Analysis of Vibrio vulnificus Infection Risk When Consuming Depurated Raw Oysters

Analysis of *Vibrio vulnificus* infection risk when consuming depurated raw oysters

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Keywords: Oyster, *Vibrio vulnificus*, dose-response, depuration, Monte Carlo, risk analysis

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ABSTRACT

A beta Poisson dose-response model for *Vibrio vulnificus* food poisoning cases leading to septicemia was used when evaluating the effect of 15°C depuration on the estimated risk of raw oyster consumption. Statistical variability sources included *V. vulnificus* load at harvest, time and temperature during harvest and transportation to processing plants, decimal reductions (SV) observed during experimental circulation depuration treatments, refrigerated storage time before consumption, oyster size, and number of oysters per consumption event. Although reaching non-detectable *V. vulnificus* levels (<30 MPN/g) throughout the year and a 3.52 SV were estimated not possible at 95% confidence, depuration for 1, 2, 3, and 4 d would reduce the warm (Jun-Sep) season risk from 2,669 cases to 558, 93, 38, and 47 cases per 100 million consumption events, respectively. At 95% confidence, 47 and 16 h depuration would reduce the warm and transition (Apr-May, Oct-Nov) season risk, respectively, to 100 cases per 100 million consumption events assumed to be an acceptable risk, while 1 case per 100 million events would be the risk when consuming untreated raw oysters in the cold (Dec-Mar) season.
Pathogens frequently present in oysters include *Vibrio* species and noroviruses \((6, 17)\). Among *Vibrio* species, 11 can cause human disease including *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* causing severe illnesses \((19)\). During warm seasons, these halophilic Gram-negative bacteria can reach high numbers in oyster harvesting areas with moderate salinity \((20)\). The CDC reports over 400 *Vibrio* illnesses each year including about 90 due to *V. vulnificus* occurring mostly during warm-weather months \((5, 25)\). Diseases caused by *V. vulnificus* are among the most severe food-borne infections and have the highest case-fatality rate in the USA \((23)\). A number of dose-response models have been used to predict the probability of illness when consumers are exposed to a given pathogen dose \((12, 27)\). Since human dose-response studies cannot be conducted, modelling of the *V. vulnificus* dose-response relationship is based on estimates of dose exposure per serving, number of servings in the susceptible population and the number of oyster-associated cases of *V. vulnificus* septicemia cases reported to the CDC \((1)\). The frequently used Beta-Poisson model \((18)\) has been used to estimate the number of *V. vulnificus* cases likely to occur when consuming raw oysters harvested in the Gulf of Mexico \((1)\) and was the model used in this study.

Depuration consists of placing live oysters in circulating seawater tanks. During treatment, the oysters’ pumping activity expels *V. vulnificus* and other contaminants from their gills and intestinal tract \((9, 22)\). To allow its reuse, seawater is filtered and then disinfected by UV, ozone or chlorine \((21)\). A 15°C treatment temperature has been recommended for the depuration of oysters.
in circulating seawater (9). A 44 h depuration time reducing *V. vulnificus* to non-detectable counts
determined using a 3-tube most probable number (MPN) procedure and 1:10 dilution, i.e., less
than 30 MPN/g is prescribed in the National Shellfish Sanitation Program (see p. 140, 3).
Increasingly high consumer expectations of quality and safety make it necessary to develop
depuration treatments that are effective for every raw oyster production lot. The design should
consider the variability of production and handling factors including harvest, transportation, post-
harvest processing, storage and other risk factors. This is not possible using deterministic
algorithms or experimental test runs in processing plants. In this study, a Monte Carlo procedure
was applied to estimate the risk of consuming raw oysters treated by depuration. Several recent
reports describe its application to evaluate the uncertainty of food safety, quality and shelf-life
estimations (10, 11, 26, 29, 31, 32). In this study, procedures were developed to estimate the
number of septicemia infection cases per 100 million oyster consumption events, and to
determine depuration times that would reduce this risk to an acceptable level defined as 100
cases (31). This study included risk factors beginning at harvest and ending when raw oysters are
consumed. The procedures used in this study estimated whether process objectives are met with a
confidence set at 95%, while considering the statistical variability of these multiple factors.

