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No 1980/6

- ☐ NEW PROPOSAL
- ☐ CONTINUING PROJECT
- ☒ FINAL REPORT
- ☐ PROGRESS REPORT

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: Viral and Bacterial Contamination and Decontamination of Oysters

ORGANISATION: CSIRO/ Health Commission of NSW

PERSON(S) RESPONSIBLE: Dr W.G. Murrell/ Mr G.R. Davey

FUNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1980/81		$24,250
1981/82		22,350
	Supplementary Grant	3,010
	TOTAL	$46,610

RELATED APPLICATIONS:

RECEIVED 23 May /1983 **DISTRIBUTED** 27 May /1983

Judith Lee
for Secretary
Fishing Industry Research Council

F139–12/81

Viral and Bacterial Contamination and Decontamination of Oysters (1980/6)

Objectives:

(a) To monitor oysters, water and sediments from selected oyster growing areas bacteriologically, virologically and physico-chemically.

(b) To study oyster purification processes on a laboratory scale for a clearer understanding of the way in which viruses are taken up and subsequently eliminated by oysters.

(c) To assess the measures adopted to ensure the safety of oysters and to assess the adequacy of current microbiological standards for oysters. To develop more effective purification processes for oysters and more efficient quality assurance if necessary.

Organisations:

CSIRO and the Health Commission of NSW

Supervisors:

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Grants:

1980/81	1981/82	Total
$24,250	$22,350	$46,610*

*including supplementary grant 1981/82 $3,010

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

SUMMARY OF FINAL REPORT

Viral and Bacterial Contamination and Decontamination of Oysters

The purpose of this study was to examine the microbial ecology of oysters, oyster growing areas and oyster purification processes. Oysters, water and sediments from three oyster growing areas were monitored virologically, bacteriologically and physicochemically over a one-year period. Purified oysters from two of these areas were also examined over the same period. These studies were supported by laboratory studies of the purification process.

The results of the estuarine studies illustrate the ability of oysters to concentrate pathogenic microorganisms from their environment and demonstrate the need for effective purification of NSW oysters. In particular, unpurified oysters collected near heavily urbanised areas were frequently unacceptably contaminated. Seasonal variations were noted in the occurrence of most of the microbial groups studied. Some of these variations appeared to be due to specific short-term weather influences, whereas others were due to broader climatic associations. For example, elevated *Escherichia coli* counts were associated with rainfall (> 10 mm) in the few days before sampling, while there were sustained changes in the patterns of isolation of *Vibrio cholerae* and *V. parahaemolyticus* from season to season. The performance of *E. coli* counts as an indicator of the potential presence of *Salmonella* in estuarine environments was good.

Laboratory studies of the elimination of poliovirus and *E. coli* from oysters showed that the purification process is capable of substantially reducing the degree of contamination of oysters with viral and bacterial pathogens which are present as a result of pollution. Microbiological studies of commercially purified oysters supported this finding. However the results

also showed that the oyster industry and regulatory authorities must not become complacent about the purification process, since purified oysters occasionally fail to comply with relevant NH & MRC microbiological standards. In addition, the purification process has little impact on the incidence of V. parahaemolyticus, with V. cholerae also being detectable in purified oysters on occasions.

This study provides a realistic basis for discussions and decisions concerning the microbiological safety of oysters and will lead to improvements in procedures for ensuring the microbiological quality of oysters. The results will help overcome the microbiological problems which have caused so many difficulties for the industry in recent years.

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

Final Report

Title

Viral and bacterial contamination and decontamination of oysters

Names of Grant Recipients

CSIRO and Health Commission of NSW (now the Department of Health, NSW).

Division

CSIRO, Division of Food Research and the Division of Analytical Laboratories, Department of Health NSW.

Period of Grant

1980-1982

Introduction

Outbreaks of oyster-borne disease and laboratory studies of the microbiological status of oysters have demonstrated clearly that oysters as harvested from several areas in NSW are not a microbiologically safe food. These public health problems have caused serious economic difficulties for the NSW oyster industry and a loss of consumer confidence in its product in recent years. Various controls have now been placed on the industry by regulatory authorities in an effort to ensure that oysters reach consumers in a microbiologically satisfactory condition. The major control has been the requirement that oysters be purified before sale in an approved purification plant.

Guidelines for oyster harvesting and purification can successfully ensure the microbiological safety of oysters only if they are based on a sound

knowledge of the microbial ecology of oysters, estuaries and purification procedures. At the time this project commenced, basic information in this area was not available. In particular, little was known about the frequency and extent of contamination of oysters with potentially pathogenic micro-organisms. Little was known of the performance under normal commercial conditions of the purification procedures being introduced in the industry. Thus, the nature and degree of microbial contamination with which purification processes must cope and with which they are able to cope were largely unknown. There were doubts about the validity of the bacterial indicator tests traditionally used to assess the cleanliness of shellfish growing areas and the effectiveness of purification processes. With such deficiencies in our knowledge, it was not possible to have full confidence in the measures adopted to control oyster hygiene.

The broad objectives of the project were to obtain a better understanding of the microbial ecology of oysters by examining selected oyster growing areas over a period of one year. Oysters, water, sediment and purified oysters from the selected areas were examined microbiologically and physicochemically. A limited number of samples from other areas were also examined. These ecological studies, together with laboratory studies of the oyster purification process performed by the applicants and others, were intended to allow an assessment of the effectiveness of measures adopted to ensure the safety of oysters as a food and to suggest improvements in those measures where possible.

