Classification and monitoring of shellfish harvesting areas in England and Wales

Classification et suivi des zones conchylicoles en Angleterre et au Pays de Galles

CHRISTOPHER RODGERS[1], DAVID LEES[1], SALLY HUDSON[2]

1. Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Fish Diseases Laboratory Weymouth, Dorset DT4 9TH, England
2. Sea Fish Industry Authority St Andrews Dock, Hull, England

Abstract

The EC Directive (91/492/EEC) on Shellfish Hygiene requires all Member States to classify their shellfish harvesting areas into one of three categories according to the degree of faecal indicator bacteria present in samples of shellfish flesh.

Historically, a limited amount of bacteriological data for England and Wales has been collected by Local and Port Health Authorities, the National Rivers Authority, the Ministry of Agriculture, Fisheries & Food and other smaller organisations involves in survey work. However, in order to provide definitive classifications before 1 January 1993, it is necessary to implement on-going monthly sampling programmes at fixed points within each shellfishery. Consequently standardised sample collection protocols and laboratory methodologies have been developed and these are discussed.

Keywords : Classification, shellfish, faecal pollution.

Résumé

La Directive européenne 91/492/EEC sur la salubrité des coquillages exige que tous les États membres classifient leurs zones d'élevage conchylicole dans l'une de trois catégories en fonction du niveau de germes indicateurs de contamination fécale présents dans des échantillons de chair de coquillage.

Historiquement, seule une quantité limitée de données bactériologiques a été recueillie par les services d'hygiène locaux et portuaires, par la National Rivers Authority, le ministère de l'Agriculture, de la Pêche et de l'Alimentation ou par d'autres organismes plus petits impliqués dans des travaux d'études. Pour établir une classification définitive avant le 1er Janvier 1993, il a été nécessaire de mettre en place des programmes permanents de prélèvement mensuel en des points fixes de chaque élevage conchylicole. Cette communication présente les protocoles d'échantillonnage et les méthodologies d'analyse normalisés mis en œuvre pour répondre à ces besoins.

Mots-clés : Classification, coquillages, pollution fécale.

INTRODUCTION

The EC Directive (91/492/EEC) on Shellfish Hygiene (Bukman, 1991) requires all Member States to classify their shellfish harvesting areas into one of three categories according to the degree of faecal indicator bacteria present in samples of shellfish flesh. Subsequent shellfish treatment regimes are determined by the classification and hence by the degree of pollution. There is, additionally, non provision for shellfish exceeding the upper limit of the most polluted category. Shellfish from such areas cannot therefore be placed on the market for human consumption and are, *de facto*, prohibited for sale.

Apart from limited historical data from some coastal areas, most of the remaining areas of England and Wales have little available monitoring information. This is due, largely, to the existing system of national controls made under the Public Health (Shellfish) Regulations. Essentially, these orders regulate the harvesting of shellfish from known polluted areas, depending on local conditions. They did not, however, stipulate any continued monitoring for possible changes in background faecal pollution in order to achieve a specified end product standard. Consequently, it has been necessary to introduce sampling programmes in most areas to meet the requirements of the Directive by 1 January 1993.

This paper describes standardised sample collection protocols and laboratory methods for use in the classification exercise.

Material and methods

Historical monitoring

The historical data was for mussels (*Mytilus mytilus*) which had been collected from fixed sampling points over a five year time frame and processed according to the most probable numbers (MPN) method of West and Coleman (1986). This information, where available, was collated and analysed. Bacterial counts were expressed as *Escherichia coli*/100 g of shellfish flesh and shown as monthly sampling occasions.

Experimental studies

i) Sample size

A single homogenate obtained from 15 mussels was tested 17 times (West and Coleman, 1986). In addition, 30 mussels and 30 oysters (*Crassostrea gigas*), obtained from the same harvesting area at the same time, were tested individually.

ii) Freeze/thaw

Mussels were collected and divided into batches of 15 animals. The first batch was tested (West and Coleman, 1986) immediately, whereas the remaining shellfish were stored at – 20°C and tested after 24 hours, 7 and 14 days. Each experiment was repeated three times.

iii) Sample preparation

Batches of 15 mussels were prepared by either stomaching (1,5 and 15 minutes) or homogenising (1 minute ; four 15 second blendings with 15 second intervals) using a Warring blender. The resultant homogenates were then tested in triplicate by the West and Coleman (1986) method.