**MATERIALS AND METHODS**

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Statistical distributions can describe: (i) *V. vulnificus* load at harvest as a function of season; (ii) time and temperature during transportation from harvest site to processing plants; (iii) kinetic *V. vulnificus* growth parameters during harvest and transportation; (iv) depuration parameters for models developed using published circulation depuration laboratory data; (v) oyster size; (vi) refrigerated storage time before consumption; (vii) *V. vulnificus* die-off during refrigerated storage; and, (vii) oysters consumed per serving.

Motes et al. (24) quantified the *V. vulnificus* (*Log (No, MPN/g oyster)*) in oysters collected from northern Gulf and Atlantic Coast sites including sites implicated in major *V. vulnificus* infection outbreaks yielding values of 3.22±0.60, 2.01±1.12, and -0.29±0.51 for the warm (Jun-Sep), transition (Apr-May, Oct-Nov) and cold (Dec-Mar) season, respectively. The USFDA Gulf Coast Seafood Laboratory described the time for the unrefrigerated oyster harvest and transportation time to processing plants using beta-PERT distributions (Table 1) (2). Data for Louisiana, Alabama, Texas and Florida (1000 values for each state) were generated using the Excel Add-in OpenPERT.xlam (https://code.google.com/p/openpert/downloads/list). These values were then grouped into cold, warm and transition season and used with the same Excel Add-in to find the min, max and ml values for each season in the four states. During harvest and transportation, oysters are exposed to air temperatures slightly higher than seawater. Since this difference
is small (1.6-3.3°C) (see p. 34, 1), oysters were assumed to be at air temperature with
normal distribution values of 13.1±4.3, 23.3±4.1, 27.2±2.0 and 16.4±5.5°C for Winter,
Spring, Summer and Fall, respectively, reported in the National Oceanic and Atmospheric
Administration/National Data Buoy Center database (1). As before, 1000 generated
temperature values were grouped to calculate the warm, transition and cold season
temperature values used in this study. Next, the V. vulnificus load for oysters arriving at the
processing plant (Log (N1, CFU/g)) was estimated using Eq. (1) where μm(T) represents the
growth rate at a random temperature T(°C) obtained from the seasonal air temperature
distribution, t(h) the unrefrigerated oyster handling time at this temperature T, and A
(= Log (N1,max, CFU/g oyster) = 6) the maximum V. vulnificus counts possible in raw oysters
(1, 7). The temperature dependence of the growth rate μm(T) in Eq. (1) was described by
Eq. (2) where T is the seasonal air temperature, k (= 0.011 Log (CFU)/(h °C)) is the V.
vulnificus growth rate above T0, and T0 (= 13°C) a threshold temperature below which V.
vulnificus does not grow (see p. 32, 1, 13).

\[
\begin{align*}
\log \left( \frac{N_1}{g_{oyster}} \right) &= \min \left( \log \left( \frac{N_0}{g_{oyster}} + \mu_m(T) \cdot t, A \right) \right) \\
\mu_m(T) &= \max \left( 0, k(T - T_0) \right)
\end{align*}
\]

(1) (2)

Data on the V. vulnificus load reduction by depuration at 15°C obtained by Chae et al. (9) by
sampling inoculated oysters every 24 h (Table 2a) was used to generate 1000 random pathogen load values at 0, 24, 48, 72 and 96 h. Quadratic models fitted to these values were used to estimate the *V. vulnificus* load after a 0 to 96 h depuration time \((t_{depuration})\). The difference between the initial *V. vulnificus* load and that after depuration was used to estimate decimal reduction \((SV)\) values achieved during that time for each of the 1,000 randomly generated datasets. \(SV\) values were then used to estimate the microbial load after depuration \((\log (N_2, \text{MPN}/\text{g oyster}))\) \((\text{Eq. 3})\).

\[
\log(N_2, \text{MPN}/\text{g oyster}) = \log(N_1, \text{MPN}/\text{g oyster}) - SV_{depuration}
\]  

\((3)\)