Three permanent estuarine sampling stations were established, at each of which a tray of oysters was set aside for the purposes of this study. These sites were in Woolooware Bay and Neverfail Bay, both in the Georges River, and in Murphy's Bay in Brisbane Water. There are substantial geographical and other differences between the sites selected. All were known to suffer pollution at times, however none was regarded as grossly polluted. The three sites

selected could be expected to yield results representative of a large number of other oyster-producing areas. Purification plants which purified oysters from Woolooware Bay and Neverfail Bay were also selected for study. Samples were collected from each of these sites on a regular basis throughout the year.

The microorganisms which were sought at each sampling from estuarine stations and commercial purification plants fell into several major categories. These included indicator bacteria (aerobic plate count, coliforms, Escherichia coli), pathogens usually introduced to the estuarine environment as a result of pollution (Salmonella, enteric viruses) and pathogens which can be considered indigenous to the estuarine environment (Vibrio parahaemolyticus, V. cholerae). Specific Australian microbiological standards exist for only two of these groups, aerobic plate count and E. coli count. Laboratory studies of the purification process were performed using E. coli as a model enteric bacterium and an attenuated strain of poliovirus type 1 as a model enteric virus.

Some minor modifications to the program originally proposed were necessary. It was intended to study a natural purification area as part of the estuarine work. However, the practice of re-laying oysters to such areas for a few days before sale was abandoned before the study commenced. As indicated in the original proposal, some of the necessary laboratory purification studies were already being conducted by the applicants at the time of the application. Although some of these studies were completed before the FIRTA grant commenced, the results have been included for the sake of completeness.

Outline of Procedures

1. Ecological Studies

a) Sampling

Estuarine sampling stations were established in Woolooware Bay and Neverfail Bay (Georges River) and in Murphy's Bay (Brisbane Water). The two purification plants selected for study were located on the shores of Woolooware Bay and Neverfail Bay and were approved by the NSW Government. Both plants were of the recirculating type, employing UV light for water sterilization. In one plant the oysters were contained within a single large pool, while the other employed several smaller tanks arranged vertically. Both plants were well designed and maintained by their operators. All studies employing commercial purification plants were performed during normal commercial operations.

Samples were collected from each of the estuarine stations every 2 weeks where possible. Because of factors such as the tides, load on laboratory facilities and availability of samples, the period between samplings was occasionally 1 week or 3 weeks or, rarely, 4 weeks. To enable all the necessary operations to be performed, the samples were collected on Tuesdays between sunrise and 11 a.m. at about low tide. The Moore swabs required for microbiological analyses were attached to each of the stations 24-48 h before sampling. The following samples were collected from the estuarine stations at each sampling: 60 oysters divided into 5 samples of 12 oysters each, 200 g of sediment, 1 L of water and 3 Moore swabs. Where possible, oyster samples were collected every 2 weeks from the 2 purification plants. Purified oysters were examined in exactly the same way as oysters from the estuarine stations. The purified oysters were usually from batches which had been harvested from Neverfail Bay and Woolooware Bay within one day of sampling from the corresponding estuarine stations. Sampling of purified oysters was not continuous throughout the year, since most plants do not operate for part of the year.

Samples were transported to the laboratory in insulated containers and testing was commenced within 1-3 h (Georges River area samples) or 24 h (Brisbane Water samples) of sample collection.

b) Physicochemical measurements

The temperature, salinity, turbidity and pH of the water at the time the samples were collected were measured. Turbidity was determined by the nephelometric method (Anon. 1976). Salinity was determined by titration (Strickland and Parsons 1972).

c) Microbiological analyses

The microbiological analyses which were performed on each type of sample are shown in Table 1. On arrival in the laboratory, a homogenate of each sample of oysters was prepared by blending the flesh of the 12 oysters with an equal weight of nutrient broth using the procedure described by Davey et al. (1982) (reprint attached). Portions of these homogenates were used for the various tests mentioned in Table 1. Sediment and water samples were tested bacteriologically without any pretreatment. Moore swabs for bacteriological examination were placed directly into appropriate media. The general methods of microbiological examination used are summarised in Table 2.

For the purposes of this study it was necessary to modify some of the methods listed in Table 2. In the examination of oysters for V. parahaemolyticus by the most probable number (MPN) method, the number of tubes tested per dilution was 3 rather than 5. In examining water by the same method, one volume of 100 ml, 5 volumes of 10 ml, and 5 volumes of 1 ml were tested. Primary monkey kidney cell cultures derived from Cynomolgus monkeys were used for detection of viruses. Several other minor modifications to the methods listed were employed.

d) Laboratory-scale purification studies

Oysters were contaminated with poliovirus and E. coli by placing them for 6 h in an aquarium containing freshly-collected estuarine water to which

suspensions of these two microorganisms had been added. After storage overnight in a moist sack, the contaminated oysters were purified in a laboratory-scale purification plant for up to 72 h. Samples were taken for microbiological analysis at various times before and during purification. The aquarium used to contaminate the oysters was a flow-through aquarium, i.e. there was a continuous inflow of freshly-contaminated water throughout the 6 h contamination period. There was also a recirculation system which ensured good aeration and mixing of the water in the aquarium. The oysters were contaminated with poliovirus and E. coli to concentrations similar to those found in oysters from polluted estuaries. The laboratory-scale purification plant was designed and operated to closely simulate the purification equipment and procedures used commercially. The plant was of the recirculating type, incorporating an ultraviolet light unit for sterilisation of the water. Both the purification tank and the aquarium used for contamination of the oysters held 60-70 oysters in a single layer and 30 L of water. E. coli was detected using methods described earlier. Poliovirus was enumerated using a plaque assay procedure (Eyles 1983) or by a most probable number (MPN) technique, both of which employed human diploid fibroblast cells as the virus detection system.

