

Study of the efficacy of a closed cycle depuration system on bivalve molluscs

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Summary

The aim of this study was to develop and apply protocols for the microbiological depuration of bivalve molluscs (*Chamelea gallina* and *Mitylus galloprovincialis*). The study was divided into the following phases: preparation of two closed-circuit tanks fitted with mechanical, chemical and biological filtration systems for the depuration of bivalve molluscs, mollusc contamination and depuration, analyses of molluscs and depuration waters (biometric and microbiological tests) and statistical processing of the experimental data. The *Escherichia coli* challenge revealed the high efficacy of the depuration system and thus the possibility of preparing the molluscs for sale in a relatively short period of time. Depuration tests against *Salmonella* Typhimurium were effective only after 72 h to 84 h. Results for *Vibrio parahaemolyticus* revealed only a small reduction in the bacterial load during the entire observation period. However, depuration times were shorter in trials with mussels: *V. parahaemolyticus* was no longer detected after 36 h to 48 h.

Keywords

Depuration, Bivalve mollusc, *Chamelea gallina*, Clam, *Escherichia coli*, Food safety, Italy, *Mitylus galloprovincialis*, Mollusc, Mussel, *Salmonella* Typhimurium, *Vibrio parahaemolyticus*.

Introduction

Background

In Italy, bivalve molluscs represent only a small proportion of the national fishing industry. However, the activities of this sector, which involve large stretches of Italian coastlines, have a very high impact from a social viewpoint, which has encouraged the Ministry of Agricultural Policies and regional authorities to implement policies to reinforce the sector and organise employees more effectively (16). The striped Venus clam (*Chamelea gallina*, Linnaeus, 1758) is one of the most popular bivalve molluscs and its high density along the Adriatic coast creates significant levels of direct and indirect employment. National production is currently around 50 000 tonnes per year.

C. gallina is a mollusc of the family *Veneridae* which is widespread in the Mediterranean. In Italy, it is relatively common, especially in the north and central Adriatic, from the more northerly of the east coasts to the Gargano promontory; it is also present in the Ionian Sea and the southern to central part of the Tyrrhenian Sea. Clams live in large groups on sand or sand and mud beds along the coastline at a depth of 0 to 15 m. Morphologically, they are almost triangular shells with valves of the same size and shape which narrow towards the top, widening out and rounded at the edges; they have a depression on the front edge near the umbo, which is small and faces forward. The valves are robust and bulge

upwards. The outer surface is covered with numerous prominent concentric ribs, often crossed by very thin, irregular radial rings. Externally, they are whitish or whitish-grey, with speckled brown dots, stripes and lines that are usually arranged in radial bands; the inside is whitish with a pale purple blotch towards the back.

Bivalves feed by filtering organic particles from surrounding water. They are thus naturally exposed to the risk of build-up of biological and chemical pollutants (pathogens, algal biotoxins and metals). Species living on the seabed, such as clams, are found in close contact with the sediment, in which pollutants are generally more concentrated than in the water. Moreover, *C. gallina* are fished within a kilometre of the coast, whereas molluscs are more exposed to pollutants from the land.

Although bivalves must be alive at time of purchase, viability is not in itself a guarantee of healthiness, as the product may become contaminated before harvesting or during the handling, transport and distribution phases. Various hygiene and health standards have therefore been implemented to guarantee product quality and thus protect public health. These standards include classification of the waters in which the bivalves live, as well as the compulsory depuration of molluscs from certain production areas in approved centres supplied with clean marine water. The safety of food production has always been a crucial aspect of seafood farming. The monitoring of molluscs and their environment has the sole objective of guaranteeing food safety through compliance with legislative hygiene and health requisites. Various EC regulations on food safety (3, 4) lay down specific hygiene parameters for bivalve molluscs for human consumption; some of these refer to microbiological levels of *Escherichia coli* and *Salmonella*, which are not correlated but the limits of which determine the product's marketability.

