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The Bacteriology and Physico-chemical Parameters of Microcosms Used for Depuration of Mangrove oyster (*Crassostrea gasar*) Harvested from the Estuary of Rivers State, Nigeria

B.J.O. Efiuvwevwere[1,*], L.O. Amadi[2]

[1]Department of Microbiology (Ofirima Complex), University of Port Harcourt, Port Harcourt, Nigeria
[2]Department of Science Laboratory Technology, Ken Saro-Wiwa Polytechnic, P.M.B 20, Bori, Rivers State, Nigeria
*Corresponding author: bjefiuvw@yahoo.com

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Abstract Changes in the physico-chemical and bacteriological attributes of mangrove oysters subjected to depuration process on daily basis for 4days in brackish and tap water microcosms were determined. There were progressive decreases in the brackish water (BW) salinity (20.3-11.5ppt), dissolved oxygen (DO) (7.0-6.0mg/l) and conductivity (32.1-19.4x10^3µS/cm) and increases in BOD (2.1-4.0mg/l) and turbidity (1.0-5.5NTU) whereas pH and temperature fluctuated between 6.6 and 7.2 and 26.5°C and 30.0°C respectively. In contrast, there were marked increases in the salinity (0.1-1.0ppt) and turbidity (0.5-1.5NTU) with decreases in DO (7.0-4.9mg/l) and BOD (3.8-1.0mg/l) whereas conductivity, pH and temperature were relatively constant in the tap water (TW) microcosm. The microorganisms isolated from the samples varied with the most heterogeneous genera; *Bacillus*, *Pseudomonas*, *Vibrio*, *Enterobacter*, *Staphylococcus*, *Escherichia*, *Proteus*, *Lactobacillus*, *Acinetobacter* and *Micrococcus* occurring in the BW and the least (7 genera) in the TW microcosm. The results indicate that changes in the physico-chemical parameters of the microcosms impacted adversely on the bacterial quality of mangrove oysters during depuration. The bacterial population dynamics and profiles obtained using the two microcosms have demonstrated the benefits of depuration on the potential health risk/safety of oysters prior to consumption.

Keywords: *Gbolokiri, depuration, microcosms, estuary, bacterial profliles*

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1. Introduction

There is growing scientific interest in understanding the physico-chemical and bacteriological profiles of estuaries or brackish water ecosystems of the Niger Delta region of Nigeria [1,2,3]. The intertidal mudflat of brackish water is the habitat of mangrove oysters. Bivalve shellfish feed by filtering large volumes of brackish water and bioaccumulate food particles [4] and microbial pathogens from the surrounding environment [5,6]. These microorganisms may present a potential health risk when consumed with raw or lightly cooked shellfish. Contaminated shellfish by industrial, sewage and other anthropogenic inputs may be cleansed by depuration as a shellfish food safety strategy [7,8].

However, the physico-chemical parameters of the habitats are also major determinants of the distribution and composition of the bacterial community [9,10,11]. The efficacy of depuration depends on a number of variables such as the initial health status of the shellfish, environmental parameters within the depuration system, the types of pathogens and level of contamination [7,12]. Currently, more attention is being given to depuration protocols [8] but with little focus on the dynamics of the physico-chemical factors during depuration.

The objective of this study was to determine the physico-chemical parameters and the bacterial profiles of mangrove oysters, brackish and tap water microcosms in a static depuration system and its potential benefits.

2. Materials and Methods

2.1. Study Site and Sample Collection

The mangrove oysters and brackish water samples were collected at both low and high tides from the Gbolokiri creek (at Rumuorlumeni) of the New Calabar River (NCR), Nigeria. The estuary consists of inter-connecting creeks that link up the lower reaches of the NCR. All samples were collected and transported to the laboratory and analyzed within 3-4hours.

2.2. Depuration of Mangrove Oysters

Mangrove oysters (200) were randomly collected and washed thoroughly under running tap water and then placed in a sterile rectangular plastic container of 50x35x28cm dimension. Oysters (20) were shucked and analysed at zero-hour bacterial load. The remaining oysters (180) were depurated in 15L of clean brackish and tap water microcosms and the water samples changed at intervals of 24-hours following the method of Chinivasagam [13] for 4days. The water samples and oysters were subjected to bacteriological and physico-chemical analyses.

