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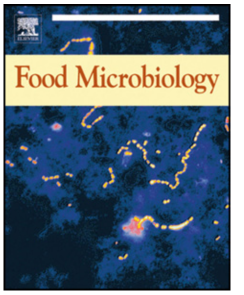

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Reductions of *Vibrio parahaemolyticus* in Pacific Oysters (*Crassostrea gigas*) by Depuration at Various Temperatures

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Running head: Reductions of *Vibrio parahaemolyticus* in oysters by depuration

ABSTRACT

Consumption of raw oysters has been linked to several outbreaks of *Vibrio parahaemolyticus* infection in the United States. This study investigated effects of ice storage and UV-sterilized seawater depuration at various temperatures on reducing *V. parahaemolyticus* in oysters. Raw Pacific oysters (*Crassostrea gigas*) were inoculated with a mixed culture of five clinical strains of *V. parahaemolyticus* (10290, 10292, 10293, BE 98-2029 and 027-1c1) at levels of 10^{4-6} MPN/g. Inoculated oysters were either stored in ice or depurated in recirculating artificial seawater at 2, 3, 7, 10, 12.5, and 15 ºC for 4 to 6 days. Holding oysters in ice or depuration of oysters in recirculating seawater at 2 or 3 °C for 4 days did not result in significant reductions ($P > 0.05$) of *V. parahaemolyticus* in the oysters. However, depuration at temperatures between 7 and 15 ºC reduced *V. parahaemolyticus* populations in oysters by >3.0 log MPN/g after 5 days with no loss of oysters. Depuration at refrigerated temperatures (7-15 ºC) can be applied as a post–harvest treatment for reducing *V. parahaemolyticus* in Pacific oysters.

Keywords: *Vibrio parahaemolyticus*, Pacific oyster, Depuration, Shellfish, Seafood safety

Highlights

Changes in levels of *Vibrio parahaemolyticus* in oysters during depuration at 2, 3, 7, 10, 12.5, and 15 ºC were studied.

Depuration of oysters at 2 or 3 ºC had no effect on reducing *V. parahaemolyticus*.

Depuration at 7-15 ºC for 5 days reduced *V. parahaemolyticus* populations in oysters by >3.0 log MPN/g.

1. Introduction

Vibrio parahaemolyticus is a foodborne pathogen that occurs naturally in the marine environments (Huq and Colwell, 1995; Su and Liu, 2007) and frequently isolated from seafood throughout the world (Hervio-Heath et al., 2002; Sujeewa et al., 2009; Costa Sobrinho et al., 2010, 2011). This pathogen is recognized as the leading cause of gastroenteritis associated with seafood consumption in the United States (Daniels et al., 2000). It is estimated that 4,500 cases of *V. parahaemolyticus* infections occur each year in the U.S. with 62% of them (2,800 cases) being associated with consumption of raw oysters (FDA, 2005). A recent *V. parahaemolyticus* outbreak in 2006 affecting 177 residents of three states, New York, Oregon and Washington, was traced to oysters harvested from Washington and British Columbia, Canada and sold nationwide (CDC, 2006).

The occurrence of *V. parahaemolyticus* in the environments and oysters is known to correlate to water temperature with the bacterium often being detected during summer months when the water temperature exceeds 15 ºC (Kaneko and Colwell, 1973; Kelly and Stroh, 1988; Duan and Su, 2005; Ristori et al., 2007; Costa Sobrinho et al., 2010). To reduce the risks of infections caused by *Vibrio* spp. associated with shellfish consumption, the U.S. National Shellfish Sanitation Program (FDA, 2009) has established time/temperature regulations that limit maximum hours of holding shellfish from harvest to refrigeration (≤10 ºC) depending on average maximum air temperature upon harvest. Hood et al. (1983) observed that levels of naturally contaminated *V. parahaemolyticus* in shellstock oysters decreased slightly when the oysters were stored at 2 and 8 ºC. A small reduction (0.39 log MPN/g) of naturally occurring *Vibrio vulnificus* was reported in shellstock oysters after being held at 4 ºC for 14 days (Cook and Ruple, 1992).

Although growth of *Vibrio* spp. in shellfish can be inhibited at refrigeration temperatures, the bacteria can multiply rapidly in shellfish once exposed to elevated temperatures (>25 ºC). Studies have shown that populations of *V. parahaemolyticus* in unrefrigerated oysters increased rapidly to 50 to 790 folds of its original level within 24 h of harvest when oysters were exposed to an elevated temperature (Gooch et al., 2002). Therefore, keeping oysters in cold chain alone may not be sufficient to eliminate risks of *Vibrio* infections associated with raw oyster consumption.