Cooling time to refrigeration temperature from depuration at 15°C was assumed short and ignored in this study. Under refrigeration, the *V. vulnificus* die-off rate is 0.041 log CFU/day and the refrigerated time before consumption follows a beta-PERT distribution with \(min, ml\) and \(max\) values of 1, 6 and 21 d \((14)\). The lognormal distribution for oyster meat weight reported by the Interstate Shellfish Sanitation Conference (ISSC)/FDA is \(\log (W, \text{g/oyster}) = 1.18 \pm 0.15\) \((1, 2)\). A published survey of metropolitan areas within 100 miles of Cedar Key in Florida encompassing 5 million residents generated data for 306 oyster consumption events \((15)\). Random sampling of this non-parametric distribution (numbers (frequency) = 1, 2, 3(9x), 4(10x), 5(14x), 6(61x), 7, 8(11x), 10(15x), 12(95x), 13, 15(5x), 17, 18(8x), 20(8x), 24(37x), 25(5x), 30(3x), 36(7x), 40(3x), 45(2x), 48(4x), 50(3x), 60) was used in this study.

The shape and scale parameter \(\alpha (= 9.3 \times 10^{-6})\) and \(\beta (= 1.1 \times 10^5)\) of the Beta distribution dose-
response model (Eq. 4), frequently used to estimate the \( V. \textit{vulnificus} \) septicemia risk probability \((P_{iii})\) for a population ingesting a given pathogen dose \((D)\) per serving published by the World Health Organization (WHO, 1), incorporate pathogenicity heterogeneity, i.e., not every ingested microorganism survives to cause an infection.

\[
P_{iii} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}
\]

Safety risk was expressed as the number of septicemia cases in 100 million oyster consumption events. Estimations were divided into 8 steps repeated 1,000 times to obtain the number of infection cases with 95% confidence (31): (1) \( V. \textit{vulnificus} \) oyster load at harvest \((\text{Log} (N_0, \text{CFU/g}))\); (2) \( V. \textit{vulnificus} \) load for oysters arriving to processing plants \((\text{Log} (N_1, \text{CFU/g}))\); (3) \( SV \) value reached after a given depuration time \((SV_{depuration})\); (4) \( V. \textit{vulnificus} \) load after depuration \((\text{Log} (N_2, \text{CFU/g}))\); (5) \( V. \textit{vulnificus} \) load at consumption \((\text{Log} (N_3, \text{CFU/g}))\); (6) \( V. \textit{vulnificus} \) dose per serving \((D, \text{CFU/serving})\); (7) infection probability \((P_{iii})\); and, (8) number of infection cases per 100 million consumption events \((N, \text{cases})\). In each step, parameter values were randomly generated using their normal, lognormal or beta-PERT distribution or by random sampling (number of oysters per consumption event). In the depuration step, 1,000 sets of \( V. \textit{vulnificus} \) load randomly generated using their lognormal distribution were fitted into quadratic expressions for \( SV \) values after depuration times between 0 and 96 h. \( V. \textit{vulnificus} \) load as a function of
depuration time, season, and handling step was analyzed by ANOVA tests. As in previous studies (30), a health risk of 100 cases per 100 million oyster consumption events at 95% confidence was used to recommend a depuration time.

RESULTS

The estimated *V. vulnificus* growth rate during harvest and transportation to processing plants was $0.159 \pm 0.022$, $0.074 \pm 0.047$, and $0.022 \pm 0.030 \ \text{Log (MPN/g oyster)/h}$ in the warm, transition, and cold season, respectively, and combining these values with random harvest and transportation time values, the estimated pathogen load increase was $1.21 \pm 0.28$, $0.57 \pm 0.38$, and $0.23 \pm 0.32 \ \text{Log (MPN/g oyster)}$, respectively. Combining the latter values with the harvest load variability yielded pathogen load values before depuration ($\text{Log } N_i$) of $4.41 \pm 0.28$, $2.59 \pm 0.38$ and $-0.09 \pm 0.32 \ \text{Log (MPN/g oyster)}$, respectively.

SV values achieved by 24, 48, 72 and 96h depuration at 15°C using quadratic models (average $R^2 = 0.95$) were $1.55 \pm 0.17$, $2.56 \pm 0.24$, $3.03 \pm 0.24$, and $2.95 \pm 0.24 \ \text{Log (MPN/g oyster)}$, respectively, showing that after 72 h the pathogen load reduction is not significant (P-value>0.05).