2.3. Bacteriological Analysis

Serial dilutions of the water samples were spread-plated in duplicate on surface-dried plate count agar (Scharlau Chemie S.A. Spain) supplemented with 1.0% NaCl [14] and incubated at 37°C for 24hours [15] to determine aerobic plate counts (APCs). Preparation of serial dilutions was carried out by blending 25g of oyster meat in 225mL 0.1N alkaline peptone water to obtain a (10^{-1}) homogenate. Serial dilutions were also prepared for oysters, brackish and tap water samples every 24hours and analysed for bacterial profiles. Total viable colonies (30-300) were enumerated as colony forming units (CFUs).

Coliforms including *Escherichia coli* colonies were determined on MacConkey agar (Oxoid) using the spread plate method and duplicate plates were incubated at 37°C for 18-24hours. *Vibrio* colonies were determined on surface-dried thiosulphate-citrate-bile-salt-sucrose agar (Lab M Ltd, UK) using spread-plate method and duplicate plates were incubated at 37°C for 18-24hours.

Identification of bacterial isolates was carried out based on their cultural, morphological and biochemical characteristics [16,17,18].

2.4. Physico-chemical Analyses of Brackish and Tap Water Samples

The brackish water (BW) samples collected at low and high tides were analysed for several physico-chemical parameters including salinity (‰), pH, turbidity and conductivity as described in Horiba U-10 Instruction Manual [19]. Dissolved oxygen (DO) and Biochemical Oxygen Demand (BOD) were determined using the Modified Azide or Winkler's method in American Public Health Association (APHA) [20]. The temperature of the water samples was determined by immersion of a hand-held mercury-in-glass thermometer. The brackish and tap water samples used for depuration were subjected to the same physico-chemical analysis.

2.5. Statistical Analysis

Data are presented as mean ± standard deviation (SD). The one way analysis of variance (ANOVA) was used to analyse obtained data for significant differences using SPSS Inc., 2007.

3. Results

The changes in salinity and DO of brackish and tap water microcosms during depuration in dry and rainy seasons are presented in Figure 1. There was an initial increase in the salinity of BW followed by decreases after day 1 of depuration in the dry and rainy seasons. Similar trends were observed with the TW sample but with much lower salinity values. The DO values of BW decreased throughout the depuration period as well as those of TW in the rainy and dry seasons respectively.