Production areas are currently classified on the basis of the *E. coli* level measured in the meat

and the shell liquor (4, 5). On the basis of the latter, areas are classified as follows:

- '1) category A: bivalve molluscs from these areas must not exceed *E. coli* levels of 230 mpn per 100 g of meat and shell liquor': molluscs can be harvested and used for direct human consumption without preventive treatment
- '2) category B: bivalve molluscs from these areas must not exceed *E. coli* levels of 4,600 mpn per 100 g of meat and shell liquor': molluscs can only be harvested and destined for human consumption following treatment in a depuration centre or relaying in an area with the microbiological, biological, chemical and physical requisites laid down for category A zones; if they are not depurated, they should undergo processing by heat treatment in an approved establishment
- '3) category C: bivalves from these areas must not exceed *E. coli* levels of 46 000 mpn per 100 g of meat and shell liquor': molluscs can be destined for direct human consumption only after at least two months relaying in an area with the microbiological, biological, chemical and physical requisites laid down for category A zones, or processing in an approved establishment after adequate heat treatment
- '4) prohibited areas: harvesting is prohibited in areas where *E. coli* levels exceed 46 000 mpn per 100 g of meat and shell liquor'.

Ready-for-sale products must also be *Salmonella*-free (2).

Depuration must be performed in Ministry of Health-approved centres and must ensure that the molluscs respond to the parameters above through excretion of residual contamination. The molluscs must be able to rapidly return to feeding via filtration and must maintain their viability.

Given the features of the habitat of *C. gallina*, the depuration of molluscs harvested from authorised areas not accepted for direct consumption (categories B and C) is clearly an

essential treatment to recover enormous quantities of molluscs that would otherwise be sold only after undergoing suitable heat treatment. *C. gallina* is particularly sensitive to environmental stress and has a notoriously low filtration capacity with respect to other bivalve molluscs; in fact, until 1992, it was not included in the legislation as a species that could be treated by depuration. On account of this factor, its adaptability to the depuration process and the real efficacy of the latter have been debated for many years. In this study, the efficacy of depuration against the pathogens *E. coli*, *S. Typhimurium* and *V. parahaemolyticus* was tested for clams of the target species against mussels of the species *Mitylus galloprovincialis* (considered as treatable by depuration and regarded as having a high filtration capacity) in order to compare the depuration efficiency of the trial protocols in species with different filtration capacities.

Depuration systems

The depuration systems currently in use can be classified as continuous flow (open) and recirculated (closed) systems. While both systems are effective, they present different benefits and drawbacks. Continuous flow systems need a greater quantity of water than closed systems. In closed systems, the seawater is reused, with replacement of 5-10% over 24 h, and it must be physically (UV) or chemically (chlorine or ozone) treated to avoid microbial contamination.

Disinfection of water for mollusc depuration is based on the use of specific chemicals, namely: chlorine, iodoform and ozone, or physical agents, such as UV and filtration (1). Depuration with chlorine was the first procedure used and although it is effective in reducing bacterial contamination, it is not as effective against enteric viruses. In addition, even at low concentrations, chlorine can affect the filtration activity of the mollusc and, consequently, residual chlorine must be removed by thiosulphate and aeration before the water is introduced into the depuration tank. Although effective against enteric bacteria, chlorine treatment is ineffective against bacterial spores, viruses and protozoa.

Furthermore, the risk of production of secondary mutagens or carcinogens during chlorination means that it is not used as a stand-alone treatment in depuration processes.

Iodoforms are used at concentrations of 0.1 mg/l to 0.4 mg/l. They have no apparent effect on mollusc activity or on their edibility and quickly reduce bacterial concentrations, but they are ineffective against enteric viruses, except at concentrations that damage the molluscs themselves (hepatitis A virus is inactivated at iodine concentrations exceeding 100 ppm).