Microbial load values after depuration are summarized in Table 2b.

*V. vulnificus* die-off during refrigerated handling time before consumption was estimated as $0.31 \pm 0.15 \ \text{Log (MPN/g oyster)}$ yielding pathogen loads for untreated oysters at consumption
of -0.38±0.6 and 4.14±0.7 Log (MPN/g oyster) in cold and warm seasons, respectively, and a significantly lower value for the transition season (2.26±1.2 Log (MPN/g oyster)) when compared to the warm season (P value < 0.05). The season effect is reflected also in V. vulnificus loads for raw oyster treated by depuration for up to 96 h (Table 2c). The pathogen dose consumed per oyster serving (D) reflects the variability in the number of oysters consumed per serving, oyster weight, and pathogen load after depuration. In the case of untreated oysters, the dose of (10.0±3.1) x 10^6 MPN/serving in the warm season was significantly higher (P value < 0.05) than the (9.7±0.4) x 10^5 and 283.1±61.7 MPN/serving in the transition and cold season, respectively. Dose values after depuration for 24 to 96 h are shown in Table 2d.

At 95% confidence, the estimated number of infection cases in 100 million consumption events for untreated oysters in the warm season was 2669 cases, while 24 and 48h depuration would reduce it to 558 and 93 cases, respectively (Table 3). The depuration time required to reach the safety target used in this study (100 cases per 100 million consumption events) was estimated to be 47h. The discrepancy between the estimated 93 and 100 cases after 48 and 47h depuration time, respectively, reflects the non-deterministic procedure used in this study. In the transition season, the number of predicted cases would be 491 for untreated oysters, reaching the 100 cases safety-target after 16h depuration, meaning that the 44h recommended depuration (see p. 126, 3) could be considered over-processing. Only 35 cases would be observed after depuration for 24h,
while a 48h depuration is likely to reduce the number of infection cases to 4. The number of cases at 95% confidence that could be expected after the recommended 44h depuration time would be 135, 8 and 0, for oysters harvested in the warm, transition and cold season, respectively (Table 3).

DISCUSSION

The U.S. FDA requires that “the dealer must demonstrate that the process reduces the level of *Vibrio vulnificus* and/or *Vibrio parahaemolyticus* ... to non-detectable (<30 MPN/gram) and that the process achieves a minimum 3.52 log reduction (see p. 140, 3).” Furthermore, *V. vulnificus* and *V. parahaemolyticus* levels must be determined following the sampling protocol (4) and microbial enumeration (see p. 345, 3) described by the National Shellfish Sanitation Program (NSSP). The USFDA/CFSAN and the ISSC state that treated oysters meeting these specified endpoint and decimal reduction levels can be labeled as "Processed to reduce *Vibrio vulnificus* to non-detectable levels" (see p. 187, 3). The 3.52 log reduction in these regulations is based on assuming extremely high *V. vulnificus* or *V. parahaemolyticus* loads observed sometimes in the Gulf Coast during summer months (100,000 MPN/g) being lowered by processing to reach non-detectable levels (<30 MPN/g) (see p. 172, 3). Although artificial seawater depuration tests showed that reduction in *V. vulnificus* counts leveled off after 48 h and that 96 h depuration cannot achieve 3.52 log reductions for (9), use of this experimental data showed that depuration would achieve large
reductions in the *V. vulnificus* infection risk reaching values below 100 cases per 100 million consumption events (Table 3).

The probability that depuration would reduce the *V. vulnificus* load to the non-detectable endpoint (<30 MPN/g) was also determined (Table 4). A 95% confidence level is typically used in process design calculations (26, 33). Oysters harvested in the cold season would meet this requirement at 95% confidence in less than 1h while in the transition and warm season it cannot be met even after 96h depuration. If pathogen loads after depuration ($N_2$) are lower than the non-detectable 30 MPN/g, the estimated infection risk at 95% confidence per 100 million consumption events estimated using Eq. (4) would be less than 27 cases per year (calculations not shown).