Results

Analysis of historical data

Analysis of limited historical information obtained from mussel harvesting areas indicates the inherent variability in count of *E. coli*. Figures 1a and 1b in particular show that an accurate and reliable trend does not emerge until an appreciable amount of data has been collected. This variability leads to difficulty in setting a classification under EC Directive 91/492, particularly with the availability of only a small amount of data.

Experimental studies

i) Sample size

Figure 2 shows the inherent variation within the MPN test. The range of values for a single homogenate, tested 17 times, was 330-3,500 *E. coli*/100 g (mean = 1,300). This supports the MPN 95% confidence limits of 300-3,100 (Anon, 1982). Figures 3a and 3b indicate the variance of *E. coli* counts from individual oysters and mussels. Both species of shellfish gave a range of 230-35,000 *E. coli*/100 g (mean for oysters = 5,400 ; mussels = 7,000). These experiments show that samples from a small number of shellfish could give misleading results when used for monitoring in support of the classification of harvesting areas.

ii) Freeze/thaw

Figure 4 shows the reduction of *E. coli* counts after 24 hours and 14 days, following storage at -20°C. The average reduction was from 4,126 at time 0 to 554 after 24 hours and 46 *E. coli*/100 g after 14 days. As a result, shellfish samples should be transported to the laboratory chilled (e.g. 4°C) and should not be frozen since freezing can lead to false low counts of *E. coli*.

iii) Sample preparation

Figures 5a et 5b show little apparent variation between counts obtained from either homogenisation or stomaching. It is concluded therefore that they are both acceptable methods of sample preparation prior to isolation of *E. coli*.

The existing data analyses and experimental observations reported here have led to the formation of standardised sampling protocols and laboratory methods for bacteriological survey work aimed at the classification of shellfish harvesting areas (table I).

Discussion

The message from historical data is that counts from shellfish flesh vary considerably with time, despite using fixed monitoring points and the most probable number (MPN) technique which includes a preliminary resuscitation step (West and Coleman, 1986) as shown in Figures 5a and 5b. Further experimental work reported here shows the inherent variability not only in the test methodology but also within the shellfish themselves. False counts of *E. coli* could lead to an inaccurate or difficult interpretation of the level of bacterial faecal pollution in shellfish. As a result the classification of harvesting areas into the wrong

Table I: Shellfish collection protocol and sample preparation for use in the classification of harvesting areas in England and Wales

1. Sample frequency A minimum survey period of one year, with at least a monthly sampling frequency, should be used. Location of fixed sample points should take into account the position of sewage discharges, tidal flows and local topographical details. Effort should be concentrated on marginal waters.
2. Sample size A minimum of 10-15 individual oysters should be taken for sampling. It may be necessary to take larger numbers of smaller shellfish in order to obtain enough material to test (e.g. 15-30 mussels; 30-50 cockles).
3. Sample composition Where shellfisheries consist of more than one species take either oysters, clams or mussels in preference to cockles.
4. Data collection It is important to record map reference co-ordinates, state of the tide and method of collection (hand picked/dredged, etc.).
5. Sample transport Transportation of samples at 4°C is recommended. Good quality insulated (25 mm thickness) picnic boxes containing up to 8 freezer packs are ideal for maintaining the correct temperature in transit. Where possible transit time should not exceed 24 hours.
6. Sample storage Shellfish should not be stored for extended periods of time. Immediate testing is preferred and shellfish must not be frozen under any circumstances before hand.
7. Sample preparation Homogenisation for 2 minutes or stomaching for at least 5 minutes are both acceptable methods for initial sample preparation.
8. Isolation of *Escherichia coli* The current recommended method is a 5-tube MPN technique employing the West and Coleman (1986) resuscitation variation. Minor modifications to this are acceptable (e.g. incorporation of MUG for the confirmatory stage).

categories, based on *E. coli* as an indicator, could lead to shellfish being placed on the market without undergoing proper treatment or relaying; this would have health implications for any potential consumer.