Oysters are filter-feeding animals, which can filter large volumes of water (up to 13 L/h) for nutrients (Loosanoff, 1958), and can accumulate microorganisms including human pathogens present in the growing environments. Many attempts have been made to develop processing technologies to reduce number of bacteria including *Vibrio* spp. in oysters for safe consumption. Post-harvest processes, including low-temperature pasteurization (Andrews et al., 2000), flash freezing followed by frozen storage (Liu et al., 2009), high-pressure processing (Kural and Chen, 2008; Kural et al., 2008; Ma and Su, 2011) and irradiation (Andrews et al., 2003), have been developed for inactivating *V. parahaemolyticus* in oysters. However, these processes either require high initial investment or operation costs and oysters are often killed during the processes, except by low-dose irradiation. A simple and cost-effective process for reducing *V. parahaemolyticus* in oysters without significant adverse effects remains to be developed.

Depuration is a controlled purification process by holding shellfish in seawater disinfected by ultraviolet (UV) light, chlorine or ozone (Blogoslawski and Stewart, 1983). The process at ambient temperatures had been reported effective in reducing *Salmonella, Esherichia coli* and coliforms but not for eliminating *Vibrio* spp. in shellfish (Vasconcelos and Lee, 1972; Son and Fleet, 1980; Eyles and Davey, 1984; Timoney and Abston, 1984). However, decreasing temperature for depuration to 15 ºC has been reported capable of reducing *V. parahaemolyticus* and *V. vulnificus* in the Gulf oysters (*Crassostrea virginica*) by 2.1 and 2.9 log MPN/g, respectively, after 48 h (Chae et al., 2009). Recently, we investigated depuration with refrigerated seawater at 5˚C for reducing *V. parahaemolyticus* in the Pacific oysters and reported that the process reduced *V. parahaemolyticus* populations in Pacific oysters by >3.0 log MPN/g after 96-144 h (Su et al., 2010). These results suggest that the efficacy of depuration for reducing *V. parahaemolyticus* in oysters depends on the temperature of operation. The objective of this study was to study temperature effects on depuration for reducing *V. parahaemolyticus* in the Pacific oysters and identify an optimal depuration for post-harvest processing of oysters without any adverse effects.

2. Material and methods

2.1. Bacterial culture preparation

Five clinical strains of *Vibrio parahaemolyticus* (10290, 10292, 10293, BE98-2029 and 027-1c1) obtained from the culture collection of the Food and Drug Administration Pacific Regional Laboratory Northwest (Bothell, WA) were used in this study. Each strain was grown in tryptic soy broth (TSB; Difco, Becton Dickinson, Spark, MD) containing 1.5% NaCl (TSB-Salt) at 37 ºC for 18-24 h. The enriched culture was streaked onto individual plates of tryptic soy agar (TSA; Difco, Becton Dickinson) supplemented with 1.5% NaCl (TSA-Salt) and incubated at 37 ºC for 18-24 h. A single colony formed on a TSA-Salt plate was transferred to TSB-Salt and incubated at 37 ºC for 4 h. Enriched cultures of *V. parahaemolyticus* were pooled into a 50-mL sterile centrifuge tube and harvested by centrifugation at 3000 x g (Sorvall RC-5B, Kendro

Laboratory Products, Newtown, CT) at 5 ºC for 15 min. Pellet cells were re-suspended in 2% salt solution to produce a culture suspension of approximately 10^{8-9} CFU/ml.

2.2. Oyster preparation

Raw Pacific oysters (*Crassostrea gigas*, diploid, 7.8 ± 0.7 cm long and 12-18 months old) were obtained from an oyster farm in the Yaquina Bay in Newport of Oregon between March and May (water temperature ranged from 9 to 14 ºC) and between September and January (water temperature ranged from 7 to 15 ºC) to avoid using oysters containing naturally accumulated *V. parahaemolyticus* in the studies. The oysters were briefly washed with tap water and placed in a tank (45 by 30 by 30 cm; Nalgene, Rochester, NY) containing aerated artificial seawater (ASW) with a salinity of 30 parts per thousand (ppt). The ASW was prepared by dissolving Instant Ocean Salt (Aquarium systems, Inc., Mentor, OH) in deionized water according to the manufacturer's instruction. After being held in ASW at room temperature for 2-4 h, oysters were used for movement study, analysis of naturally-accumulated *V. parahaemolyticus* and inoculation with *V. parahaemolyticus*.

