver > 3.52 reductions of *V. parahaemolyticus* in oysters after 5 days of process.

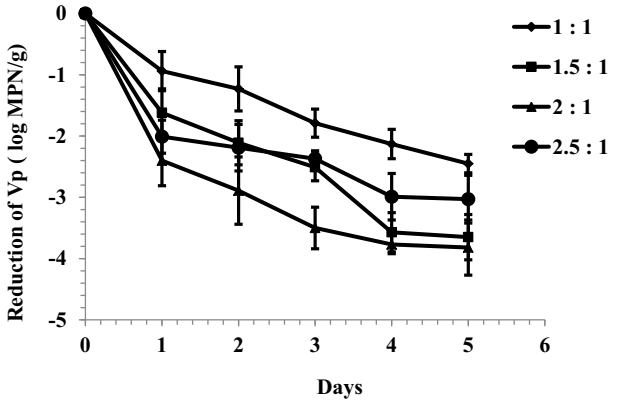

Fig. 1. Effects of artificial seawater (ASW) to oyster ratios on *Vibrio parahaemolyticus* survival during depuration at 12.5 °C. Values were reported as means of six determinations ± standard deviation.
Legend "1:1" represents "40 L of ASW: 40 oyster"; Legend "1.5:1" represents "60 L of ASW: 40 oyster"; Legend "2:1" represents "80 L of ASW: 40 oyster"; Legend "2.5:1" represents "100 L of ASW: 40 oyster".

3.2. Comparison of depuration efficacy for purging V. parahaemolyticus at water to oyster ratios of 1.5:1 and 2:1 L of ASW/oyster

Triplicate studies were performed at 12.5 °C to compare the efficacy of *V. parahaemolyticus* purging from oysters during depuration at water to oyster ratios of 1.5:1 and 2:1. Depuration of oysters in 60 L (1.5:1 L of ASW/oyster) yielded 3.44 and 3.38 log MPN/g reductions of *V. parahaemolyticus* in oysters after 4 days and 3.58 to 3.64 log (MPN/g) after 5 days (Table 2). Greater reductions of *V. parahaemolyticus* in oysters of 3.68–3.88 log (MPN/g) were achieved after 4 days of depuration in 80 L of ASW (2:1 L of ASW/oyster).

3.3. Confirmation of the efficacy of depuration at 2:1 L of ASW/oyster

Further studies were conducted to verify the efficacy of depuration as a post-harvest process to achieve at least 3.0 and 3.52 log (MPN/g) reduction of *V. parahaemolyticus* by depurating oysters in 70 L or 80 L of ASW at 12.5 °C with a water to oyster ratio of 2:1. The survival of *V. parahaemolyticus* in each oyster was determined daily up to five days. Results confirmed that all the oysters achieved greater than the mandated 3 and 3.52 log (MPN/g) after 2 and 4 days depuration process, respectively (Table 3).

3.4. Dynamics of purging V. parahaemolyticus from oysters during depuration at 2:1 L of ASW/oyster

Seven additional depuration trials were performed at 2:1 L of ASW/oyster and indicated that the concentration of *V. parahaemolyticus* in oysters decreased in a nonlinear manner (Fig. 2). The depuration curve was fitted to a one phase decay model with a coefficient of determination (R^2) of 0.933. The rate of *V. parahaemolyticus* removal from oysters during depuration was 0.95 ± 0.04 d^{-1} (mean ± SEM) with a 95% confidence interval from 0.86 to 1.03 d^{-1}. The total log reduction of *V. parahaemolyticus* during the 5 days of depuration was 3.70 ± 0.04 log (MPN/g) with a 95% confidence interval from 3.62 to 3.79 log (MPN/g). The time for a 3 log reduction of *V. parahaemolyticus* was 1.75 days with a 95% confidence interval from 1.65 to 1.85 days. A 3.52 log reduction of *V. parahaemolyticus* was achieved in 3.17 days with a 95% confidence interval from 2.92 to 3.54 days. Levels of < 100 and 30 MPN/g were achieved after 4 and 5 days, respectively.