An analysis of the effectiveness of the recommended depuration time (44 h) showed that only 23% and 50% of oysters harvested in the warm and transition seasons, respectively, would reach the non-detectable *V. vulnificus* level (i.e., below 30 MPN/g), while 100% would reach it in the cold season (Table 4). At 95% confidence, the use of the recommended 44 h depuration time would result in 135, 8, and 0 cases in the warm, transition, and cold season, respectively (Table 3). Thus, in the summer season it would be too short while for other seasons it would be too long. The microbial risk analysis of HPP-treated oysters completed by Serment-Moreno et al. (31) showed that 4 cases of *V. vulnificus* infection cases (95% confidence) per 100 million consumption events could be expected from the consumption of oysters harvested in the warm season if treated at 250
MPa for 2 min at 1°C with no cases expected in other seasons.

The number of oysters consumed per serving was obtained from central and north-central Florida surveys (15) where the number consumed per oyster serving could be higher than in inland states. Population inference errors may also occur when consumers recall consumption events (15) and consumers eating oysters more frequently may tolerate higher pathogen loads (28). Moreover, *V. vulnificus* in oysters were assumed to correspond to equally virulent strains (16). Although samples below detectable level were assigned a half-way value between 0 and the detection value, this can be ignored since the number of oyster samples below detection level was reported to be small (i.e., 2 of 24 samples, 24).

This study used reported experimental data (8) focusing on *V. vulnificus* oyster depuration. The same procedure could be applied to reduce the risks of other pathogens by depuration, or to analyze other oyster treatment technologies by modifying only the SV estimation. The procedure would be most effective when using data for individual processors or at least individual harvest regions. This would reduce statistical variability and lower the treatment intensity recommended (depuration time/temperature, pressure level/holding time, etc.). The positive effect of reducing statistical variability has been previously shown (10, 29).

In conclusion, the *V. vulnificus* infection risk associated with raw oyster consumption was quantified to estimate a recommended depuration time. The 44h depuration set independently of
oyster harvest season, non-detectable endpoint (<30 MPN/g), and 3.52 *V. vulnificus* decimal reduction, were analyzed using a Monte Carlo protocol. The analysis included the variability of the seasonal oyster pathogen load at the point of harvest, the time and local temperature during oyster harvest and transportation to oyster processing facilities, time that oysters are kept refrigerated after processing and before consumption, and the size and number of oysters consumed per serving. For untreated oysters, the *V. vulnificus* infection risk at 95% confidence is exceedingly low in the cold season but unacceptably high in the warm and transition season. An acceptable risk, defined in this study as 100 cases per 100 million consumption events, could be achieved with 95% confidence by oyster depuration for 47 and 16 h in the warm and transition season, respectively.

**ACKNOWLEDGMENTS**

The authors thank critical reviews by PhD Candidates M.C. Rosas González and L.E. García Amézquita. Financial support from the Tecnológico de Monterrey (Research Chair Funds CAT-200) for the Emerging Technologies and Nutrigenomic Research Groups, Fondo Nacional de Ciencia y Tecnología de Chile (FONDECYT Grant 1110569), Research Office of Universidad Andres Bello (DI-275-13/R 2013), Fondo de Fomento al Desarrollo Científico y Tecnológico (FONDEF) CA13I10077, and Formula Grants no. 2011-31200-06041 and 2012-31200-06041 from the USDA National Institute of Food and Agriculture is gratefully acknowledged.
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<table>
<thead>
<tr>
<th>Location</th>
<th>Winter (Jan-Mar)</th>
<th>Spring (Apr-Jun)</th>
<th>Summer (Jul-Sept)</th>
<th>Fall (Oct-Dec)</th>
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<tr>
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<td>ml = 9</td>
<td>ml = 12</td>
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<td>ml = 8</td>
<td>ml = 7</td>
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</table>
Table 2. *Vibrio vulnificus* load and consumption dose of untreated and depurated oyster