Ultraviolet radiation is an effective disinfection method that is used widely for water purification in depuration systems. The UV rays inactivate enteric bacteria and viruses and protozoan and bacterial spores without producing toxins or other chemical residues. The drawback of UV radiation is that under certain conditions, some micro-organisms can repair their cell damage and reactivate: in bacteria especially, the mutagenic effects of UV radiation can be repaired by exposure to wavelengths in the visible range (320-400 nm). Systems using UV lamps for disinfection must use filters to remove the suspended material as the presence of suspended particles limits the penetration of the UV rays and reduces the efficiency of the treatment. For this reason, the method has very high running costs.

The use of ozone disinfection has become increasingly popular in recent years. Ozone acts on bacteria through a combination of protein oxidation, alteration of the molecular structure and enzyme inhibition. Viruses are destroyed in the same way, although their lack of a cell membrane facilitates oxidation of their proteins.

After depuration, the water is directed into the tanks holding the mussels, which are able to excrete their accumulated micro-organisms through their natural filtration system. The combination of various water treatment mechanisms boosts the efficiency of depuration and reduces the time required to complete the process. The depuration times currently used (12-24 h) are based on bacteriological parameters, although it has

often been demonstrated that virus clearance times are longer than those required for faecal and pathogenic bacteria. It has also been amply demonstrated that the presence of virus is not always correlated with the presence of faecal bacteria. Finally, the *Vibriosis* should not be forgotten: many *Vibrio* species form part of the native aquatic bacterial flora and approximately half of these species have been implicated in infections in humans or aquatic animals. Some *Vibrio* infections are considered to be important for public health because they cause diseases that require quarantine and notification of the World Health Organization (e.g. *V. cholerae*), are known to cause high mortality rates (e.g. *V. vulnificus*) or because they cause a high number of food poisoning cases in temperate countries (e.g. *V. parahaemolyticus* in Japan).

These considerations motivated the choice of depuration system tested here (closed system fitted with chemical and physical water treatment mechanisms) and the pathogens used for contamination (*S. Typhimurium*, *E. coli* and *V. parahaemolyticus*).

Materials and methods

Trial specimens

Sampling methods

Clams were taken 200 m from the mouth of the Rio Vivo (south Termoli coast) and the mouth of the Trigno (north Termoli coast). Samples were taken by boats fitted with hydraulic dredges. The mussels used in the depuration tests were collected from natural banks near the mouth of the Rio Vivo and from the Lagmar mussel farm (north Termoli coast). The specimens (mean size: 25–32 mm for clams and 5–8 cm for mussels) were taken to the laboratory in refrigerated containers (4°C) and inspected.

Preparation of trial specimens (screening and acclimatisation)

Dead or damaged specimens were discarded and the remainder were divided into aliquots of 100 clams or 50 mussels. Before the trial, specimens were acclimatised in aquariums with artificial seawater (Instant Ocean

Aquarium System, salinity: 35‰) to confirm their viability and filtration capacity (Fig. 1).

Bacteriological examination

Samples taken from the same site as the trial specimens underwent bacteriological analysis (*E. coli*, *S. Typhimurium* and *V. parahaemolyticus*) to evaluate the initial load of each pathogen and thus determine contamination loads.



Figure 1
Acclimatisation tank

Sample contamination

Bacterial strains used

E. coli ATCC 25922, *S. Typhimurium* ATCC 14028 and *V. parahaemolyticus* ATCC 17802 were used for the trial.

Contamination method

Sample contamination was conducted in 8 l (0.12 m³) tanks filled with artificial seawater (Figs 2 and 3), which were inoculated with specific bacterial suspensions to reach the following concentrations:

- 1.87*10⁴; 3.75*10⁴ *E. coli*/ml
- 9.37*10³; 1.87*10⁴ *S. Typhimurium*/ml
- 9.37*10³; 1.87*10⁴; 3.75*10⁴ *V. parahaemolyticus*/ml.

Samples were exposed to contamination for 60–70 h and then transferred to the depuration systems.