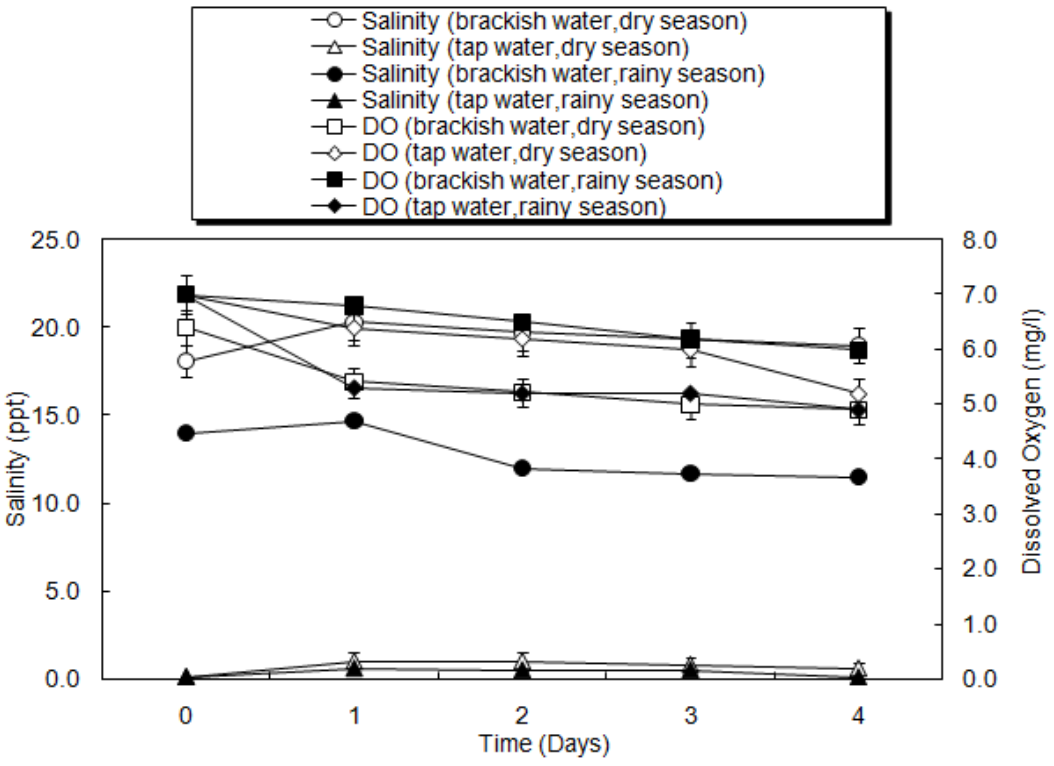

Figure 1. Changes in salinity and DO in brackish and tap water microcosms collected seasonally for oyster depuration. Shaded symbol = Rainy season samples; Unshaded symbol = Dry season samples. Values represent the mean of four determinations. Error bars represent standard deviations of four determination

The pH values were relatively stable in both the BW and TW microcosms during depuration (Figure 2). There was some degree of disparity with the conductivity values of BW during depuration; a high peak was observed on day 3 in the dry season as against absence of peak in the rainy season (Figure 2). The conductivity values were higher during the dry season than in the rainy season.

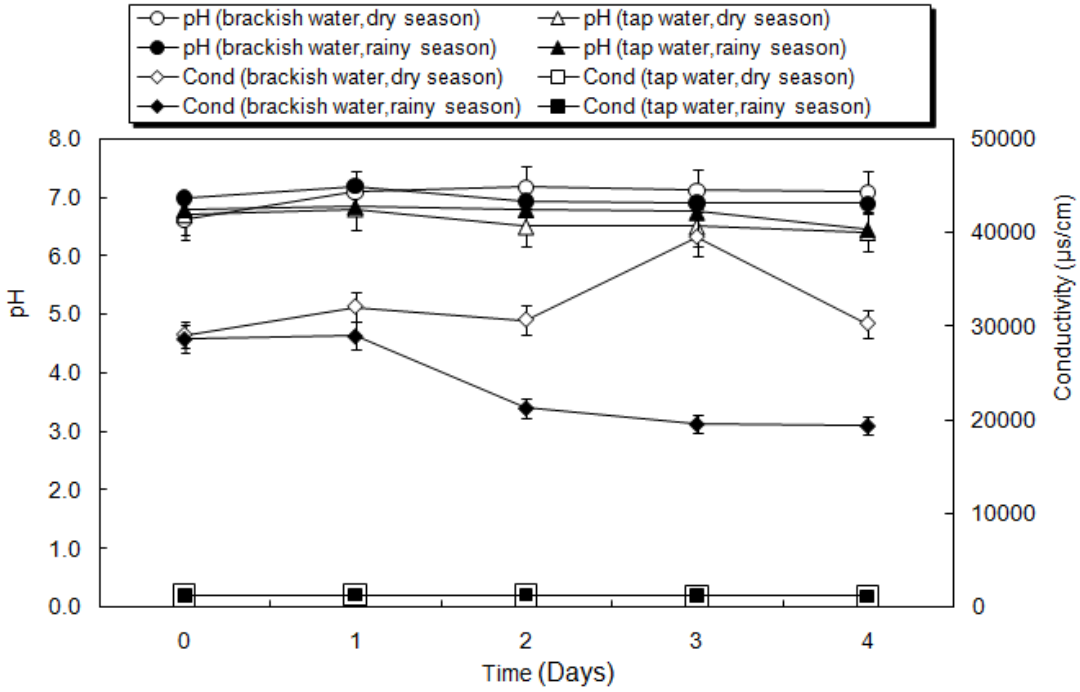

Figure 2. Changes in pH and conductivity of brackish and tap water microcosms collected seasonally for oyster depuration. Shaded symbol = Rainy season samples; Unshaded symbol = Dry seasons samples Values represent the mean of four determinations. Error bars represent standard deviations of four determinations.

The BW temperature was higher during the dry season than during the rainy season but was relatively stable during depuration (Figure 3). Similar findings were observed with the TW (Figure 3). There was a sharp increase in BW turbidity as opposed to TW during depuration in the rainy and dry seasons respectively.