Results

1. Permanent Estuarine Sampling Stations

   a) Physicochemical data

Samples were collected from the Woolooware Bay and Neverfail Bay stations on 25 occasions from 24 February 1981 to 2 March 1982 and from Murphy's Bay on 21 occasions from 2 April 1981 to 23 March 1982. Temperature and salinity data are shown in Table 3 and Figure 1. The pH values of the water were in the ranges 7.3-7.9 (Woolooware Bay, Neverfail Bay) and 7.2-8.3 (Murphy's Bay) and the means were 7.6 (Woolooware Bay, Neverfail Bay) and 7.8 (Murphy's

Bay). Turbidity was consistently low in Murphy's Bay, never rising above 10 NTU (nephelometric turbidity units). Turbidity was more variable in the Georges River, rising to 39 and 82 NTU on occasions in Woolooware and Neverfail Bays respectively.

b) Aerobic plate counts

Aerobic plate counts on oysters were always well within the NH & MRC limits. Aerobic plate counts on samples from Murphy's Bay were, in general, slightly lower than those from the Georges River sites, however there was little difference between the three sites in the range of counts recorded. Geometric means of the five counts performed per sampling ranged from 1.3×10^2 - 2.2×10^4/g. Counts on individual samples ranged from 2.0×10^1 - 4.0×10^4/g. Rises in aerobic plate counts were frequently recorded at times when salinity fell and there was a sustained lowering of aerobic plate counts during July, August and September at all three stations.

c) Escherichia coli

A high proportion of oyster samples from all three estuarine sampling stations failed to comply with the NH & MRC standard for E. coli (not more than 2.3 E. coli/g in 4 out of 5 sub-samples and not more than 7/g in the remaining sub-sample). Oysters failed to comply with the standard at 11/25, 16/25 and 7/21 samplings from Neverfail Bay, Woolooware Bay and Murphy's Bay respectively and at 34/71 samplings overall. Very high counts were recorded at all 3 sites on occasions. Maximum mean E. coli counts were (range of 5 individual counts in brackets): Neverfail Bay 84/g (24-270/g), Woolooware Bay 168/g (40-400/g), Murphy's Bay 70/g (36-102/g). E. coli was not detectable in oysters at only 4/25, 5/25 and 7/21 samplings from Neverfail Bay, Woolooware Bay and Murphy's Bay respectively. Nearly all of these occasions were between early July and early October when E. coli levels were generally low at all 3 stations (see Figure 2 for E. coli data from Neverfail Bay station).

E. coli counts in water and in oysters generally followed the same trends, with counts in oysters usually being substantially above those in water. This illustrates the ability of oysters to concentrate indicator and pathogenic bacteria from the surrounding water (see Figure 2, a similar pattern was observed at other stations). The ratio of the mean *E. coli* count in oysters to the *E. coli* count in water was calculated for each sampling at which both sample types contained *E. coli*. The ratio was in the range 0.2-0.9 on 5 occasions, 1-10 on 19 occasions, 10.1-50 on 14 occasions, and > 50 on 5 occasions. The geometric mean of these ratios was 7.6.

E. coli counts in water samples were frequently high at the Georges River stations, for example, counts were above 1 *E. coli*/ml on 14 occasions. Maximum counts recorded in water were > 20/ml (Woolooware Bay), 17/ml (Neverfail Bay) and 0.7/ml (Murphy's Bay). High *E. coli* counts were also recorded in some sediment samples. *E. coli* was detected in sediment samples (maximum counts in brackets) at 11/25 (1100/g), 14/25 (180/g) and 6/21 (20/g) samplings from Neverfail Bay, Woolooware Bay and Murphy's Bay respectively. Overall, *E. coli* was rarely undetectable at the three sampling stations. There were only 6 samplings (4 at Murphy's Bay, 2 at Woolooware Bay) at which *E. coli* was not detectable in any sample.

d) Coliforms

Only 14 of the 141 samples tested did not contain detectable coliforms, most of the negative samples occurring in July, August and September. In general the trends in coliform counts followed trends in *E. coli* counts, although coliforms were detectable more frequently and the counts were usually higher. Maximum counts recorded were 7.0×10^3/g (Woolooware Bay), 3.5×10^2/g (Neverfail Bay) and 4.6×10^1/g (Murphy's Bay).

e) *Salmonella*

Salmonellae were isolated from 3 of 211 oyster samples, 7 of 70 water samples (Moore swabs) and 1 of 71 sediment samples collected from the

permanent sampling stations. Most of the isolates were obtained from Neverfail Bay. A wide range of serotypes was detected (Table 4). The performance of *E. coli* counts as an indicator of the potential presence of *Salmonella* in the estuarine environments studied was good, since salmonellae were isolated only from samples with elevated *E. coli* counts (Table 4). Batches of oysters which contained salmonellae also failed to comply with the NH & MRC standard for *E. coli* in oysters.

f) *Vibrio parahaemolyticus*

V. parahaemolyticus was detectable in a high proportion of oyster samples from all three permanent sampling stations (Table 5). There was a seasonal pattern to the isolations (Figure 3). Almost all oyster samples collected during the summer were positive for *V. parahaemolyticus*, while most were negative during winter, especially late winter (Table 5). The highest concentrations of *V. parahaemolyticus* in oyster flesh were also recorded during summer (Neverfail Bay 100/g, Woolooware Bay 220/g) or, in Murphy's Bay, early autumn (100/g). During late autumn, winter, and early spring, oyster samples which contained *V. parahaemolyticus* carried low numbers of the organism (less than 5/g).

There was a similar seasonal variation in the detection of *V. parahaemolyticus* in water samples (Table 6). *V. parahaemolyticus* was detectable in nearly all water samples collected during late spring, summer and early autumn, and less frequently during the colder months. Murphy's Bay showed a slight variation from this pattern, in that *V. parahaemolyticus* was not detectable in water during spring. The concentration of *V. parahaemolyticus* in water was generally low, with only 3 of the 69 samples tested containing more than 1.6/ml, the upper quantitative limit of the test. Numbers detected during the colder months were generally less than 0.1/ml. *V. parahaemolyticus* concentrations in oysters were usually substantially above those in the water.