In addition to the need for correct sampling and laboratory methodologies, it is also recognised that accurate information arising from good communication is also important to the interpretation of data. Consequently, the establishment of regional shellfish liaison committees, comprised of regulatory bodies and public health laboratory representatives, has been encouraged. This has led to a co-ordinated approach to classification and a pooling of resources with conse-

quent savings in time, money and manpower. Representatives of the shellfish industry are also being consulted and invited to take part in the programmes where necessary. These steps should ensure that a comprehensive amount of data can be gathered and used to classify the shellfish harvesting areas of England and Wales.

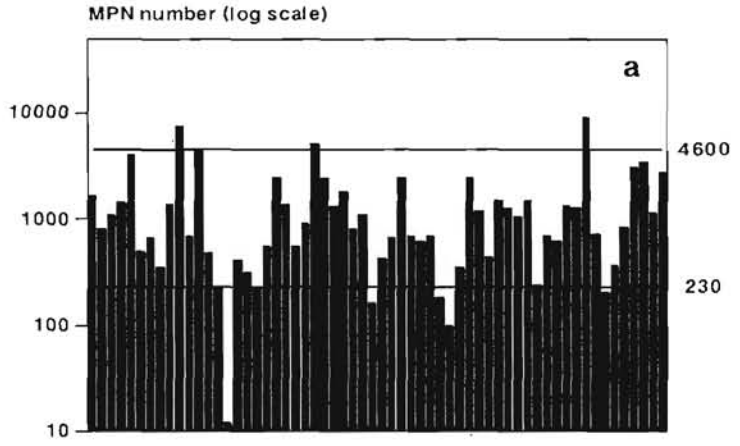

Figure 1a: *Escherichia coli* counts from fixed sampling points-category B area

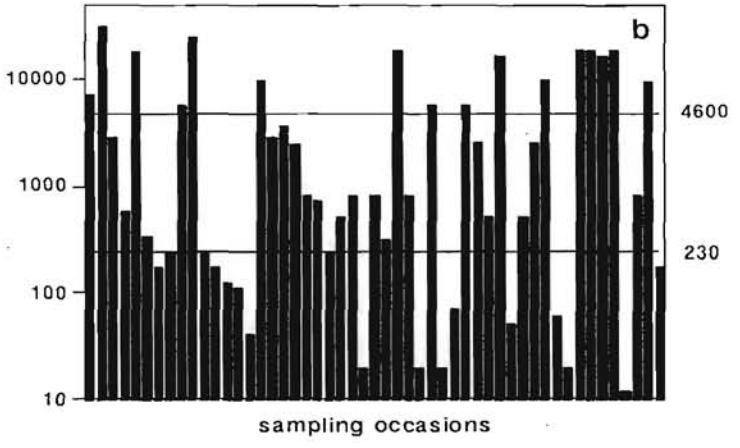

Figure 1b: *Escherichia coli* counts from fixed sampling points-category C area

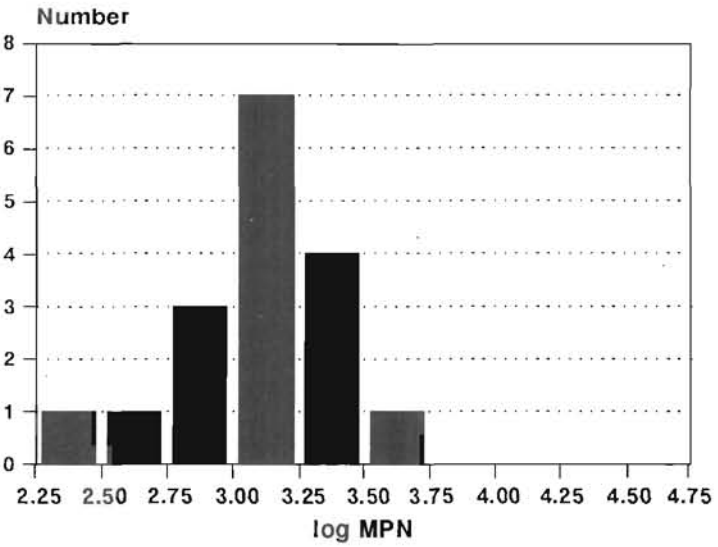

Figure 2: Inherent variation within the most probable numbers test

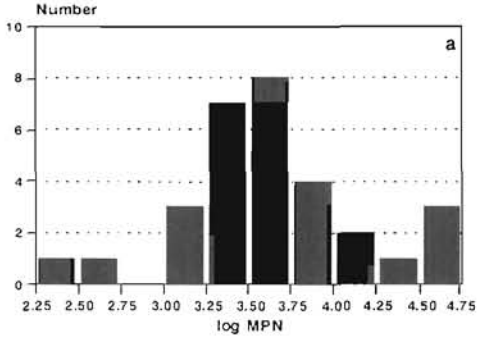