2.3. Determination of oyster movement

Oyster movement in ASW (15 L) at various temperatures was studied using a Gape Ometer (Pacific Shellfish Institute, Olympia, WA) with eight oysters each time. The Gape Ometer consists of four rectangular bars each containing an electronic device capable of measuring the distance between the surface of the bar and a magnetic sensor. For determination of oyster movement, two oysters were glued to each bar with a magnetic sensor attached to the upper shell of each oyster. The oysters were placed in a polystyrene foam cooler containing ASW being circulated using a pump (Mini-jet 606, Aquarium systems, Italy) at 320 L/h through a water chiller (EU-CL85, AquaEuroUSA, Gardena, CA) at 3, 7, 10, 15 or 20 ºC. The distance between the rectangular bar and magnetic sensor was recorded every 5 minutes for 24 h in a computer. Changes in the distance (>0.05 cm) indicated the movement of oysters.

2.4. Inoculation of oysters with *V. parahaemolyticus*

For each experiment, 45 oysters were exposed to *V. parahaemolyticus* cocktail at a level of 10^{4-5} CFU/ml in freshly prepared ASW (20 L). Accumulation of *V. parahaemolyticus* in oysters was conducted according to previously published procedures in aerated ASW at room temperature overnight (16-18 h) with water being circulated (15 L/h) (Su et al., 2010).

2.5. Ice treatment and low temperature depuration

Oysters inoculated with *V. parahaemolyticus* were either stored with ice covered in a chest cooler for 4 days or depurated with ASW at various temperatures (2, 3, 7, 10, 12.5 and 15 ºC) for 4 to 6 days. For depuration study, the oysters were held in 60 L of ASW in a laboratory-scale recirculating (1500 L/h) system equipped with a 15 W Gamma UV sterilizer (Current-USA Inc., Vista, CA), a water chiller (Delta Star, Aqua Logic, Inc., San Diego, CA) and a temperature regulator capable of controlling water temperature between 2 and 15 ºC with an accuracy of ± 0.5 ºC. The depuration was conducted at 2 and 3 ºC for 4 days and at 7, 10, 12.5 and 15 ºC for 6 days. Survival of oysters during depuration was monitored daily. Oysters which opened shells during the process and did not close upon touch were considered dead and discarded.

2.6. Microbiological analysis

Concentrations of *V. parahaemolyticus* in oysters before and after inoculation as well as during ice storage or depuration were determined using the three-tube most-probable-number (MPN) methods (Kaysner and DePaola, 2004). Five oysters were randomly picked for analysis at each test time. Each oyster was shucked with a sterile knife and shucked oyster meat was homogenized with an equal volume of sterile alkaline phosphate buffer saline (PBS; pH 7.4) at high speed for 1 min using a two-speed laboratory blender (Waring Laboratory, Torrington, CT). Twenty-five grams of oyster homogenate sample (1:2 dilution) was mixed with 100 ml of PBS to prepare 1:10 dilution sample suspension. Additional 10-fold dilutions of the sample suspension were prepared using PBS. All sample dilutions were individually inoculated into 3 tubes of alkaline peptone water (APW). Inoculated APW tubes were incubated at 37 ºC for 16-18 h. A loopful (3-mm inoculating loop) of each enriched APW from positive (turbid) tubes was streaked onto thiosulfate-citrate bile salt-sucrose (TCBS; Difco, Becton Dickinson) plates and incubated at 37 ºC for 18-24 h. Formation of colonies that were round and green or bluish on the plates were considered positive for *V. parahaemolyticus*. Concentrations of *V. parahaemolyticus* were determined using 3 tube MPN table by converting the number of APW tubes that were positive for *V. parahaemolyticus*. Results were reported as the mean of five determinations. The efficacy of the UV sterilizer in inactivating *V. parahaemolyticus* cells released from oysters into the recirculating water was analyzed for *V. parahaemolyticus* daily by plating water samples on TCBS plates followed by incubation at 37 ºC for 24 h.

2.7. Statistical analysis

Results of microbiological tests were converted to $\log_{10}$ values before being analyzed with ANOVA and Tukey’s test using SPSS 13.0 software (Chicago, IL, USA). Significant differences among means of each treatment over time were established at a level of $P < 0.05$. Reductions of *V. parahaemolyticus* in oysters over time during depuration were estimated by linear regression with coefficient of determination (R^2).