Table 2
Vibrio parahaemolyticus levels during depuration with 1.5:1 or 2:1 artificial seawater (ASW) to oyster ratios.

Days	1.5:1 (60 L ASW/40 oysters)			2:1 (80 L ASW/40 oysters)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	5.17 ± 0.23A[a]	5.32 ± 0.18A	5.06 ± 0.28A	5.22 ± 0.41A[a]	5.23 ± 0.47A	5.15 ± 0.37A
1	3.40 ± 0.26B(1.78[b])	3.42 ± 0.19B(1.90)	3.45 ± 0.23B (1.62)	2.39 ± 0.29B(2.83[b])	2.86 ± 0.21B(2.37)	2.75 ± 0.31B (2.40)
2	2.66 ± 0.37C(2.51)	2.64 ± 0.35C(2.68)	2.95 ± 0.43C (2.11)	1.99 ± 0.16B(3.23)	2.36 ± 0.26C(2.87)	2.25 ± 0.49B (2.89)
3	2.12 ± 0.14C(3.05)	2.21 ± 0.31C(3.12)	2.55 ± 0.44C (2.51)	1.77 ± 0.32C(3.45)	1.66 ± 0.35D(3.57)	1.65 ± 0.36BC (3.50)
4	1.73 ± 0.23CD(3.44)	1.95 ± 0.11C(3.38)	1.49 ± 0.34D (3.57)	1.54 ± 0.19D(3.68)	1.35 ± 0.22D(3.88)	1.38 ± 0.39C (3.77)
5	1.59 ± 0.20D(3.58)	1.68 ± 0.28D(3.64)	1.41 ± 0.25D (3.65)	1.28 ± 0.21E(3.94)	1.30 ± 0.29D(3.93)	1.33 ± 0.40C (3.82)

[a] Values were reported as means of six determinations ± standard deviation. Data with different letters in the same column are significantly different ($P < 0.05$).
[b] Reduction of *V. parahaemolyticus* (log MPN/g).

The log reduction achieved at a specific time can be calculated using the one phase decay model and fitted parameters. For example, the log reduction at 2 days would be:

$$= (0 \text{ to } -3.70 \text{ log/g}) * \exp(-0.95*2) + -3.70 = -3.15 \text{ log/g}$$

4. Discussion

The current study investigated the influences of seawater to oyster ratio on depuration for purging *V. parahaemolyticus* in raw oysters. The water to oyster ratios tested in this study ranged from 1.0 to 2.50 L of ASW per oyster (40 oysters in 40, 60, 80 and 100 L ASW). When the water to oyster ratios ranged from 1.0 to 2.0 L of ASW per oyster, the effectiveness of depuration was positively related to the water to oyster ratio. However, the effectiveness decreased when the water to oyster ratio reached 2.5:1 compared with the 2:1 ratio. Our hypothesis is that too much water might reduce the dissolved oxygen in the system that negatively affected oysters' biological activities including the pumping rate.

The temperature (12.5 °C) and salinity of ASW (30 ppt) for depuration process in this study were selected based on previous studies (Chae et al., 2009; Su et al., 2010; Phuvasate et al., 2012; Phuvasate and Su, 2013). The results showed that the effectiveness of the depuration process for purging *V. parahaemolyticus* from oysters was greatly affected by the ratio of water to oyster. A ratio of 2 L of ASW per oyster was optimal for purging of *V. parahaemolyticus* during depuration and previously optimized conditions of temperature (12.5 °C) and salinity (30 ppt). NSSP mandated reduction of 3.0 logs for PHP was reliably achieved in two days. While the NSSP mandate of a 3.52 logs for PHP with an added safety labeling claim was achieved in four days, the additional requirement to reduce levels below 30 MPN/g was achieved after five days. The < 100/g performance standards mandated by Japan and Canada were achieved in all but one trial within three days and always in four days. For a labeling claim purpose, post-harvest processing needs to reduce *V. parahaemolyticus* level in oysters to non-detectable (< 30 MPN/g) and achieve a minimum 3.52 log reductions (FDA, 2017). Below 100 MPN/g is an international performance standard for *V. parahaemolyticus* control currently applied in other countries, such as Canada and Japan (FAO/WHO, 2016).