Figure 3a: Variation of *Escherichia coli* counts in individual oysters

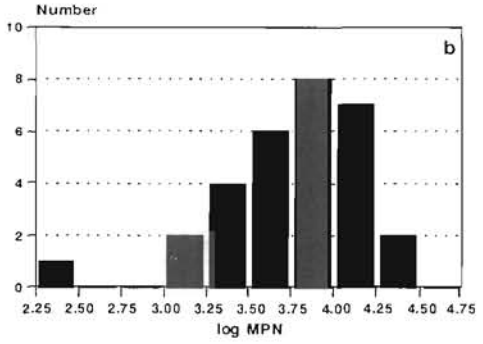

Figure 3b: Variation of *Escherichia coli* counts in individual mussels

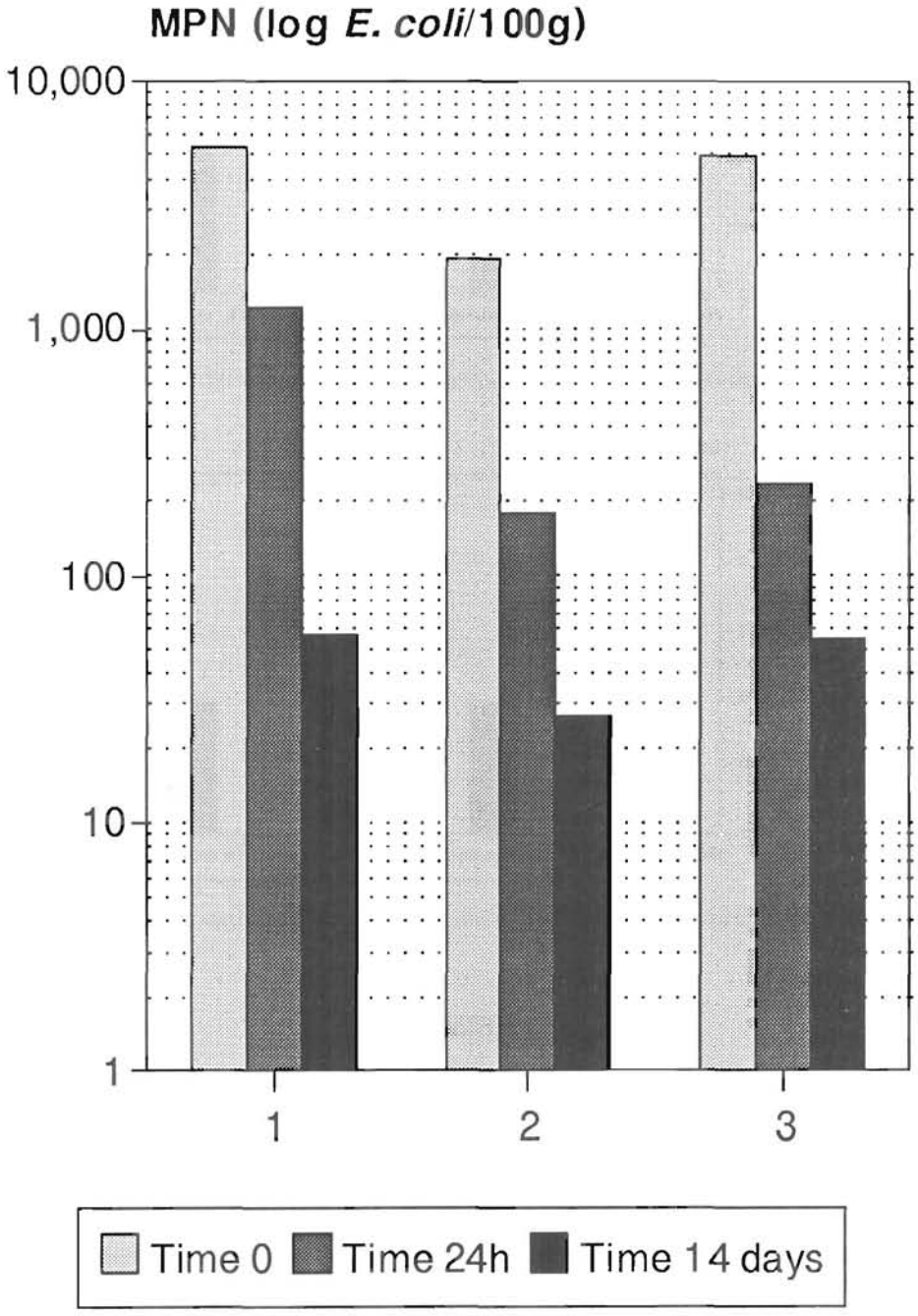

Figure 4: Reduction in *Escherichia coli* counts after freezing

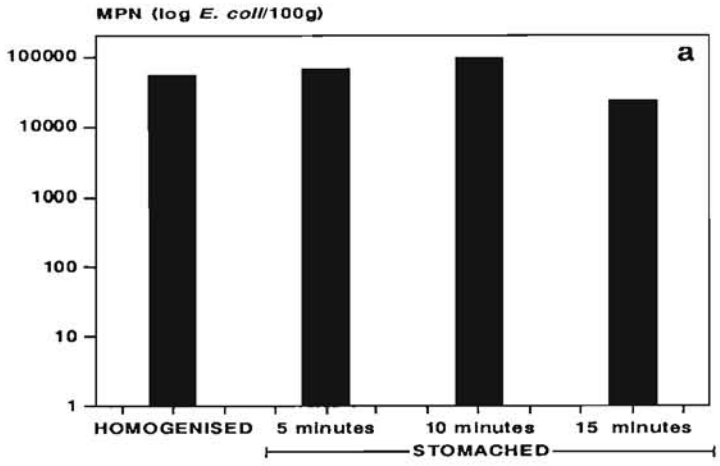

Figure 5a: Comparison of homogenising and stomaching-high contamination

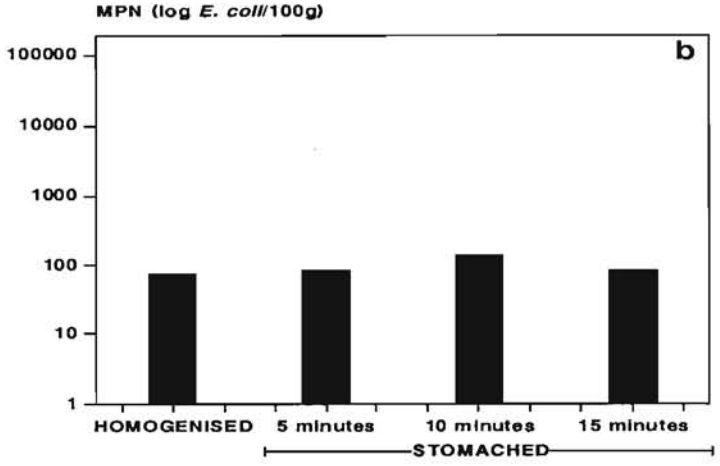

Figure 5b: Comparison of homogenising and stomaching-low contamination

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