3. Results and Discussion

The movement of oysters in seawater at temperatures between 3 and 20 ºC is reported in Table 1. Oyster movement was rarely detected when oysters were held at 3 ºC but the movement was more frequently observed when oysters were exposed to temperatures at 7 ºC or higher. Most gape distances recorded for oyster movement were smaller than 0.50 cm. However, oyster gape could be as big as 1.25 cm. Although it is not clear whether shell movement measured by gap distances is associated with the water pumping activity of oysters, this study demonstrated that oysters were able to acclimate to environments with temperatures as low as 7 ºC and exhibited water-pumping activity (Figure 1).

It has been reported that the Gulf oysters (*Crassostrea virginica*) could pump water at rates of 4 and 7 L/h at 15 and 20 ºC, respectively, but the rates gradually decreased to <1 L/h when water temperature decreased to 10 ºC (Loosanoff, 1958). In this study, we observed that only a few oysters (25%) showed minimal water-pumping activity when water temperature dropped to 3 ºC (Table 1). While the water-pumping activity is reduced when exposed to low temperature (<10 ºC), oysters were capable of acclimating to new environments and slowly resume water-pumping activity to a degree similar to that observed at 20 ºC (Figure 1). However, the time required for oysters to resume water-pumping activity upon exposure to low-temperature environments may vary among oysters. In this study, the water temperatures at the times of harvesting oysters ranged from 7 to 15 °C. It is believed that oysters were able to acclimate quickly to the depuration temperatures (7-15°C) similar to those of the growing environments and resume normal water-pumping activity. Although no experiment was conducted with oysters harvested in the summer months when water temperature may increase to around 20 °C (Duan and Su, 2005), it is hypothesized that oysters would acclimate quickly to a depuration temperature of 12.5 or 15 °C. Such a hypothesis will need to be verified by conducting depuration of oysters with naturally accumulated *V. parahaemolyticus* in a summer month.

There was no naturally accumulated *V. parahaemolyticus* detected (<3 MPN/g) in oysters used in this study. This is probably because the seawater temperature of the Yaquina Bay was not higher than 15 °C when oysters were harvested. Duan and Su (2005) previously reported low levels of *V. parahaemolyticus* in oysters harvested from the Yaquina Bay (3.6-43 MPN/g) during summer months (June – August). This was in accordance with a survey conducted in the U.S. reporting geometric mean densities of *V. parahaemolyticus* in retail Pacific oysters were usually low (<3 MPN/g) year round except in the summer (39 MPN/g) (Cook et al., 2002). However, a recent survey reported that densities of *V. parahaemolyticus* in retail oysters harvested from the Pacific regions varied greatly and ranged from non-detectable (50%) to greater than 4 log MPN/g (1.7%) (DePaola et al., 2010). High levels (>10,000 cells/g) of *V. parahaemolyticus* have also been reported in retail oysters harvested from the Mid-Atlantic and Gulf regions of the U.S. (DePaola et al., 2010), in china (Chen et al., 2010) and in Brazil (Costa Sobrinho et al., 2011). Presence of high levels of *V. parahaemolyticus* in retail oysters is a health concern because densities of *V. parahaemolyticus* could increase rapidly by 1.7 and 2.9 log CFU/g in oysters after being exposed to 26 ºC for 10 and 24 h, respectively (Gooch et al. 2002). Therefore, keeping oysters at low temperatures after harvest is critical in preventing rapid growth of *V. parahaemolyticus* in oysters before consumption.

However, this study found that storing whole oysters covered with ice had little effects on the reduction of *V. parahaemolyticus* in oysters. The densities of *V. parahaemolyticus* in whole oysters decreased slightly (<0.8 log MPN/g) during four days of storage in ice, but the reductions were not significant (P > 0.05) (Table 2). This is similar to previous reports of observing a small reduction (about 0.73 log MPN/g) of *V. vulnificus* in shellstock Gulf oysters stored in ice for 7 days (Cook and Ruple, 1992) and about 1 log MPN/g reduction of *V. vulnificus* in Pacific oysters after 7 days of storage at 0.5 ºC (Kaysner et al., 1989). These findings indicate that *V. parahaemolyticus* and *V. vulnificus* can survive in whole oysters stored in ice or at near freezing temperature, and storing oysters in ice is not a means to inactivate these pathogens.