Although the levels of V. parahaemolyticus in oysters declined during the colder months, it was present consistently in the environment of the 3 sampling stations. Oysters, water and sediment were all negative for V. parahaemolyticus at only 2 of a total of 69 samplings. V. parahaemolyticus was present in a high proportion of sediment samples throughout the year (Table 6), with little variation being observed between stations.

g) Vibrio cholerae

A total of 20 of 211 oyster samples, 21 of 70 water samples (Moore swabs) and 8 of 71 sediment samples collected at the three permanent sampling stations were positive for V. cholerae. There were no marked differences between the three stations in rates or patterns of isolation. During the period studied there were substantial seasonal variations in the incidence of V. cholerae in all types of sample, with the highest proportion of positive samples occurring in the autumn (Table 7). V. cholerae was detected at these stations much less frequently than V. parahaemolyticus. At 44 of the 71 samplings, oysters, water and sediment were all negative for V. cholerae. There was no evidence of persistence of V. cholerae in sediments throughout the year. Although the techniques used for the detection of V. cholerae were qualitative, the data suggest that its concentration in oysters was low for much of the year. When present in oysters, V. cholerae was detected in only one of the triplicate oyster samples tested per station at each sampling, except in early autumn when all three samples were positive on occasions.

On two occasions, O1 serotypes of V. cholerae were isolated from water, once from Woolooware Bay (27/4/81) and once from Murphy's Bay (6/1/82). Both were biotype eltor, serotype Ogawa, and neither produced heat-labile cholera toxin. Twenty four non-O1 isolates were forwarded to the Cholera Reference Laboratory for serotyping. There were 7 different serotypes and 17 non-typable strains among these isolates.

h) Viruses

No viruses were detected in the samples collected during this study. Because of international problems with the supply of monkey kidney cell cultures a small proportion (15%) of samples, all collected during the last few months of the study could not be tested satisfactorily for the presence of viruses. This finding is discussed in more detail later.

2. Purified Oysters

The performance of two purification plants, as assessed by the microbiological quality of the oysters they produced, was similar. An exception was a very bad failure of the purification process experienced by one of the operators once during this study. On this occasion the aerobic plate counts, coliform counts and E. coli counts of the purified oysters were all extremely high. V. parahaemolyticus and V. cholerae were also present in the purified oysters. This failure appeared to be due to the UV lamps, since no other fault in the equipment or its operation was evident. The lamps, which were near the end of their recommended lives, were replaced and the problem did not recur. The results obtained at this sampling have not been included in the summary below.

In order to compare the microflora of purified and unpurified oysters, the results of analyses of purified oysters have been compared with those of unpurified oysters collected from the corresponding estuarine stations at about the time the purified oysters had been harvested. Purified oysters were tested on 28 occasions. The aerobic plate counts of purified oyster samples (geometric mean of all samples 3.6×10^2/g) were generally lower than those of unpurified oysters (geometric mean 1.1×10^3/g). Coliforms and E. coli were also detectable less frequently and at generally lower levels in purified oysters (Table 8). Aerobic plate counts never approached the NH & MRC limit. Four batches of purified oysters failed to comply with the NH & MRC

standard for E. coli in oysters, while corresponding samples of unpurified oysters failed to comply on 13 occasions. Salmonellae or viruses were not detected in any of the 81 samples of oysters tested from the two purification plants.

Purified oysters frequently contained V. parahaemolyticus (Table 8). Counts in both purified and unpurified oysters were usually relatively low, since the two purification plants selected for study were not operating at the time maximum levels of V. parahaemolyticus were detected in the estuary. V. cholerae was detected in 2 samples of purified oysters and 6 of the corresponding samples of unpurified oysters. Purified oysters were positive for V. cholerae at samplings at which unpurified oysters were also positive. The presence of both vibrios appeared to be independent of the concentration in purified oysters of all the other microbial groups studied.

3. Samples from other Areas

Bacteriological examinations were performed on oysters from areas other than the 3 permanent sampling stations and from other purification tanks on 40 occasions. It was not possible to sample from these other sources on a systematic basis. As in previous studies performed by the Health Department, the results showed that areas not substantially affected by urbanisation are much less likely to produce heavily contaminated oysters than urbanised areas such as Georges River or Brisbane Water. Otherwise the observations concerning microbial ecology are consistent with those already described.

4. Laboratory-scale Purification Studies

Table 9 shows the results of 3 experiments in which oysters contaminated in the laboratory with both poliovirus and E. coli were subsequently purified in the laboratory. Neither poliovirus nor E. coli was detected in oysters purified for 24 h or longer in experiments A and B. In experiment C

poliovirus and E. coli were removed from the oysters more slowly than in previous experiments, although the concentrations of both contaminants were eventually reduced to undetectable levels. Poliovirus remained detectable after 48 h, the purification time used commercially, whereas E. coli was not detectable after 48 h. Experiment C differed from the two previous experiments in that it was performed under circumstances which might affect the efficiency of the purification process adversely. Because of heavy overnight rain between the contamination and purification phases of the experiment, the water in the purification tank was markedly less saline than the water to which the oysters had been exposed previously (Table 10).

The substantial reduction in E. coli concentrations in oysters demonstrated in the laboratory-scale purification plant were confirmed using commercial plants (Table 11). It is undesirable to deliberately introduce human enteric viruses into commercial food handling areas, thus similar experiments using poliovirus were not feasible.