Depuration

Depuration system used

The depuration systems consisted of 197 × 72 × 45 cm tanks fitted with a wool/Perlon pre-filter and hyperactive carbon filter, an active biological filter using *Lithothamnium calcareum* algae and a UV sterilisation plant (Fig. 4).



Figure 2
Chamelelea gallina contamination

Figure 5 shows the layout of the system and its various components.

The pump takes water from the tank containing the biological filter (*L. calcareum* algae) and pumps it into the UV sterilisation unit and then on through the bubbler to reach the tank. It then crosses to the chamber fitted with the wool/Perlon and the carbon filters, through the serpentine cooling unit (in which ozonation also takes place) and back to the tank containing the biological filter. The water is then taken back up by the pump and re-circulated. The water temperature is regulated by thermostat.

Specimens were placed in a single layer on a plastic grill to avoid contact with the bottom of

the tank and thus minimise recontamination (Fig. 6).

The chemical and physical parameters of the water (temperature, salinity, dissolved oxygen) in the aquarium were checked regularly.

Sampling

Water samples were taken every 24 h to check that the depuration system was functioning correctly. Mollusc samples were collected before being placed in the depuration tank and then every 6 h to 12 h, so as to measure the bacterial load over time and thus establish the depuration times necessary before marketing the product. Samples were processed immediately using the methods reported below.

Escherichia coli, *Salmonella* Typhimurium and *Vibrio parahaemolyticus* counts in molluscs and water

Molluscs

The presence and counts of *E. coli* were tested by culturing in a liquid medium containing chromogen (5-bromo-4-chloro-3-indolyl beta-D-glucuronide) and calculating the most probable number (mpn) after incubation at both 37°C and 44°C (9). *Salmonella* spp. were evaluated using the procedure (mpn) reported in the Food and Drug Administration's



Figure 3
Mytilus galloprovincialis contamination



Figure 4
Trial depuration system

Bacteriological analytical manual (6). *V. parahaemolyticus* was analysed using an in-house method (mpn) involving enrichment in alkaline peptone water, subculture at 6 h to 8 h, inoculation on selective medium (thiosulfate-citrate-bile salts-sucrose: TCBS) and biochemical confirmation with individual and miniaturised tests (API 20E/API 20NE).

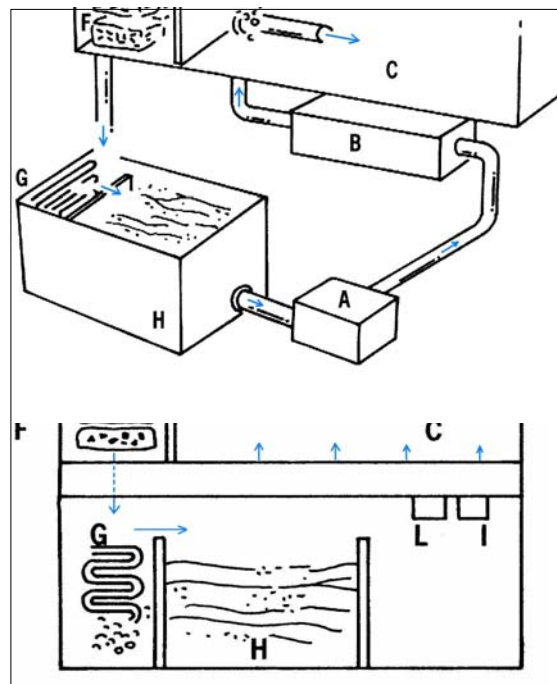
Water

Salmonella spp. and *E. coli* levels were evaluated according to International Standardization Organisation (ISO) 6340/1995 (8) and *Istituto di Ricerca sulle Acque-Consiglio Nazionale delle Ricerche* (IRSA-CNR) analytical methods for water (10).

Results

Bacteriological examination of trial specimens

Analysis of specimens taken from the sampling site revealed low *E. coli* values (<230 mpn/100 g) in all cases. *S. Typhimurium* and *V. parahaemolyticus* were absent in all samples.