<table>
<thead>
<tr>
<th>Sample</th>
<th>Untreated*</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Reduction during depuration test at 15°C**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>5.52 ± 0.16</td>
<td>3.49 ± 0.23</td>
<td>2.76 ± 0.19</td>
<td>2.67 ± 0.29</td>
<td>2.23 ± 0.20</td>
</tr>
<tr>
<td>b) Load after depuration for (t = 0) (untreated) to 96 h, Log ((N_{2:t}, MPN/g oyster))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm</td>
<td>4.41±0.28i</td>
<td>2.89±0.7</td>
<td>1.87±0.7</td>
<td>1.41±0.7</td>
<td>1.48±0.7</td>
</tr>
<tr>
<td>Transition</td>
<td>2.59±0.38i</td>
<td>0.95±1.2</td>
<td>-0.07±1.2</td>
<td>-0.54±1.3</td>
<td>-0.46±1.2</td>
</tr>
<tr>
<td>Cold</td>
<td>-0.09±0.32i</td>
<td>-1.63±0.6</td>
<td>-2.65±0.6</td>
<td>-3.11±0.6</td>
<td>-3.03±0.62</td>
</tr>
<tr>
<td>c) Load after depuration and refrigerated storage, Log ((N_3, MPN/g oyster))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm</td>
<td>4.14±0.7</td>
<td>2.59±0.7</td>
<td>1.58±0.7</td>
<td>1.12±0.7</td>
<td>1.19±0.7</td>
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<tr>
<td>Transition</td>
<td>2.26±1.2</td>
<td>0.71±1.2</td>
<td>-0.31±1.2</td>
<td>-0.77±1.2</td>
<td>-0.69±1.2</td>
</tr>
<tr>
<td>Cold</td>
<td>-0.38±0.6</td>
<td>-1.95±0.6</td>
<td>-2.96±0.6</td>
<td>-3.43±0.6</td>
<td>-3.35±0.6</td>
</tr>
<tr>
<td>d) Dose when consuming raw oysters, (D, MPN/serving)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm</td>
<td>10.0±3.1x10^6</td>
<td>3.0±0.29x10^5</td>
<td>3.1±0.53x10^4</td>
<td>1.1±0.43x10^4</td>
<td>1.4±0.16x10^4</td>
</tr>
<tr>
<td>Transition</td>
<td>9.7±0.4x10^5</td>
<td>3.3±0.04x10^4</td>
<td>3.6±0.07x10^3</td>
<td>1.1±0.06x10^3</td>
<td>1.3±0.02x10^3</td>
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<tr>
<td>Cold</td>
<td>283.1±61.7</td>
<td>8.0±0.57</td>
<td>0.83±0.11</td>
<td>0.29±0.09</td>
<td>0.37±0.03</td>
</tr>
</tbody>
</table>

(*) Untreated load corresponds to the Log N1 value obtained in the previous calculation step
(**) Data obtained from Chae et al. (9)
Table 3. Probable number of septicemia infection cases by consuming raw oysters treated by depuration for $t = 0$ (untreated) to 96 h and recommended depuration time ($t_{depuration}$)\(^1\)

<table>
<thead>
<tr>
<th>Season</th>
<th>Untreated</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>44 h(^2)</th>
<th>$t_{depuration}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm</td>
<td>2669</td>
<td>558</td>
<td>93</td>
<td>38</td>
<td>47</td>
<td>135</td>
<td>47</td>
</tr>
<tr>
<td>Transition</td>
<td>491</td>
<td>35</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Cold</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(^3)</td>
</tr>
</tbody>
</table>

(1) depuration time to reach N = 100 cases per 10\(^8\) consumption events
(2) # of cases at 95% confidence expected after the recommended 44h depuration time
(3) depuration treatment is not recommended for cold season
Table 4. Probability that the *V. vulnificus* load in raw oysters is reduced to <30 MPN/g by depuration for $t = 0$ to 96 h and recommended depuration time ($t_{30\text{MPN/g}}$) to reach this load with 95% confidence

<table>
<thead>
<tr>
<th>Season</th>
<th>Untreated</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>44 h</th>
<th>$t_{30\text{MPN/g}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm</td>
<td>0%</td>
<td>3%</td>
<td>31%</td>
<td>55%</td>
<td>50%</td>
<td>23%</td>
<td>&gt;96 h</td>
</tr>
<tr>
<td>Transition</td>
<td>17%</td>
<td>66%</td>
<td>88%</td>
<td>95%</td>
<td>94%</td>
<td>50%</td>
<td>72 h</td>
</tr>
<tr>
<td>Cold</td>
<td>99%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>0 h</td>
</tr>
</tbody>
</table>

(1) Currently recommended depuration time