Discussion

The investigation described here is the most comprehensive study of the microbial ecology of oysters and oyster production in Australia performed so far. The results provide a more realistic basis for discussions and decisions concerning the microbiological safety of oysters and will lead to improvements in procedures for ensuring the microbiological quality of oysters. Some improvements, for example to microbiological methodology and standards, have already been initiated. The study has also identified some areas of concern to the industry which should be investigated further.

The results of the examinations for microorganisms which are usually present as a result of pollution of waterways, principally E. coli and Salmonella, reinforce the need for effective purification of oysters produced in NSW. In particular, oysters produced near heavily urbanised areas are

frequently unacceptably contaminated. Oysters from areas less affected by urbanisation are less likely to be of unacceptable quality, nevertheless oysters from such areas do fail to comply with NH & MRC standards on occasions.

Seasonal variations were noticed in the occurrence of most of the microbial groups studied. Some of these were probably due to specific weather influences. For example, elevated E. coli counts were strongly associated with rainfall (> 10 mm) in the few days immediately before sampling. Other variations were related to the normal environmental behaviour of certain microorganisms (e.g. V. parahaemolyticus). The results of the study may have been influenced to some extent by the dry weather which occurred during much of the study period. This factor was likely to have decreased the level of pollution.

The two potentially pathogenic vibrios which were studied, both of which are considered native to estuarine environments, appeared to be quite different to each other and different to the other organisms studied in their ecological associations. V. parahaemolyticus, which was detected most frequently in summer, persisted in the environment of the sampling stations throughout the year. The incidence of V. cholerae was highest in autumn and it was not detectable for long periods during winter and spring. It is generally accepted that V. cholerae is adapted to areas of lower salinity than V. parahaemolyticus. For example, V. cholerae was detected only at stations where the salinity was 17°/₀₀ or less in one extensive study in the USA. It is interesting to note that the average salinity in the areas studied here was around 30°/₀₀ and salinities below 25°/₀₀ were recorded rarely.

Before the present study, V. cholerae 01, the group which includes the aetiological agent of pandemic cholera, had not been detected in the environment in NSW, nor had V. cholerae been isolated from Australian shellfish. Although they may be pathogenic by other mechanisms, the V. cholerae 01

strains detected here did not produce cholera toxin. However, this study provides no assurance that toxigenic strains of V. cholerae are not present in oyster-producing areas of NSW. Toxigenic strains are isolated regularly from Queensland waterways.

Although the failure to isolate viruses from the samples tested is encouraging from the oyster industry's point of view, this finding must be considered carefully since the widely publicised outbreaks of viral gastro-enteritis in 1978 showed that oyster-producing areas, particularly in the Georges River, do suffer viral contamination. The frequency with which elevated concentrations of E. coli were observed and the detection of Salmonella on occasions during this study are strong evidence that all three estuarine stations were unacceptably polluted from time to time. However, human enteric viruses do not necessarily enter estuaries at the same time as these bacteria. E. coli and salmonellae may have many sources, including industrial effluents, tip leachates, agricultural runoff, wildlife, urban stormwater and treated or untreated sewage. Human enteric viruses are likely to come from only one source, human excreta. Thus, the major source of viral contamination in the areas studied is treated or untreated sewage.

Human enteric viruses are most likely to enter these estuaries from the sewage system during prolonged heavy rain. During such periods the sewage system cannot cope with the greatly increased flows and partially treated or untreated sewage may be released into estuaries. During dry weather the likelihood of viral pollution reaching oyster-producing areas is much lower. The present study was performed during a severe drought, whereas the outbreaks of gastroenteritis in 1978 were associated with prolonged heavy rain. There was no period of prolonged rainfall in Sydney during the study period which compared with that experienced in 1978.

It is possible, although very unlikely, that the virological methodology used was not sufficiently sensitive for its task. The methodology used for

the isolation of viruses from oysters has been used successfully in several studies in other laboratories and was used successfully by the grant recipients to isolate viruses from oysters which caused gastroenteritis during the 1978 outbreaks (Eyles et al. 1981). At that time, viruses were detected only in oysters which had actually caused gastroenteritis, suggesting that the pollution which caused the outbreaks might have been particularly restricted in time or place.

The laboratory studies of the elimination of poliovirus and E. coli from oysters showed that the purification process is capable of substantially reducing the degree of contamination of oysters with viral and bacterial pathogens which are present as a result of pollution. Thus, the process can substantially reduce the risk that oysters will transmit illness. Bacteriological studies conducted at the University of NSW using pathogens such as salmonellae are in agreement with this finding (Son and Fleet 1980). The microbiological studies of commercially purified oysters described here also support these findings, in that they showed that most batches of purified oysters are much less likely to contain detectable coliforms or E. coli than are oysters taken directly from the estuary and that salmonellae are rarely found in purified oysters.

The microbiological studies of commercially purified oysters also showed that the oyster industry and regulatory authorities must not become complacent about the efficiency of the purification process. The process failed to produce oysters which complied with the appropriate NH & MRC standard on 5 occasions. Although some of the reasons for failures of the purification process can be inferred from microbiological data, we lack the basic physiological knowledge of the Sydney rock oyster necessary to fully explain these problems. One failure appeared to be caused by the equipment problem mentioned earlier and one was almost certainly caused by a very high load of E. coli in the incoming oysters (20-240/g). On two other occasions failures

occurred because one or two samples contained high concentrations of E. coli while the others from the same batch contained few, if any, detectable E. coli. Such failures may be due to a few damaged or weakened oysters failing to function properly during purification. It must be remembered that the purification plants studied here were challenged severely, much more severely than many other plants.