Depuration of oysters in ASW at 2 or 3 ºC did not result in significant reductions of *V. parahaemolyticus* in oysters, though slightly greater reductions were observed at 3 than at 2 ºC (Table 2). The limited reductions of *V. parahaemolyticus* in oysters during storage in ice or depuration at near freezing temperatures were probably related to minimal biological activity of oysters at such low temperatures. The investigation of oyster gaping at 3 ºC indicated that the majority (75%) of oysters did not show shell movement for 24 h (Table 1). The lack of biological activity plus physiological variability among oysters might be the factors contributing to inconsistent reductions of *V. parahaemolyticus* in oysters observed under such processes.

Increasing temperature of depuration to 7 ºC and higher significantly increased reductions of *V. parahaemolyticus* in oysters. Densities of *V. parahaemolyticus* in the laboratory-inoculated oysters were significantly (P < 0.05) reduced by 1.9-2.0 log MPN/g after one day of depuration at temperatures between 7 and 15 ºC (Table 3). The reductions increased to 2.8-2.9 log MPN/g in oysters after four days of processes. All processes were able to yield >3.0 log MPN/g reductions of *V. parahaemolyticus* in oysters after five days. Analysis of water samples collected during the depuration processes did not find viable cells of *V. parahaemolyticus* in water (<1 CFU/10 mL), indicating the UV sterilizer worked functionally to inactivate *V. parahaemolyticus* cells released from oysters into the water.

Many studies have indicated that depuration at ambient water temperatures had little effects on reducing *Vibrio* spp. Ren and Su (2006) found no apparent change in levels of *V. parahaemolyticus* or *V. vulnificus* in artificially contaminated oysters depurated in ASW (salinity; 29.6 ppt) at ambient temperature for up to 24 h. Limited reductions of *V. parahaemolyticus* (1.2 log MPN/g) and *V. vulnificus* (2.0 log MPN/g) were observed in the Gulf oysters after depuration with a UV sterilizer at 22 ºC for 48 h (Chae et al., 2009). Tamplin and Capers (1992) reported that levels of *V. vulnificus* accumulated naturally in Gulf oysters increased by 5 log MPN/g after depuration in UV-sterilized water at 23 ºC for the same period of time. The ineffectiveness of depuration at ambient temperatures for reducing levels of *V. parahaemolyticus* or *V. vulnificus* in oysters might be due to multiplication of *Vibrio* cells in oyster tissues at warm temperatures. Our previous study reported that depuration with refrigerated seawater at 5 ºC for 96-144 h reduced *V. parahaemolyticus* populations by >3.0 log MPN/g in the Pacific oysters without significant fatality of the oysters (Su et al. 2010). This study showed that depuration at 7-15 ºC for five days could also achieve the same degree of reduction (>3.0 log MPN/g) of *V. parahaemolyticus* in oysters with no mortality (data not shown).

Linear estimates of reductions of *V. parahaemolyticus* in oysters during depuration at various temperatures (7-15 ºC) indicated similar R^2 values (0.60-0.61) for depurations at 7 and 10 ºC, which were slightly lower than those observed from depurations at 12.5 (0.66) and 15 ºC (0.70) (Figure 2). This might be due to variability in the biological activity of oysters at lower water temperatures (7 and 10 ºC), resulting in a slight difference in bacterial reductions throughout a depuration process. The reduction models suggested that the processes of oysters at 7, 10, 12.5 and 15 ºC could achieve a 3.52-log reduction of *V. parahaemolyticus*, a guideline for post harvest processing of shellfish established by the National Shellfish Sanitation Program (FDA, 2009), within 148, 164, 137 and 155 h, respectively. The longer times (148-164 h) estimated for depuration processes at 7, 10 and 15 ºC than 137 h at 12.5 ºC could be due to higher contamination levels (5.9-6.3 log MPN/g than 4.8 log MPN/g) of *V. parahaemolyticus* in oysters It has been reported that efficacy of depuration could be influenced by the loads of pathogens in oysters. A depuration at 16-18 ºC for 36 h reduced *Salmonella* spp. in oysters by 2-3 log CFU/g while a 72-h process was required to achieve reductions by 3-4 log CFU/g (Son and Fleet, 1980).

In conclusion, reducing temperature for depuration enhanced the efficacy in reducing *V. parahaemolyticus* in oysters. However, the biological activity of oysters appeared to be minimized when oysters were exposed to temperatures below 5 ºC. Depuration of oysters at temperatures between 7 and 15 °C can be applied as a post-harvest treatment for reducing contamination of *V. parahaemolyticus*. Further studies are needed to validate the efficacy of the process in reducing *V. parahaemolyticus* accumulated naturally in oysters.