- A) Pump
- B) UV sterilisation unit
- C) Tank
- D) Bubbler
- E) Wool/Perlon filter
- F) Carbon filter
- G) Serpentine cooling unit
- H) Biological filter
- I) Ozonation air pump
- L) Ozonator

Figure 5
The depuration system



Figure 6
Mytilus galloprovincialis specimens undergoing depuration

Escherichia coli trials

Trials with *E. coli* revealed that clam depuration times depended on the initial level of contamination. In tests 1 and 2, depuration was performed for samples simulating a category C origin (*E. coli* <46 000 mpn/100 g)

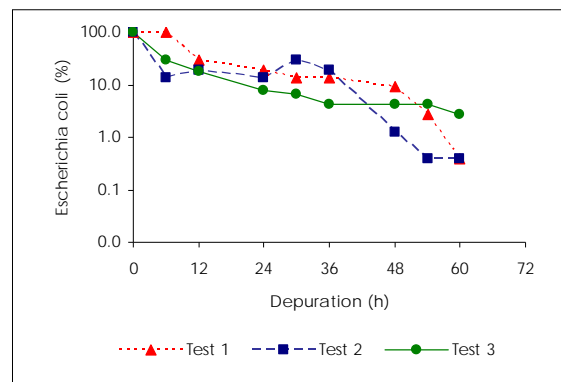
(Table I), while test 3 involved the depuration of category B samples (*E. coli* <4 600 mpn/100 g). Bacterial counts over time (in absolute and percentage terms) (Table I; Fig. 7) revealed a considerable reduction in bacterial load (including 90-99%) in all three trials. *E. coli* values <230 mpn/100 g were found after 48-60 h in samples simulating a category C origin, and after 24 h in category B samples. These levels, which make the product marketable, were maintained up to the end of the trial, demonstrating the efficacy of the depuration process and the consequent possibility of marketing products that were still fresh.

Results for mussels revealed *E. coli* loads of under 230 mpn/100 g after only 6-12 h of depuration. Mussels therefore showed a greater depuration capacity than clams. Furthermore, after depuration lasting 48 h, the bacterial load had been reduced by 99.5%.

Salmonella Typhimurium trials

Tests conducted for *S. Typhimurium* simulated the depuration of clam samples with baseline bacterial loads of between 430 mpn/g and 930 mpn/g. On the basis of the bacterial counts over time (in absolute and percentage terms) (Table II; Fig. 8), clams can be considered as free from *Salmonella* after 72-84 h depuration. In percentage terms, the load reduction was 99.9%.

A) Clam trials



B) Mussel trials

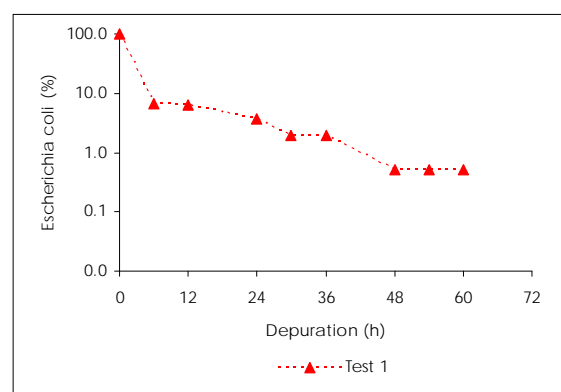


Figure 7 Percentage variation in *Escherichia coli* load against the baseline during clam and mussel trials

Table I Effect of depuration on molluscs contaminated with *Escherichia coli*

Depuration (h)	Test 1 ^(a)	<i>Escherichia coli</i>			Test1 ^(a) <i>Mytilus galloprovincialis</i> (mpn/100 g)
		Test 2 ^(a, b) <i>Chamelea gallina</i> (mpn/100 g)	Test3 ^(a)		
0	18 000	18 000	1 700	3 500	
6	18 000	2 400	490	230	
12	5 400	3 500	310	220	
24	3 500	2 400	130	130	
30	2 400	5 400	110	68	
36	2 400	3 500	70	68	
48	1 700	220	70	<18	
54	500	70	70	<18	
60	70	70	45	<18	

