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Fecal coliform accumulation and depuration in the oyster *Crassostrea gigas*

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Abstract

Experiments on fecal coliform accumulation and depuration in the oyster *Crassostrea gigas* were performed under two seasons (winter, summer), under various conditions of bacterial concentration (from 10^1 to 10^3 CFU ml^{-1}) and suspended matter (10 to 50 mg l^{-1}). Contamination process in the bivalve is mainly influenced by the bacterial density in the seawater. Influence of suspended matter concentration was less effective. Maximal bacterial accumulation was reached within 30 min. in summer (18 °C) and 5 hours in winter (11 °C). Concerning depuration process a 10 fold decrease of initial contamination required 3 hours and a 100 fold decrease was achieved within 10 hours. Time required for depuration was mainly dependent on the initial bacterial concentration in the oyster.

Introduction

Edible bivalve are often reported to be responsible for human diseases of bacterial origin, due mainly to pathogen enteric bacteria (PRIEUR et al. 1990, WEST 1989). Those health hazards essentially result from the accumulation and retention of bacteria in the bivalve tissues. Bacterial enrichment in the bivalve has been reported for a wide diversity of bivalves, generally from studies in uncontrolled natural conditions.

The aim of the present study was to obtain quantitative data about accumulation, retention and release of bacteria in the edible oyster *Crassostrea gigas*, and to establish the influence of environmental parameters on accumulation and depuration processes under controlled conditions.

Seasonal incidence was tested by two series of experiments conducted respectively in winter with seawater temperature from 9.7 to 12 °C and in summer with seawater temperature from 16 °C to 19 °C. Environmental parameters experimentally controlled during the survey were respectively the bacterial density

in seawater (with fecal coliform concentration ranging from 10 to 10^3 ml^{-1}) and particle load in seawater (with suspended matter concentration ranging from 10 to 50 mg l^{-1}).

Material and methods

The experimental study was performed in a laboratory microcosm in conditions close to natural conditions. This system, described more precisely by BEUCHER et al. (in preparation) allowed alternating contamination and depuration non-inferring periods without turbulence. Independent measurements were performed from 12 identical experimental tanks.

Oysters were contaminated by a seawater-sewage mixture (primary sewage). Bacterial contamination was carried out during a 24 hours period. Depuration was obtained by putting the system under running pure seawater for 24 to 42 hours. Bacterial contamination in seawater and oysters was followed through the enumeration of fecal coliforms in seawater and oyster whole-tissue, sampled periodically during each cycle. Suspended matter in seawater was measured in parallel.

Five oysters showing filtration activity were analysed for each sampling time to follow individual variability. Seawater and oyster samples were homogenized in a "stomacher" mixer. Fecal coliforms were enumerated in MFC medium by pour-plate method or membrane filtration technique.

This experimental study has been conducted according to a defined experimental schedule allowing data to be statistically analysed by ANOVA (BEUCHER et al. in preparation).

Results and discussion

Twelve complete experiments (contamination and depuration) have been realized: 6 in winter, 6 in summer. For each seasonal survey three contamination levels in the seawater were used during contamination period (high contamination: hC, medium contamination: mC, low contamination: lC). They were combined with two suspended matter levels in seawater (high concentration: hSM, low concentration: lSM).

Kinetics of contamination and depuration in both seawater and oyster tissues are represented in Fig. 1 (winter) and Fig. 2 (summer).

Contamination profiles in the oyster show an increasing contamination followed by a more or less steady equilibrium level. Two main conclusions can be infered from these profiles: the time necessary to reach this steady state and the enrichment factor in the oyster tissue. According to CABELLI and HEFFERNAN (1970a), this factor is the ratio between bacterial number per gram of bivalve tissue and bacterial number per ml of seawater at the equilibrium state. Depuration experiments allowed to conclude in terms of feature of depuration profile and time necessary to reach an undetectable bacterial contamination in the oyster tissue. In parallel with suspended matter and bacteriological measurements the oyster activity has been surveyed and expressed as cumulative percentages of oysters displaying a filtration activity (BEUCHER et al. in preparation).