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Table 1

Movement of oysters in artificial seawater at various temperatures for 24 h.

Temperature (ºC)	Total number of oysters	Number of oysters with the maximum gape (cm) range					
		No movement [a]	0.06 to 0.25	>0.25 to 0.50	>0.50 to 0.75	>0.75 to 1.00	>1.00
3	8	6	2	-	-	-	-
7	8	3	5	-	-	-	-
10	8	1	2	2	1	2	-
15	8	2	3	1	-	1	1
20	8	1	4	3	-	-	-

[a] Recorded gape distances were not greater than 0.05 cm.

Table 2

Changes of *Vibrio parahaemolyticus* populations ($\log_{10}$ MPN/g) in laboratory-contaminated oysters during ice storage and depuration at 2 and 3ºC.

Time (day)	Ice storage [a]	Depuration temperature (ºC) [b]	
		2	3
0	4.72 ± 0.25[c] A	5.62 ± 0.74 A	5.50 ± 0.69 A
1	4.27 ± 0.74 (0.45)[d] A	5.49 ± 0.15 (0.13) A	5.06 ± 0.46 (0.45) AB
2	4.04 ± 0.45 (0.68) A	5.29 ± 0.99 (0.33) A	3.58 ± 0.94 (1.92) AB
3	3.97 ± 0.54 (0.75) A	5.47 ± 1.13 (0.15) A	3.51 ± 1.08 (1.99) B
4	4.15 ± 0.73 (0.57) A	4.91 ± 1.07 (0.71) A	4.19 ± 1.57 (1.31) AB

[a] Study was conducted in May.

[b] Studies were conducted in March.

[c] Values were reported as means of five oyster samples ± standard deviation. Data with the same letter in the same column were not significantly different ($P > 0.05$).

[d] Reduction ($\log_{10}$ MPN/g) of *V. parahaemolyticus* after treatments.

Table 3

Changes of *Vibrio parahaemolyticus* populations (log_{10} MPN/g) in laboratory-contaminated oysters during depuration at 7, 10, 12.5, and 15ºC.

Time (day)	Temperature [a] (ºC)			
	7	10	12.5	15
0	5.91 ± 0.30[b] A	6.30 ± 0.18 A	4.83 ± 0.69 A	6.30 ± 0.18 A
1	4.04 ± 0.44 B (1.87)[c]	4.30 ± 0.42 B (2.00)	2.96 ± 0.33 B (1.87)	4.36 ± 0.29 B (1.93)
2	3.68 ± 0.33 BC (2.23)	3.95 ± 0.45 BC (2.35)	2.35 ± 0.75 BC (2.27)	3.87 ± 0.38 BC (2.43)
3	3.31 ± 0.42 BC (2.60)	3.78 ± 0.27 BC (2.51)	2.44 ± 0.13 BC (2.39)	3.80 ± 0.39 BC (2.50)
4	3.07 ± 0.66 CD (2.84)	3.37 ± 0.25 CD (2.93)	1.86 ± 0.39 BC (2.96)	3.40 ± 0.27 CD (2.90)
5	2.63 ± 0.43 D (3.28)	3.25 ± 0.44 CD (3.05)	1.50 ± 0.83 BC (3.33)	2.98 ± 0.38 D (3.32)
6	2.50 ± 0.52 D (3.40)	3.01 ± 0.51 D (3.29)	1.59 ± 0.62 C (3.43)	3.04 ± 0.14 D (3.26)

[a] Studies were conducted in September (7 ºC), January (10 ºC) and December (12.5 and 15 ºC).

[b] Values were reported as means of five oyster samples ± standard deviation. Data with the same letter in the same column were not significantly different ($P > 0.05$).