Potentially pathogenic bacteria which are considered indigenous to estuarine environments (e.g. V. parahaemolyticus, V. cholerae) appear to present problems quite different to those presented by the pathogens introduced with pollution discussed above. The purification process had little impact on the incidence of V. parahaemolyticus, with V. cholerae, including an O group 1 serotype, also detectable in purified oysters on occasions. Earlier data, obtained using oysters contaminated with V. parahaemolyticus in the laboratory, indicated that V. parahaemolyticus were readily cleansed from oysters during depuration (Son and Fleet 1980). Clearly, V. parahaemolyticus which become associated with oysters under natural conditions behave quite differently to those introduced to oysters in the laboratory. The data suggest that the purification process, as presently practised, cannot be expected to eliminate either V. parahaemolyticus or V. cholerae from oysters. The numbers of V. parahaemolyticus detected in purified oysters were probably insufficient to cause illness in consumers unless subsequent handling procedures allowed growth of the organism. Less is known of the pathogenicity of V. cholerae.

The information about the microflora of commercially purified oysters obtained during this study supports the view that microbiological standards for oysters should be modified. A bacteriological standard originally devised to apply to oysters taken directly from an estuary can have little relevance for oysters which have been subjected to a process whose only purpose is to substantially modify the microflora of oysters. Standards for the presence of

E. coli and other enteric microorganisms in these two categories of shellfish are attempting to do two different things. One is attempting to provide some assurance that a waterway is safe for oyster harvesting, the other is providing some assurance that an important food processing operation is functioning properly. Regulatory authorities may also need to accept more readily the presence in oysters of certain potential pathogens which are native to estuarine environments. The data also suggest that coliform counts may be useful in monitoring the efficiency of purification. Coliforms are present more often and in higher numbers than E. coli and appear to be reduced in numbers quite substantially when the purification process is operating efficiently. The data obtained during this study will assist the NH & MRC to revise microbiological standards for oysters.

The results obtained during this study give the oyster industry and regulatory authorities a much clearer view of the microbial ecology of oysters and of the microbiological problems confronting the industry. The results show that the purification process is capable of improving substantially the microbiological quality of oysters. However, the performance of the process must be monitored carefully. The results indicate that purification equipment must be carefully maintained and operated and that more care should probably be taken with the handling of oysters before and after purification. Oysters should not be harvested for purification at times when pollution is likely to be heavy, for example when breakdowns in the sewerage and drainage system occur. If there is complacency within the industry, purification failures will probably lead to outbreaks of oyster-borne disease as in other countries where purification is practised. The study has also identified a potential problem with vibrios. Because of the serious consequences for the industry of an outbreak of oyster-borne illness caused by V. cholerae, it is essential that this problem be studied more fully. Such a study, funded by FIRTA, is now in progress. In some respects the analysis and discussion of results

presented in this report should be considered preliminary. The results will be published in a suitable form, with the permission of FIRC, when a statistical analysis of some of the correlations mentioned earlier is completed. Some of the findings have already been published (attached).

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Table 1

Microbiological examinations performed at each sampling

	Oysters					Water	Sediment
	a	b	c	d	e		
Aerobic plate count	x	x	x	x	x		
Coliforms	x	x					
E. coli (MPN)[b]	x	x					
E. coli (DP)[c]	x	x	x	x	x	x	x
Salmonella			x	x	x	S[a]	x
V. parahaemolyticus			x	x	x	x	x
V. cholerae			x	x	x	S	x
Viruses	x	x				S	x

[a] A Moore swab was used for the analyses marked S.

[b] Most probable number method.

[c] Direct plate method.

Table 2

General methods of microbiological examination

Examination	Sample Type	Method Type	Method Reference[a]	Sensitivity or Sample Size[b]
Total plate count	Oysters	Pour plate	1	5/g
Coliforms	Oysters	MPN[c]	1	0.4/g
E. coli	Oysters	MPN	1	0.4/g
	Oysters	Direct plate	2	1/g
	Sediment	Direct plate	2	3/g
	Water	Membrane filter	3	0.01/ml
Salmonella	Oysters	Qualitative	1	25 g
	Sediment	Qualitative	1	25 g
	Water	Qualitative	1	Moore swab
V. parahaemolyticus	Oysters	MPN	1	0.6/g
	Sediment	Qualitative	1	25 g
	Water	MPN	1	0.01/ml
V. cholerae	Oysters	Qualitative	4	25 g
	Sediment	Qualitative	4	25 g
	Water	Qualitative	4	Moore swab
Viruses	Oysters	Qualitative	5	10 oysters
	Sediment	Qualitative	6	50 g
	Water	Qualitative	5	Moore swab

[a] References:

1 Standards Association of Australia (1980)
2 Anderson and Baird Parker (1975)
3 Anon. (1969)
4 Davey et al (1982)
5 Metcalf and Stiles (1968)
6 Hurst and Gerba (1979)

[b] Expressed as theoretical maximum sensitivity per gram or millilitre of sample for quantitative methods and as sample size for qualitative methods.

[c] Most probable number

Table 3

Temperature and salinity ranges and means

	Temperature (°C)		Salinity (°/₀₀)	
Station	Range	Mean	Range	Mean
Woolooware Bay	12.2-28.0	19.5	29.3-37.6	33.1
Neverfail Bay	12.0-28.0	20.2	4.7-36.3	29.0
Murphy's Bay	12.4-26.2	19.1	25.3-35.6	32.7

Table 4

Detection of *Salmonella* at the estuarine sampling stations

Station	Date	Sample	Serotype	E.coli count[a]
Woolooware	17/11/81	water	give	> 20
Murphy's	20/10/81	oysters	adelaide	3-12
		water	anatum	74
Neverfail	13/4/81	water	infantis	21
			singapore	
	26/5/81	oysters	warragul	93
	9/6/81	water	warragul	20
		sediment	oranienburg	1000
	20/10/81	water	eimsbuettel	1580
	1/12/81	oysters	infantis	59
	19/1/82	water	havana	90

[a] *E.coli* count of the corresponding sample (*E.coli*/g oyster or sediment; *E.coli*/100 ml water).