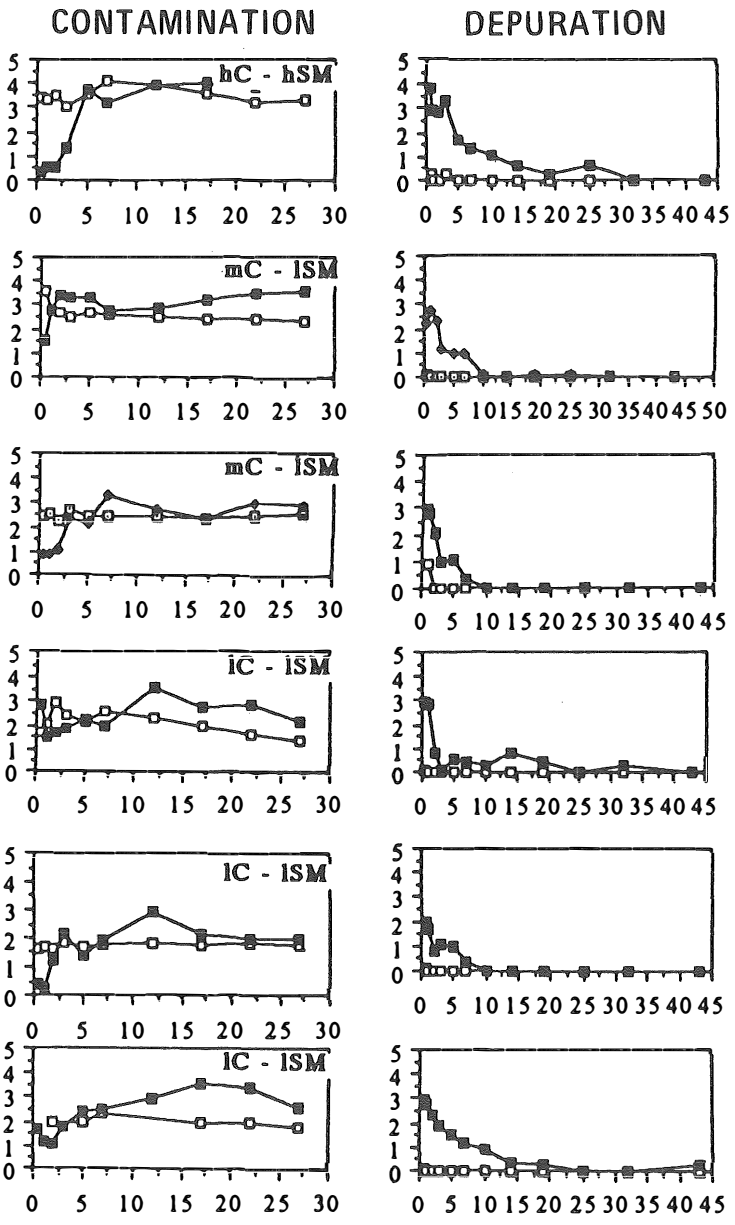

Fig. 1. Kinetics of contamination and depuration in seawater (□) and oysters (■)during winter. Abscissa: time in hours. Ordinate: log·(CFU per ml of seawater or per g of bivalve tissue).
High contamination: hC, medium contamination: mC, low contamination: lC, high concentration of suspended matter: hSM, low concentration of suspended matter: lSM.