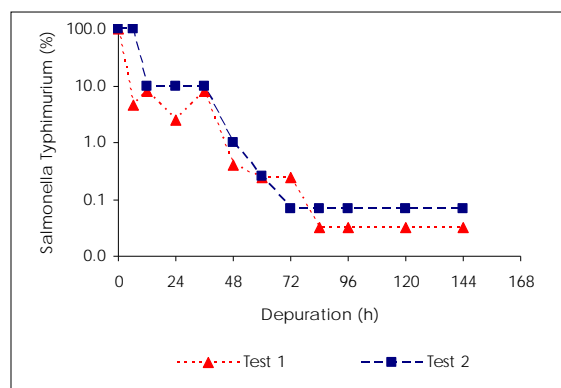
a) load inoculated in the contamination tank: 1.87 *10⁴/ml
 b) load inoculated in the contamination tank: 3.75 *10⁴/ml

Table II
Effect of depuration on molluscs contaminated with *Salmonella* Typhimurium

Depuration (h)	<i>Salmonella</i> Typhimurium			
	Test 1 ^(a) <i>Chamelea gallina</i> (mpn/g)	Test 2 ^(b)	Test 1 ^(a) <i>Mytilus galloprovincialis</i> (mpn/g)	Test 2 ^(b)
0	930	430	230	230
6	43	430	230	430
12	75	43	9.3	46
24	23	43	2.3	<0.3
36	75	43	<0.3	<0.3
48	3.8	4.3	<0.3	<0.3
60	2.3	1.1	<0.3	<0.3
72	2.3	<0.3	<0.3	<0.3
84	<0.3	<0.3	<0.3	<0.3
96	<0.3	<0.3	<0.3	<0.3
120	<0.3	<0.3	<0.3	<0.3
144	<0.3	<0.3	<0.3	<0.3

a) load inoculated in the contamination tank: $9.37 \cdot 10^3$ /ml
b) load inoculated in the contamination tank: $1.87 \cdot 10^4$ /ml

A) Clam trials



B) Mussel trials

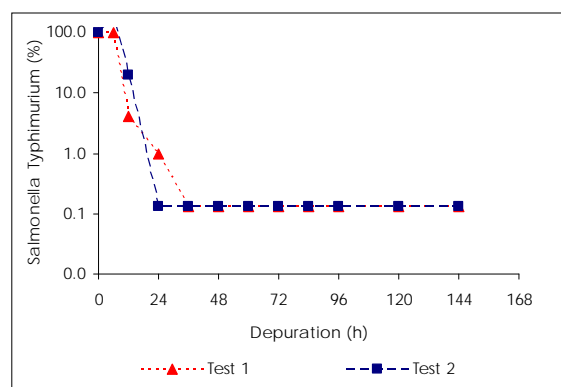


Figure 8
Percentage variation in *Salmonella* Typhimurium load against the baseline during clam and mussel trials

Results for mussels revealed *S. Typhimurium* loads of below 0.3 mpn/g after 24-36 h of depuration. After 48 h depuration, the bacterial load was reduced from the baseline 230 mpn/g by 99.9%.

Vibrio parahaemolyticus trials

Tests conducted with *V. parahaemolyticus* simulated the depuration of samples with baseline bacterial loads of between 2 400 mpn/100 g and 9 000 mpn/100 g (Table III). Results for clams revealed only a small reduction in the bacterial load during the entire observation period. In tests 1 and 2, a decrease of approximately 75% in bacterial load was seen after 24 h (Fig. 9A). These values remained relatively constant throughout the subsequent observation period. In contrast, tests on mussels showed values of less than 0.3 mpn/100 g after 36-48 h depuration. These values also remained almost constant throughout the observation period that followed. In percentage terms, the drop was between 99.5% and 99.9% after 36-48 h (Fig. 9B).

