



Impact of feeding on oyster depuration efficacy under conditions of high salinity and low temperature



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ARTICLE INFO

Keywords:

Oyster
Depuration efficacy
Feeding regimes
Vibrio
Salinity
Temperature

ABSTRACT

Feeding regimes of laboratory-cultured algae reportedly maintain meat quality as well as reduce mortality of oysters during prolonged depuration. However, feeding regimes are costly and their impact on depuration efficacy is not understood. The impact of a feeding regime of laboratory-cultured *Chaetoceros gracilis* (added at a manufacturer-recommended dose of 1.4×10^9 cells/oyster/day) on depuration efficacy for batches of Pacific oysters collected from four sites in Japan in 2016 and 2017 was evaluated. Depuration was done in filtered seawater (FSW) under conditions of low temperature and high salinity (17 °C and 28 to 31 ppt respectively). Indicators of depuration efficacy (*Vibrio parahaemolyticus* and *V. vulnificus* counts, condition index as well as mortality) for oyster batches that were fed were compared with those for batches that were not fed over a depuration period of 14 days. Generally, feeding did not significantly affect removal of *V. parahaemolyticus* and *V. vulnificus*, condition index or mortality of oysters ($P > .05$). However, for one batch of oysters in an exceptionally poor physiological state (condition index < 32), condition index improved significantly after 14 days of depuration with feeding. From the results it was concluded that under conditions of low temperature and high salinity, feeding does not have a significant impact on depuration efficacy unless initial condition index of the batch was very low. This is the first study to show that maintaining high salinity and low temperature during oyster depuration eliminates the need for feeding regimes.

1. Introduction

Vibrio parahaemolyticus caused 56.7% (1931 cases) of food-borne *Vibrio* species infections reported to the Centers for Disease Control and Prevention (CDC) between 1997 and 2006 (Iwamoto et al., 2010). Outbreaks of *V. parahaemolyticus* are often linked to consumption of raw or under-cooked oysters (Su and Liu, 2007). Similarly, *V. vulnificus* continues to receive a lot of research attention because of its association with serious foodborne illnesses linked to consumption of raw oysters in the warmer months of the year (Deng et al., 2015; Larsen et al., 2013; Larsen et al., 2015). To ensure safety of oysters for raw consumption, several post harvest procedures such as depuration, refrigeration, wet storage and antifouling have been considered (Richards, 1988; Son and Fleet, 1980).

Depuration is a process of holding filter-feeding shellfish in clean seawater, typically for periods of 24 to 48 h, to enable release of pathogens and other contaminants (Blogowaski and Stewart, 1983; Drake

et al., 2007). Depuration is a standard procedure for reducing the risk of food poisoning due to *Escherichia coli* (Lee et al., 2008). Although the National Shellfish Sanitation Program prescribes a depuration time of 44 h for reducing *V. vulnificus* to non-detectable levels, this period is reported to be too short during the warmer months of the year (Deng et al., 2015). Recently modifications of the conventional depuration process have shown increasing potential to improve its efficacy. Such modifications include prolonged (over 44 h) depuration, low temperature (15 °C) depuration, and high salinity (above 25 ppt) depuration (Chae et al., 2009; Larsen et al., 2013; Larsen et al., 2015). However, the successful application of any post harvest procedure that improves microbial safety of oysters intended for raw consumption requires that the oysters remain physiologically healthy. Unfortunately, there is concern in the shellfish industry that prolonged depuration procedures starve the oysters and result in damages to nutritional composition as well as organoleptic characteristics. This concern has been justified by studies on condition index and fatty acid profiles of bivalves during

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<https://doi.org/10.1016/j.aquaculture.2018.10.009>

Received 5 July 2018; Received in revised form 5 October 2018; Accepted 6 October 2018

Available online 07 October 2018

0044-8486/ © 2018 Published by Elsevier B.V.

prolonged depuration (Larsen et al., 2013; Ruano et al., 2012). To address this problem, maintaining oyster physiology during prolonged depuration by providing a shellfish diet has been suggested (Larsen et al., 2015; Lee et al., 2008).

As depuration has historically focused on the reduction of *E. coli* over short periods of time (24 to 48 h), there is little information on how a combination of prolonged depuration and feeding affects microbial quality, physiology and mortality of oysters. Moreover, most of the reports on the efficacy of prolonged depuration for the reduction of *Vibrio* spp. are based on results from laboratory experiments using artificially contaminated or deliberately temperature-abused oysters. The applicability of studies based on artificial contamination of oysters has been questioned as laboratory-induced microbial contaminants depurate faster than naturally acquired microbial contaminants (Richards, 1988; Steslow et al., 1987). For practical application it is desirable that observations are done with naturally contaminated oysters (oysters exposed to microbial contamination in their natural habitats). The objective of this study was to evaluate the effect of feeding on *V. parahaemolyticus* and *V. vulnificus* populations, condition indices as well as mortality of naturally contaminated oysters during prolonged depuration under conditions of low temperature (15 °C) and high salinity (28 to 31 ppt).