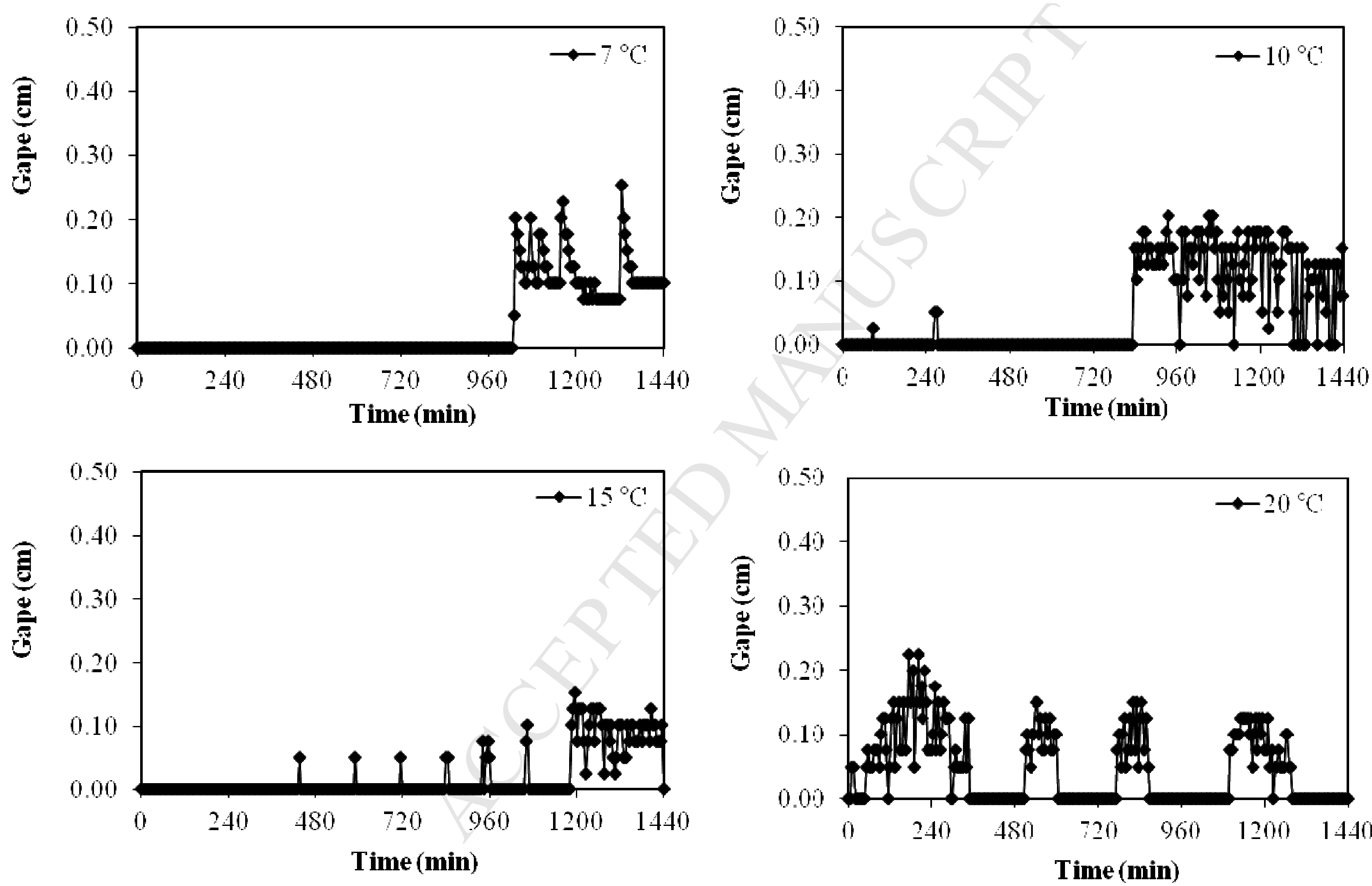
[c] Reduction (log_{10} MPN/g) of *V. parahaemolyticus* after treatments.

Figure Captions

Figure 1. Oyster movement in artificial seawater at various temperatures.