Table 5

Detection of *V. parahaemolyticus* in oysters at each permanent sampling station according to season

	Neverfail	Woolooware	Murphy's	Overall
Summer	100[a]	94	93	96
Autumn	86	81	83	83
Winter	40	31	0	22
Spring	50	61	39	50
Overall	71	70	50	64

[a] Proportion (%) of samples examined which were positive for *V. parahaemolyticus*. 13-21 samples were tested per station per season.

Table 6

Detection of *V. parahaemolyticus* in water and sediment according to season.

Season	Sediment[a] (% positive)	Water[a] (% positive)
Summer	65	94
Autumn	67	33
Winter	69	38
Spring	83	39
Whole year	71	51

[a] Proportion (%) of samples examined which contained *V. parahaemolyticus*. Each value represents 16-18 samples.

Table 7

Samples positive for *V. cholerae* by season and sample type

	Oysters[a]		Water[b]		Sediment[c]		All samples	
	No	%	No	%	No	%	No	%
Summer	4	7	4	22	1	5	9	10
Autumn	14	26	12	67	5	28	31	34
Winter	2	4	4	25	0	0	6	8
Spring	0	0	1	6	2	11	3	3
Whole year	20	9	21	30	8	11	49	14

[a] 46-57 samples/season

[b] 16-18 samples/season

[c] 16-19 samples/season

Table 8

Coliform, *E. coli* and *V. parahaemolyticus* levels in purified and unpurified oysters.

	Count Range (/g oyster)	% of Samples[a]	
		Unpurified	Purified
Coliforms	ND[b]	8	54
	0.4-5	58	37
	5.1-50	26	9
	> 50	8	0
E. coli	ND	41	79
	1-2.3[c]	21	12
	2.4-7	18	4
	7.1-50	18	5
	> 50	3	0
V. parahaemolyticus	ND	32	54
	0.6-5	64	40
	5.1-50	4	6

[a] Number of samples tested ranged from 53 to 135

[b] Not detectable

[c] Ranges are based on NH & MRC standard.

Table 9

Elimination of poliovirus and *E. coli* from oysters during laboratory purification

Depuration time (h)	Poliovirus concentration (MPN/g oyster flesh)			*E. coli* concentration (MPN/g oyster flesh)		
	Expt A	Expt B	Expt C	Expt A	Expt B	Expt C
0	9.8	8.6	9.2	15.8	9.8	9.2
6	1.4	1.0	2.2	1.0	< 0.4	9.8
24	0[a]	0	+[b]	< 0.4	< 0.4	1.6
48	0	0	+	< 0.4	< 0.4	< 0.4
72	0		0	< 0.4	< 0.4	< 0.4

[a] Poliovirus not detected in 10 oysters

[b] Poliovirus detected in 10 oysters

Table 10

Environmental conditions during purification experiments A, B and C (Table 9).

	Contamination		Purification tank	
Experiment	Temperature (°C)	Salinity (°/oo)	Temperature (°C)	Salinity (°/oo)
A	14.7-19.3	33	19.1-24.4	33
B	21.0-24.5	32	19.2-24.6	34
C	18.0-20.9	31	18.0-24.8	21

Table 11

Elimination of *E. coli* from oysters during commercial purification (oysters contaminated in the laboratory)