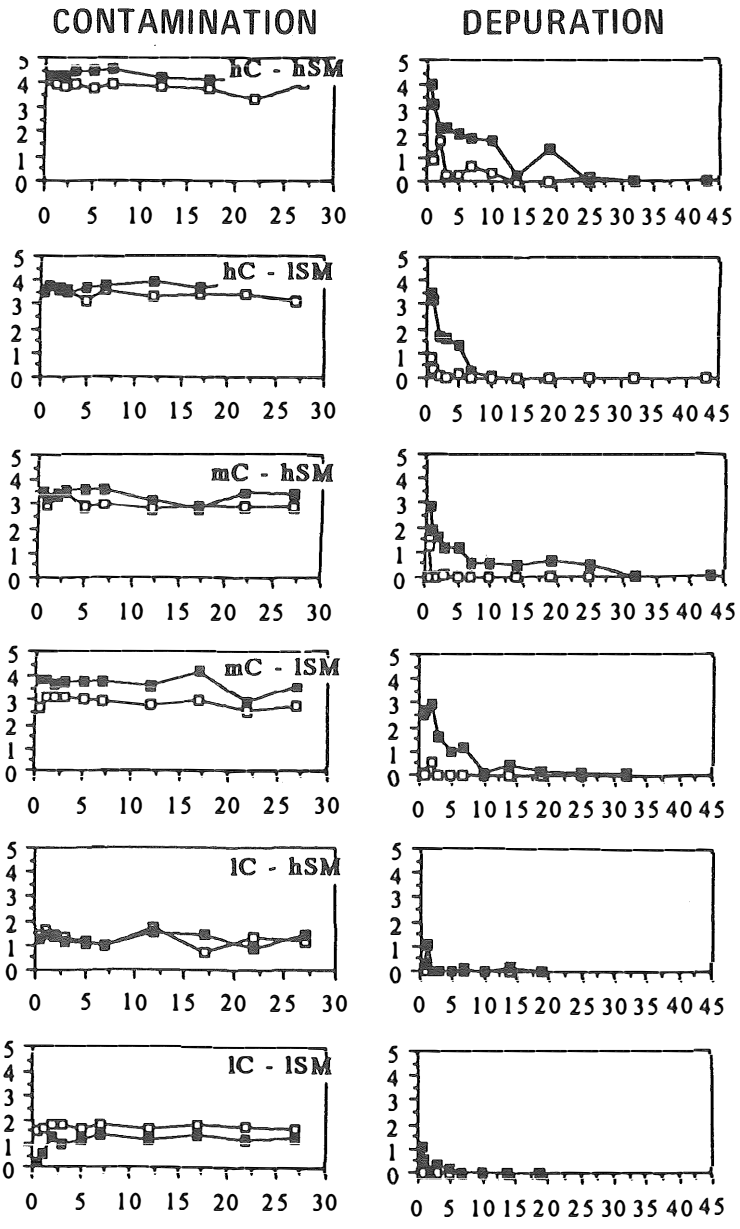

Fig. 2. Kinetics of contamination and depuration in seawater (□) and oysters (■) during summer. Abscissa: time in hours. Ordinate: log (CFU per ml of seawater or per g of bivalve tissue).
High contamination: hC, medium contamination: mC, low contamination: lC, high concentration of suspended matter: hSM, low concentration of suspended matter: lSM.

Statistical analysis of all the results concluded that the bacterial contamination in the oyster depended for 80 to 90 % on the bacterial density in seawater.

The season markedly influences oyster filtration activity. Such a reduction of bivalve filtration rate at low temperature had often been mentioned (CABELLI and HEFFERMAN 1970a, BAYNE 1973, 1976, BAYNE and NEWELL 1983). Consequently a maximal contamination in the oyster is reached within less than 30 min. in summer, versus 5 hours in winter. Furthermore the bacterial enrichment in oyster tissue (as defined previously) is higher in winter (E = 6) than in summer (E = 2). Literature data concerning bacterial enrichment by bivalves are ranging in quite a wide scale: from 3 to 50 (PRIEUR et al. 1990). This enrichment depends on the bacterial group considered (DELATTRE and DELESMONT 1981, PLUSQUELLEC et al. 1983), the bivalve species (CABELLI and HEFFERNAN 1970b) and the experimental conditions (PLUSQUELLEC et al. 1990). Consequently the enrichment values presented here are only representative of our experimental conditions: enumeration of fecal coliforms after experimental contamination of oyster by natural sewage.

A weak but significant interaction between bacterial contamination and suspended matter has been shown, confirming CABELLI and HEFFERNAN (1970a) findings, who estimated that the enrichment factor was correlated to the ratio between *Escherichia coli* and total ingested particles. Consequently, the increase of suspended matter in seawater at steady bacterial concentration leads to a reduced bacterial enrichment.

In terms of depuration process, profiles of Fig. 1 and 2 show a rather homogeneous trend. The depuration rate decreases with time. As a consequence, a 10 fold reduction of initial contamination of oyster is achieved within 3 hours under pure running seawater whereas 10 hours are necessary to reach a 100 fold reduction.

The oysters depuration delay is dependent on the bacteriological technique used. In the present study, when all the experiments are grouped, the average time to obtain absence of fecal coliform in one gram of oyster is 27 hours. This time is markedly dependent on the contamination of the oyster at the initiation of depuration period. In addition, we have to emphasize that analyses were performed only with bivalves displaying a filtration activity. In winter, some contaminated oysters did not present filtration activity during depuration period over 27 hours, and so, one can assume that, at low temperature, very long depuration periods are necessary to obtain depurated oysters.

Consequently, quantitative data (contamination level) and qualitative data (filtration activity) have to be both considered to estimate health hazards related to an oyster population.

The previous data have been used to conduct a further study in conditions close to natural conditions with alternation of contamination and depuration periods interrupted by emersion periods. The results of this study will be combined to the present data for a more complete and synthetic presentation of contamination and depuration process in the oyster *Crassostrea gigas* (BEUCHER et al. in preparation).

Acknowledgements

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