Specimen mortality during trials

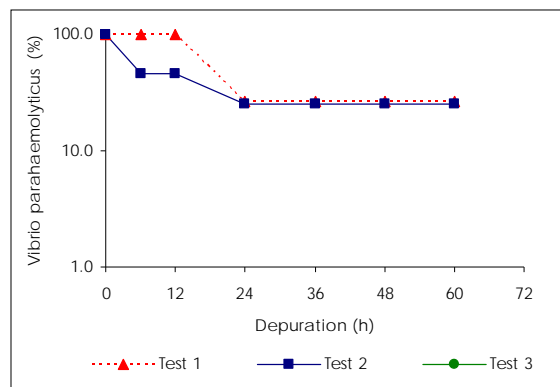
Mortality was found to be zero or close to zero during all trials.

Table III
Effect of depuration on molluscs contaminated with *Vibrio parahaemolyticus*

Depuration (h)	<i>Vibrio parahaemolyticus</i>					
	Test 1 ^(a)	Test 2 ^(b) <i>Chamelea gallina</i> (mpn/100 g)	Test 3 ^(c)	Test 1 ^(a)	Test 2 ^(b) <i>Mytilus galloprovincialis</i> (mpn/100 g)	Test 3 ^(c)
0	9 000	5 200	2 400	5 400	5 400	2 400
6	9 000	2 400	2 400	230	230	2 100
12	9 000	2 400	2 200	92	110	230
24	2 400	1 300	2 200	30	92	36
36	2 400	1 300	2 200	<0.3	230	30
48	2 400	1 300	2 200	<0.3	<0.3	<0.3
60	2 400	1 300	2 200	<0.3	<0.3	<0.3

a) load inoculated in the contamination tank: 9.37×10^3 /ml
 b) load inoculated in the contamination tank: 1.87×10^4 /ml
 c) load inoculated in the contamination tank: 3.75×10^4 /ml

A) Clam trials



B) Mussel trials

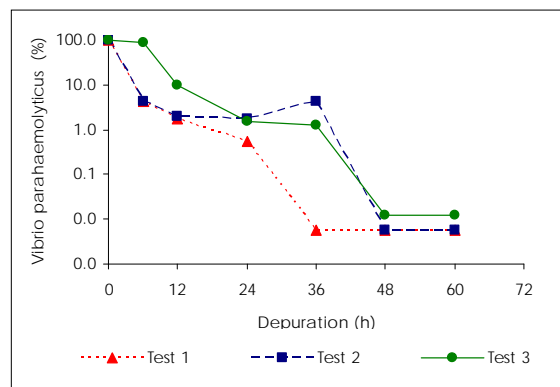


Figure 9
Percentage variation in *Vibrio parahaemolyticus* load against the baseline during clam and mussel trials

Circuit function

Salinity, temperature and oxygen values showed the following minimal variations:

- salinity $36 \pm 1\text{‰}$
- temperature $14 \pm 1^\circ\text{C}$
- dissolved oxygen 8 ppm.

All water samples taken from the depuration circuit were pathogen-free. This demonstrated the highly efficient depuration of the re-circulated water.

Discussion

These tests revealed marked differences in the efficacy of depuration of the various pathogens studied. Further differences were found by comparing the tests conducted with clams and with mussels.

The results showed that *E. coli* can be depurated from *C. gallina*. However, the time required varies significantly, depending on the initial level of contamination. In fact, clams from category B zones were depurated rapidly, reaching the market values authorised by current legislation (*E. coli* ≤ 230 mpn/100 g) in less than 24 h, while clams from category C zones required 2-3 times longer. Depuration plants could thus use shorter and economically more advantageous depuration times for clams from category B zones. In contrast, the protracted depuration time for products from category C zones would require a greater

economic outlay. The usefulness of the treatment required by current legislation, consisting of extended depuration (≥ 2 months) in an approved relaying area, is therefore confirmed.