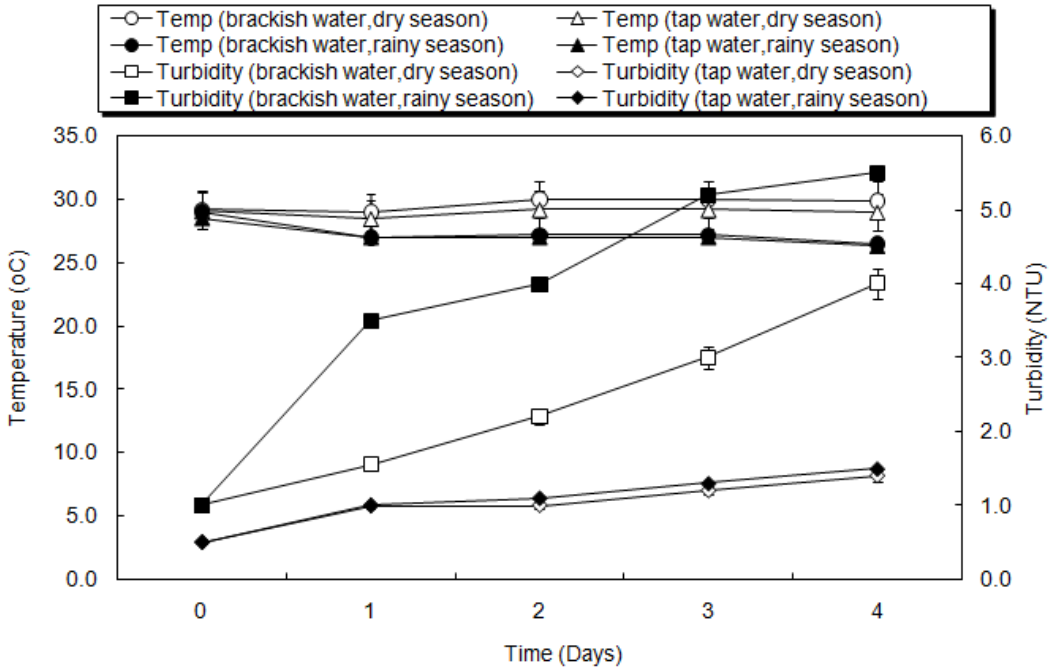

Figure 3. Changes in temperature and turbidity of brackish and tap water microcosms collected seasonally for oyster depuration. Shaded symbol = Rainy season samples; Unshaded symbol = Dry seasons samples. Values represent the mean of four determinations. Error bars represent the standard deviations of four determinations

There were initial sharp decreases in BOD values for both samples and thereafter followed by progressive increases after day 1 for the BW and decreases for the TW in BOD values for both seasons respectively (Figure 4).