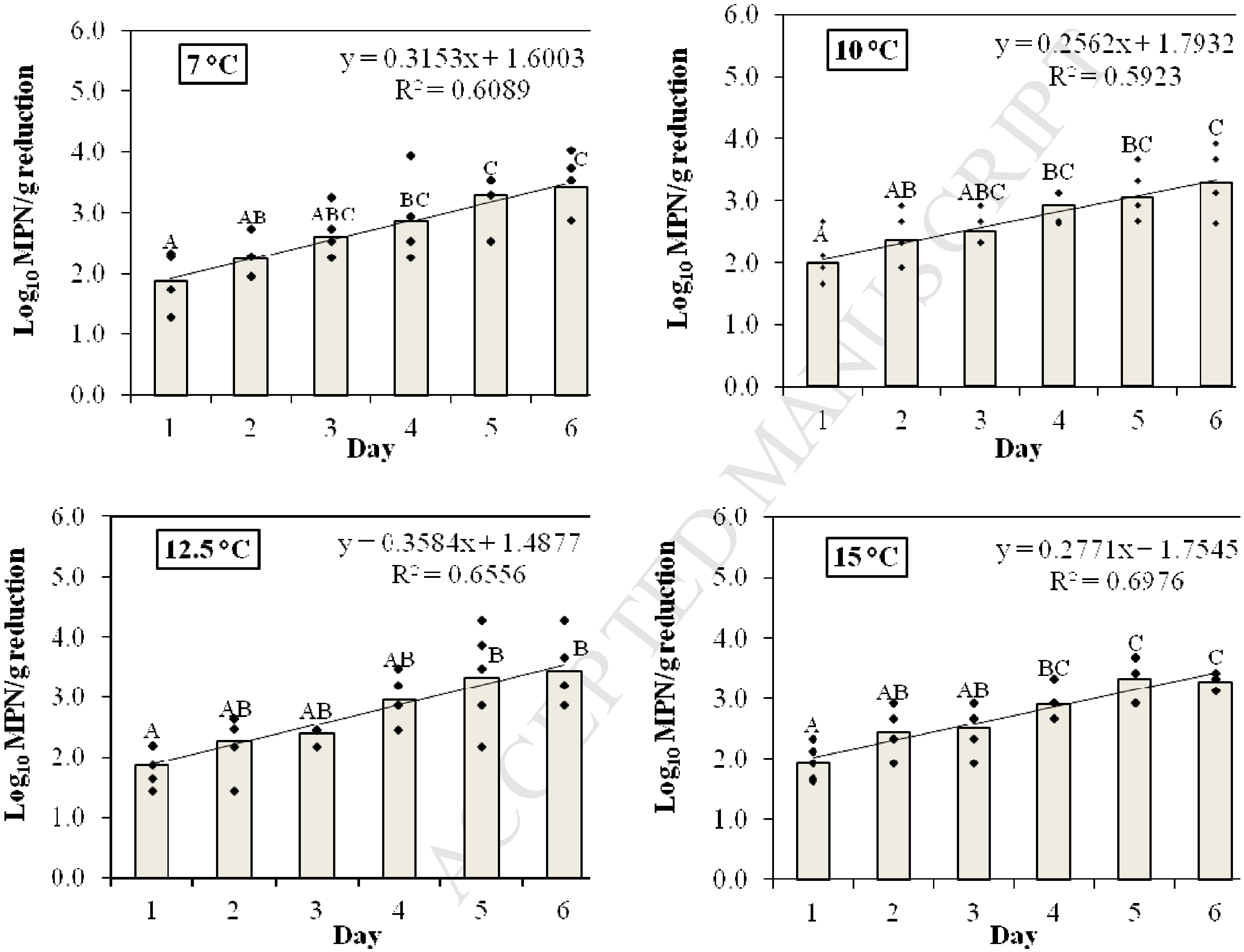
Figure 2. Reductions (Log_{10} MPN/g) of *V. parahaemolyticus* in laboratory-contaminated oysters during depuration at 7, 10, 12.5 and 15 ºC. Data were reported as mean values of reductions determined from five separate oysters. The means with the same letter observed at the same depuration temperature were not significantly different ($P > 0.05$). A linear prediction for the reduction of *V. parahaemolyticus* in oysters was sketched over time.

Figure 1



7 °C
10 °C
15 °C
20 °C
Gape (cm)
Time (min)
0.00
0.10
0.20
0.30
0.40
0.50
0
240
480
720
960
1200
1440

Figure 2


7 °C
y = 0.3153x + 1.6003
R² = 0.6089
10 °C
y = 0.2562x + 1.7932
R² = 0.5923
12.5 °C
y = 0.3584x + 1.4877
R² = 0.6556
15 °C
y = 0.2771x − 1.7545
R² = 0.6976
Log10 MPN/g reduction
Day
A
AB
ABC
BC
C
B
0.0
1.0
2.0
3.0
4.0
5.0
6.0
1
2
3
4
5
6

Highlights

1. Changes in levels of *Vibrio parahaemolyticus* in oysters during depuration at 2, 3, 7, 10, 12.5, and 15 ºC were studied.

2. Depuration of oysters at 2 or 3 ºC had no effect on reducing *V. parahaemolyticus*.

3. Depuration at 7-15ºC for 5 days reduced *V. parahaemolyticus* populations in oysters by >3.0 log MPN/g.