2. Materials and methods

2.1. Samples used in this study

In this study, a total of seven batches of Pacific oysters (10 to 17 cm long) from two prefectures in Japan were used. Two batches of oysters were received from each of two sampling areas in Hiroshima Prefecture in Japan, in June and in late July 2016. Another batch of oysters was received from a sampling area in Oita Prefecture in Japan in January 2017. Finally, one more batch of triploid Pacific oysters was received from each of two sampling areas in Oita Prefecture in Japan, in late July 2017. Only oysters that had been reared at the harvesting sites for more than two weeks prior to the experiment were used. The oysters were harvested in the morning and transported in a refrigerated container to a research station in Oita Prefecture, Japan within 24 h of harvest. Upon arrival at the station (the next morning), dead oysters were discarded while live ones were cleaned to remove debris. The oysters were then loaded into plastic perforated baskets in a single layer. For depuration treatments, the baskets were placed in 200 L tanks to achieve a suspension of 2 cm off the bottom. The number of live oysters in each batch (at Day 0 of sampling) is shown in Table 1.

Table 1

Oyster mortalities during prolonged depuration (14 days at 5 °C and salinity of 28 to 31 ppt) with and without feeding.

Oyster batch	Percentage mortalities during depuration (Number of oysters at Day 0 are in parentheses)	
	With feeding	Without feeding
Hiroshima Area 1 in June 2016	2.1 (96)	1.9 (96)
Hiroshima Area 2 in June 2016	5.2 (96)	4.2 (104)
Hiroshima Area 1 in late July 2016	1.6 (78)	6.4 (78)
Hiroshima Area 2 in late July 2016	3.9 (125)	3.9 (125)
Oita Area 1 in late July 2017	0.4 (245)	7.4 (217)
Oita Area 2 in late July 2017	1.0 (200)	1.0 (200)

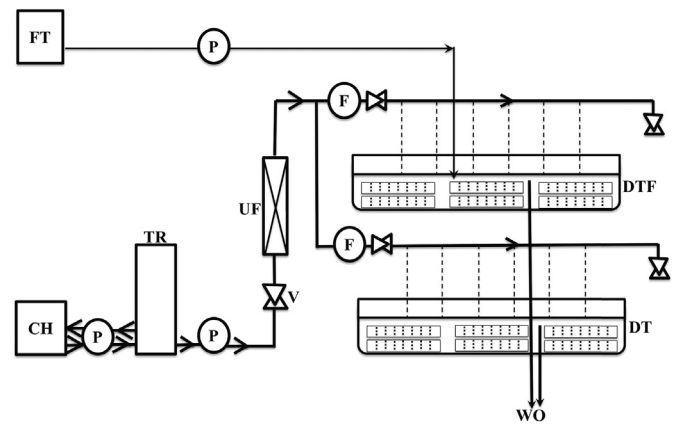


Fig. 1. Experimental set up for the depuration process. After temperature regulation in the chiller (CH) and the temperature regulation tank (TR), sea water flowed through a UF membrane (UF) to become filtered sea water which flowed to the depuration tanks (DT, and DTF) and then out of the system through a water outlet (WO). Depuration tank (DT) contained oysters that were not fed. Depuration tank (DTF) contained oysters that were to be fed through a connection from the feed tank (FT). Water flow was enabled, controlled and monitored by pumps (P), valves (V) and flow meters (F) respectively.

2.2. Depuration procedures

For depuration, oysters were placed in filtered seawater (FSW) for 14 days at an average temperature of 17 °C. To prepare FSW, seawater was membrane filtered using a UF membrane (TORAY CP20-1010-B) and caused to flow through 2001 depuration tanks (750 × 1500 × 215 mm) at a flow rate of 3.5 l/min. Used water was not recirculated. Oysters for depuration were arranged in perforated trays and placed in the depuration tanks. For feed-supplemented depuration, artificially cultured *Chaetoceros gracilis* (Yanmar YCG-10) was added to the depuration tanks to maintain a concentration of 1.4×10^9 cells/oyster/day according to the manufacturers instructions. The flow rate, water salinity, water temperature, and contamination (*V. parahaemolyticus* and *V. vulnificus* populations) in source water as well as feedstock were monitored during depuration at 0, 1, 2, 4, 7, and 14 days. Oxygen levels were not measured. An overview of the experimental set up is shown in Fig. 1.

2.3. Assessing the effect of feeding on *Vibrio* spp. populations during depuration

Based on reports on the occurrence and ecology of *V. parahaemolyticus* (DePaola et al., 1990; Kaneko and Colwell, 1973), analysis of the effect of feeding on *V. parahaemolyticus* was limited to batches of oysters harvested in summer. Similarly, analysis of the effect of feeding on *V. vulnificus* was limited to batches of oysters harvested in summer of 2017. To assess the effect of feeding on *V. parahaemolyticus* and *V. vulnificus*, 5 randomly selected oysters from each treatment were analyzed for *Vibrio* spp. at 0, 4, 7, and 14 days. Populations of *V. parahaemolyticus* and *V. vulnificus* in oysters were determined based on the standard three-tube most-probable-number (MPN) procedure described in the U.S. Food and Drug Administration's Bacteriological Analytical Manual with slight modifications (U.S. Department of Agriculture, 2008). For microbial analysis, 5 oysters were randomly chosen from each of the depuration treatments. Oyster shell content was transferred to sterile bags (Eiken Kizai Co. LTD Code No. T17500) forming a pool of 5 oysters. Shell contents were manually pulverized and mixed with phosphate buffered saline (PBS) to make a 1:1 homogenate. From this homogenate a filtrate of 2 ml was added to 8 ml of PBS to make a 1:10 dilution. Aliquots of 1 ml each were sequentially transferred to tubes containing 9 ml of PBS to create three more levels of dilutions

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