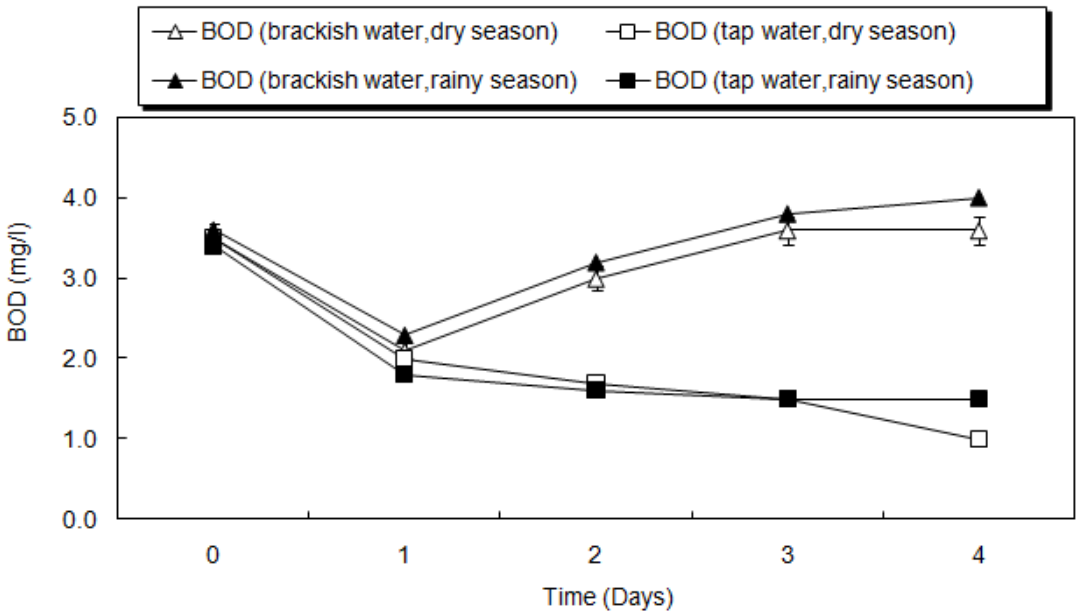

Figure 4. Changes in BOD of brackish and tap water microcosms collected seasonally for oyster depuration. Shaded symbol = Rainy season samples; Unshaded symbol = Dry seasion samples. *Values represent the mean of four determinations. Error bars represent standard deviations of four determinations*

In general, there were decreases in bacterial loads between days 0 and 1 followed by progressive increases except for *E. coli* (Table 1 and Table 2). Evidently, the APCs, CCs and VCs were higher for the dry season than those for the rainy season for both BW and TW. Comparatively, the APCs of oysters depurated in TW microcosm were higher than those of BW irrespective of seasons.

Table 1. Bacterial population (cfu/g) dynamics of oysters depurated in brackish and tap water microcosms during rainy season

		Total viable counts (cfu/g)			
Day	Sample	APC	CC	EC	VC
0	RO	$2.80x10^4$	$1.60x10^2$	$1.10x10^2$	$0.30x10^2$
1	BW	$7.00x10^2$	$1.00x10^2$	ND	ND
	TW	$5.00x10^2$	$3.00x10^2$	ND	ND
2	BW	$2.20x10^3$	$8.00x10^2$	ND	$1.40x10^2$
	TW	$1.15x10^2$	$8.60x10^3$	ND	ND
3	BW	$3.50x10^3$	$2.73x10^2$	ND	$4.50x10^2$
	TW	$2.70x10^5$	$2.88x10^5$	ND	$1.90x10^3$
4	BW	$1.40x10^5$	$6.30x10^4$	ND	$3.00x10^2$
	TW	$1.70x10^6$	$1.00x10^5$	ND	$1.38x10^3$

ND = Not dtected; RO = Raw oyster; BW= Brackish water; TW= Tap water; APC = Aerobic plate counts; CC = Coliform counts; EC = Escherichia coli counts; VC = Vibrio counts. Values represent the mean of four determinations.

Table 2. Bacterial population (cfu/g) dynamics of oysters depurated in brackish and tap water microcosms during dry season

0	RO	$2.45x10^5$	$1.42x10^4$	$2.00x10^2$	$2.95x10^3$
1	BW	$1.60x10^4$	$1.55x10^3$	ND	$3.50x10^2$
	TW	$2.80x10^4$	$1.04x10^3$	ND	$5.70x10^2$
2	BW	$1.81x10^4$	$2.85x10^3$	ND	ND
	TW	$1.33x10^5$	$1.86x10^4$	ND	$2.00x10^2$
3	BW	$1.73x10^5$	$1.05x10^4$	ND	$4.10x10^2$
	TW	$3.00x10^6$	$1.90x10^5$	ND	$2.30x10^3$
4	BW	$2.25x10^6$	$2.49x10^4$	ND	$1.70x10^3$
	TW	$1.95x10^7$	$2.11x10^5$	ND	$6.30x10^4$

ND = Not dtected; RO = Raw oyster; BW= Brackish water; TW= Tap water; APC = Aerobic plate counts; CC = Coliform counts; EC = Escherichia coli counts; VC = Vibrio counts. Values represent the mean of four determinations.

The bacteria isolated from the samples are shown in Table 3. The most diverse bacterial genera occurred in the brackish water while the least diverse was observed in the tap water microcosms.