In all cases, it should be noted that the depuration times revealed by this study were highly dependent on the characteristics of the system used, in this case a closed system, running on water with chemical and physical properties under constant control. Moreover, the specimens were laid out in much lower densities than would normally be found in the bins in mollusc depuration centres. The ideal living conditions within the depuration system are demonstrated by the low mortality rate during the trial.

The influence of the chemical and physical properties of the depuration water on the viability of *C. gallina* has also been observed in previous studies. Serratore *et al.* (16) reported that after 24 h depuration, mortality was zero in closed-circuit systems, in contrast with 15.5% in the open-circuit systems.

With respect to *S. Typhimurium*, clams required long depuration times, with values equating to the absence of *Salmonella* after 72-84 h. Various authors have reported that depuration of *Salmonella* spp. requires a longer period of time than *E. coli* depuration (11, 17). Some authors have suggested that this difference might be explained by the chemical and physical properties of the depuration water (15) which were controlled in our trials.

The behaviour of clams in the presence of *V. parahaemolyticus* is particularly significant. Samples undergoing depuration seemed to excrete a low bacterial load, suggesting that this pathogen might curtail their filtration capacity. A comparison of tests with similar baseline loads of *V. parahaemolyticus* and *E. coli* (1 200 mpn and 1 700 mpn/100 g, respectively) revealed that clams were rapidly depurated of *E. coli*, with a reduction in bacterial load of approximately 95% after 24 h, in contrast with the limited clearance of *V. parahaemolyticus*.

Similar observations have been made in other bivalve species: the Eastern oyster (*Crassostrea virginica*) takes longer to be depurated of *Vibrio*

spp. than of *E. coli* and *S. Tallahassee* (13). However, mussels appear to be more sensitive to the depuration treatment, as they were free of *V. parahaemolyticus* in our tests after 36-48 h.

Given the considerable differences in the speed with which *E. coli* and *V. parahaemolyticus* are depurated from clams, the reduction in *E. coli* numbers cannot be used to extrapolate the reduction in the number of Vibrios. This demonstrates that *E. coli* is an inadequate microbiological indicator of the healthiness of molluscs, not only because it is not correlated with the natural presence of Vibrios (14), but also because it is unable to demonstrate the general depuration capacity of a given species. Furthermore, while the risk of faecal contamination can obviously be reduced by harvesting molluscs from areas that are not contaminated by urban effluent, different preventive measures need to be implemented for Vibrios which are naturally present in the sea. The results presented here confirm those found in previous studies which noted that high, medium and low levels of *V. parahaemolyticus* could be a public health risk (7, 18). Mead *et al.* (12) showed that infections due to the *Vibrio* genus are one of the major causes of epidemics linked with the consumption of bivalves. Surveys of diseases encountered in the United States in 1997-1998 found a level of mortality associated with *Vibrionaceae* infections that was 10 times higher than that with *Salmonella* and *E. coli*.

Conclusions

This study demonstrates that clams (*C. gallina*) can be depurated of *E. coli*, a comforting conclusion at a time in which the areas in which this species is harvested, usually classified as category A, are suffering heavy contamination from fresh water run-off due to climate change and floods, resulting in their downgrading to category B. The trial also confirms the need for longer depuration times for other pathogens, namely *Salmonella* spp. and *V. parahaemolyticus*. While consumer safety is guaranteed by the prohibition of harvesting from an area that gives positive results for *Salmonella* until the source of contamination is

cleared, this is not the case for *V. parahaemolyticus*. Current legislation does not specify any limits for this native halophilic bacterium which is a normal constituent of the mollusc flora, due in part to lack of data on the infectious dose in humans and the lack of

reliable test procedures. For the time being, the only way to protect the public against contamination with *V. parahaemolyticus* is to recommend that molluscs be cooked before eating.

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