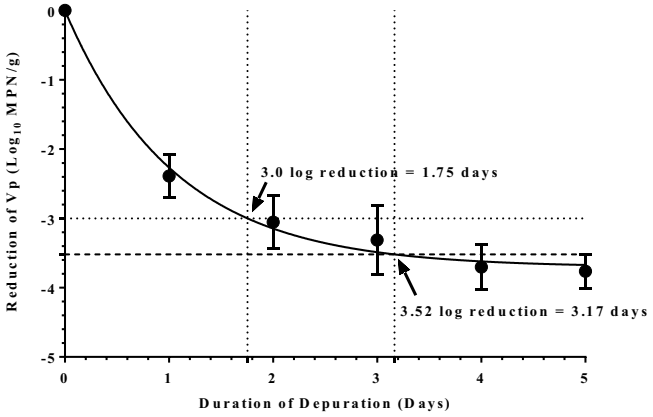

Fig. 2. Log change (log MPN/g) of *Vibrio parahaemolyticus* (Vp) in oysters during depuration at 12.5 °C at 2:1 of water/oyster ratio. Values are means ± standard deviations of MPN of 42 determinations in 7 trials).

Depuration has a long history as a postharvest treatment for reducing sewage-associated bacterial contaminants (coliforms, *Esherichia coli* and *Salmonella*) in a variety of shellfish species sold alive including oysters, clams, mussels, cockles and scallops. Depuration is widely applied in many countries such as Australia, France, Italy, Spain and the United Kingdom to remove microbiological contaminants originating from sewage (Lee et al., 2008). However, depuration has not yet been practiced in shellfish industry as a postharvest treatment for controlling natural flora of shellfish, such as *V. parahaemolyticus*, because the conventional depuration process at ambient temperatures effective for

Table 3
Confirmation of the efficacy of *Vibrio parahaemolyticus* (Vp) reduction in individual oysters depurated with artificial seawater (ASW) at 2:1 of water/oyster ratio.

ASW/oyster ratio	Vp Level (log MPN/g)						Vp Reduction (log MPN/g)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
2:1 (70 L ASW/35 oysters)	5.66	3.32	2.66	2.20	1.81	1.88	0	2.34	3.00	3.46	3.85	3.78
	5.38	3.20	2.32	1.97	1.58	1.81	0	2.18	3.06	3.41	3.80	3.57
	5.38	3.04	2.08	1.97	1.45	1.63	0	2.34	3.30	3.41	3.93	3.75
	5.08	3.04	2.08	1.97	1.43	1.63	0	2.04	3.00	3.11	3.65	3.45
	4.99	2.86	1.88	1.88	1.30	1.46	0	2.13	3.11	3.11	3.69	3.53
	4.88	2.56	1.63	1.80	0.87	1.46	0	2.32	3.25	3.08	4.01	3.42
2:1 (80 L ASW/40 oysters)	5.38	3.32	1.88	1.63	1.63	1.58	0	2.06	3.50	3.75	3.75	3.80
	5.38	3.08	1.81	1.63	1.58	1.46	0	2.30	3.57	3.75	3.80	3.92
	5.32	2.45	1.63	1.56	1.58	1.46	0	2.87	3.69	3.76	3.74	3.86
	5.18	2.32	1.54	1.54	1.56	1.43	0	2.86	3.64	3.64	3.62	3.75
	5.08	2.32	1.43	1.43	0.87	1.32	0	2.76	3.65	3.65	4.21	3.76
	4.88	2.20	1.32	0.57	0.86	1.30	0	2.68	3.56	4.31	4.02	3.58

reducing sewage-associated pathogens has little effect on reducing *V. parahaemolyticus* in shellfish (Vasconcelos and Lee, 1972; Son and Fleet, 1980; Eyles and Davey, 1984; Timoney and Abston, 1984; Ren and Su, 2006).