Table 3. Bacterial flora isolated from tap water, oyster and brackish water samples during depuration

	Samples		
Bacteria	Tap water	Oyster	Brackish water
Pseudomonas spp	+	+	+
Vibrio spp	+	+	+
Proteus spp.	+	+	+
Bacillus spp.	+	+	+
Staphylococcus aureus	+	+	+
Escherichia coli	+	−	+
Lactobacillus spp	+	+	+
Enterobacter aerogenes	−	−	+
Micrococcus spp	−	+	+
Acinetobacter sp	−	+	+

4. Discussion

The results of this investigation indicate that appreciable changes occurred in the physico-chemical parameters, bacterial profiles of the microcosms and the oyster samples (Figure 1-Figure 4 and Table 1-Table 3). The progressive decreases in salinity during depuration of oysters in both microenvironments may be related to the ability of bivalves to assimilate substances from their environment, digest and discharge some in form of pseudofaeces [21,22]. The decreases in DO in both microcosms during depuration may not be unconnected with the presence of materials of high organic content leading to oxygen depletion (Figure 1). The occurrence of DO deficit is indicative of deoxygenation rate due to higher biological decomposition of organic matter compared with reoxygenation from the microenvironment or may be due to the presence of oxidizable minerals in the microcosms of stagnant water which has been reported

by previous workers [23,24,25]. The DO of not less than 2.0mg/l has been recommended [22] as conducive for depuration of bivalve. Thus, the DO values observed in the depuration microcosms were adequate for the oysters.

The relatively stable pH values of both microenvironments may be attributed to the activities of microorganisms which make their environment more alkaline by generating ammonia through amino acid degradation or production of acidic or basic metabolic waste products [7,26,27].

The increase or fluctuation in conductivity values of the brackish water microenvironment may be due to the concentration of ions by evaporation, coupled with increased mineralization of organic matter in the stagnant unagitated water. On the other hand, the relatively stable conductivity values of TW microenvironment could be attributed to poor conductivity and ionic concentration while those of the BW reflect the status of inorganic constituents [25]. The concentrations of ions of metals and nonmetals have been earlier reported to affect the metabolism of bacteria and higher organisms in brackish water [28]. This may partly explain the shorter shelf-life of oysters in TW than in BW during the depuration.

Similarly, the consistent temperatures observed throughout depuration suggest their probable beneficial effects on the ecology of oysters and their sustainability. But the progressive increases in turbidity of both water bodies may not be unrelated to the metabolic activity of organisms and the discharge of pseudofaeces in the microenvironment. In addition, the natural status of the water used for depuration may contribute to changes in turbidity. Lio-Po [22] had earlier reported 77NTU as the maximum allowable turbidity in a depuration system and the results obtained in this work are lower and hence adequate for the physiological activities of the oysters.

The observed increases in BOD (Figure 4) in the BW microenvironment could be related to rapid biological depletion of organic matter as a result of proliferation of diverse bacterial groups (Table 3). In contrast, the decrease in BOD of TW microcosm may not be unconnected with the composition of the medium and the bacterial content. Consequently, the poor activity of oysters in TW may be related to the influence of physico-chemical parameters and these may explain the higher bacterial loads in TW than in BW microcosms (Table 1 and Table 2).

Depuration has been one the strategies used in eliminating oyster-associated bioindicators, microbial pathogens and viruses [6,8,28] and therefore constitutes a veritable tool for assessing and achieving microbial safety of oysters. The APCs in oysters depurated in BW and TW microcosms during the dry season were comparatively higher than in the rainy season (Table 1 and Table 2) most probably due to evaporation effects. The apparent decreases in APCs following day 1 of depuration were indicative of the beneficial effects associated with purification of contaminated shellfish [7,27,29]. The increases in APCs after day 1 may be attributed to selective mechanism which operates in the gut and intervalvular fluid of oysters that favours the retention and multiplication of some microorganisms such as gram-positive cocci and *Vibrio* species [30,31].

The present work has also shown that coliforms, especially *E. coli* were not selectively retained in the gut but were readily eliminated from the oyster during depuration such that *E. coli* became undetectable after day 1 (Table 1 and Table 2). This corroborates the findings earlier reported by other workers [31,32]. The reduction in APCs and CCs in BW microcosms than in TW used for oyster depurations in both rainy and dry seasons (Table 1 and Table 2) further confirms the beneficial impacts of BW in terms of enhanced microbial safety.

5. Conclusions

The physico-chemical parameters of the microcosms influenced the diversity and composition of bacterial flora as well as the activity and viability of the oysters. However, this study has provided baseline information on the range of physico-chemical parameters needed to understand the dynamics of bacterial communities available in the depuration system. It also showed that the conditions affecting oyster activity during depuration in the microcosms drastically affect the profiles and manner of bacterial elimination.

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