The maximum average pumping rate of oysters can reach 13 L/h at temperatures of 28–30 °C compared to 5.4 L/h at 12–14 °C (Loosanoff, 1958). However, higher ambient temperatures promote faster *V. parahaemolyticus* growth and higher levels in oyster tissues. The ability of *Vibrio* spp. to colonize shellfish is a major difference from sewage associated bacteria and limits the use of conventional depuration as a means for eliminating *V. parahaemolyticus* (Chae et al., 2009). Therefore, it is very important to identify optimal depuration conditions that retard multiplication of *V. parahaemolyticus* while providing physiological conditions for oysters' filter-feeding activity and purging contaminants.

Several previous studies have helped us understand how operation temperature and salinity affect the efficacy of depuration for purging *V. parahaemolyticus* from oysters (Chae et al., 2009; Su et al., 2010; Phuvasate et al., 2012). These studies confirm that depuration at an ambient water temperature (22 °C) has limited effects on reducing *V. parahaemolyticus*, while decreasing the water temperature to a range from 5 to 15 °C enhances the efficacy of depuration. Furthermore, the efficacy of depuration for purging *V. parahaemolyticus* at 5 °C is similar to that at 7, 10, 12.5 and 15 °C. These results suggest that depuration at temperatures between 5 and 15 °C allows oysters to effectively purge *V. parahaemolyticus* with greater than a 3.0 log reduction after depuration for 5 days. Therefore, effective depuration could be performed at any temperature between 5 and 15 °C. Considering depuration efficacy and energy conservation, 12.5 °C was adopted for further studies thereafter (Phuvasate and Su, 2013; Shen and Su, 2017) and this study.

Depuration times depend on starting concentrations. Harvest levels are generally lower than starting levels (at zero hour of depuration) in the current study but could be higher depending on post-harvest temperature abuse. In the current study, the harvest levels of *V. parahaemolyticus* in sample oysters were undetectable. Prior to depuration, a practical bioaccumulation approach was applied in this study to achieve the target levels of 10,000 MPN/g or greater as indicated in the National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish. The required initial level (≥10,000 MPN/g) can also be achieved through naturally occurring *Vibrio* levels in shellfish or time/temperature abuse (FDA, 2017).

The depuration kinetics of laboratory grown bacteria may differ from resident microflora. However, similarly rapid purge rates have been observed in natural settings. Nordstrom et al. (2004) reported that *V. parahaemolyticus* levels increased in Pacific oysters in Hood Canal Washington during intertidal exposure but returned to background levels within a single overnight tidal cycle. Further studies are needed to validate the efficacy of the depuration process for reducing naturally accumulated *V. parahaemolyticus* in Pacific oysters according to the National Shellfish Sanitation Program (NSSP) Guide recognized by the US FDA.

The current study was conducted in a re-circulating system. But the approach may be employed in other depuration systems (i.e. flow through system) and adapted to commercial depuration practices and other bivalves (i.e. mussels and clams) for *Vibrio* control. Of course, the conditions may need to be verified or adjusted by further studies based on the depuration system, bivalve species, and other factors. In addition, this study was conducted in a lab scale re-circulating system. A further feasibility study by using this commercial size system may be conducted in the near future.

5. Conclusion

The efficacy of *V. parahaemolyticus* depuration in oysters was affected by water to oyster ratio used for the process. Depuration at 12.5 °C with a water to oyster ratio of 2:1 for 2 days achieved > 3.00 log reduction of *V. parahaemolyticus* as mandated for PHP of Pacific oysters. Extending the depurating process to four days reduced levels below 100 MPN/g and would meet the most stringent international performance standards. The NSSP mandate for a labeling claim for PHP of a 3.52 log reduction and levels < 30 MPN/g was achieved after five days.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2018.10.005.

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