	E. coli concentration (cfu/g oyster)	
Trial	Before Purification	After Purification
X	34.8	< 0.5
Y	95.5	< 0.5

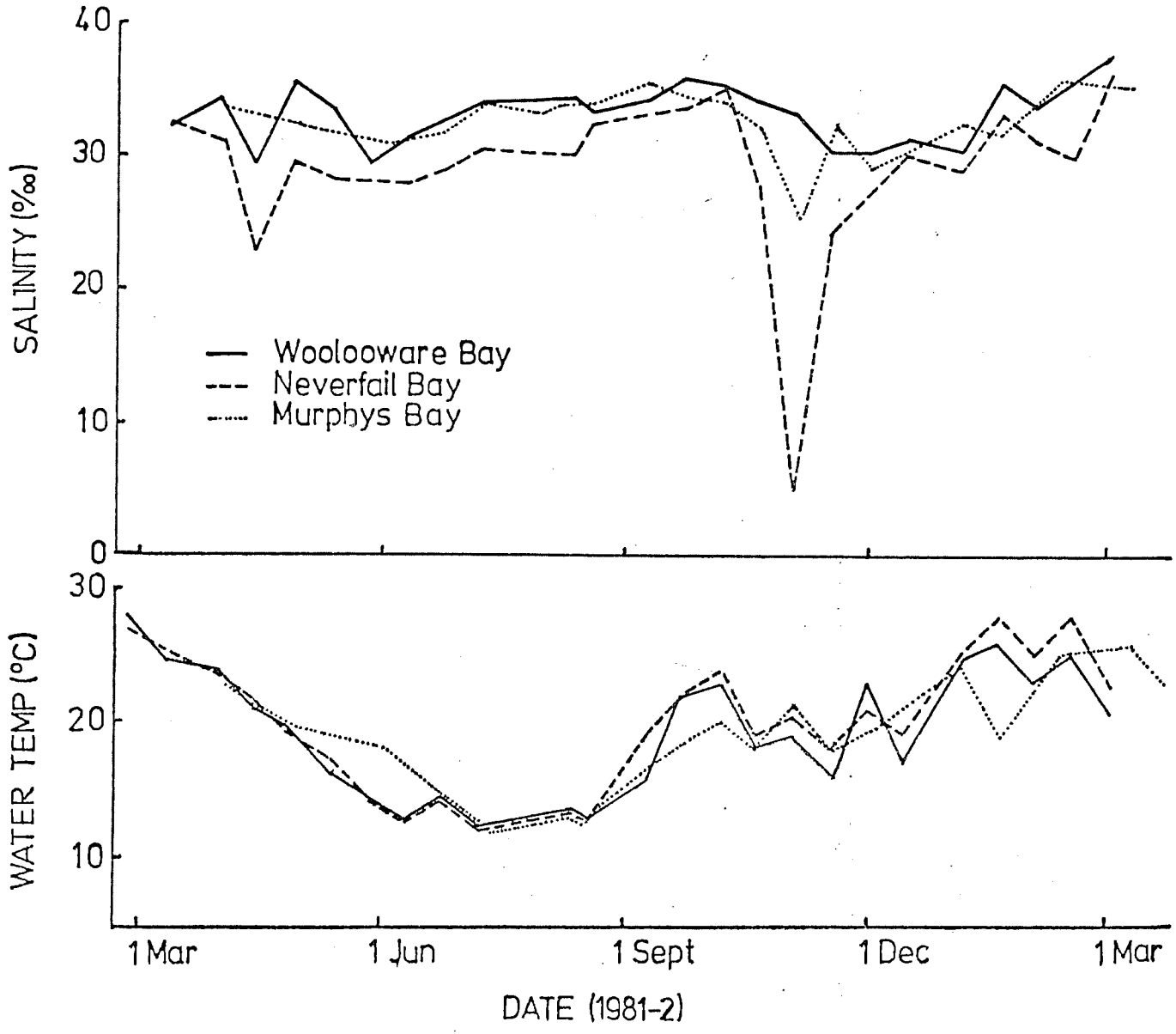

Figure 1
Water temperature and salinity at the permanent estuarine sampling stations

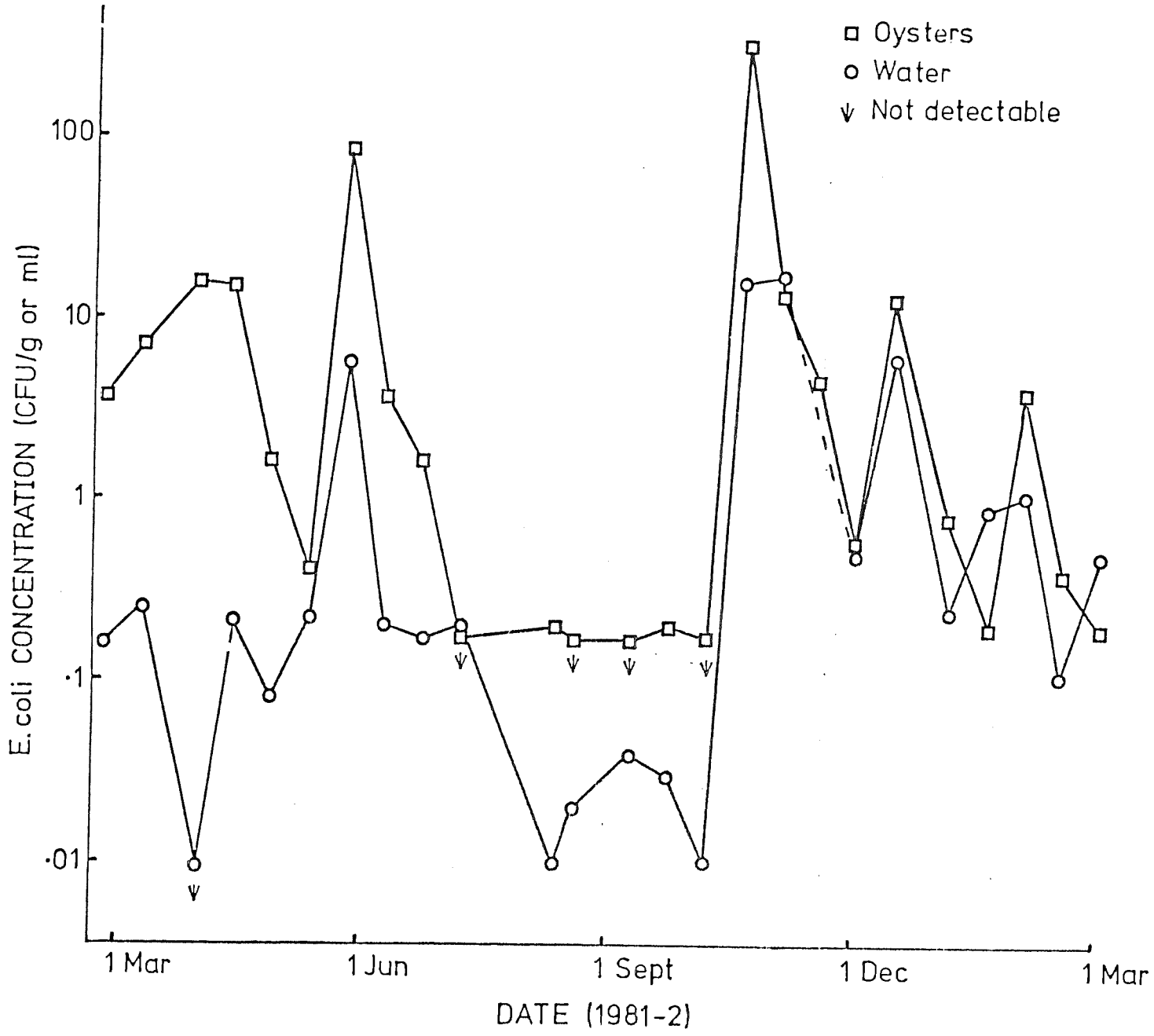

Figure 2
Mean E. coli counts in oysters and water at the Neverfail Bay sampling station

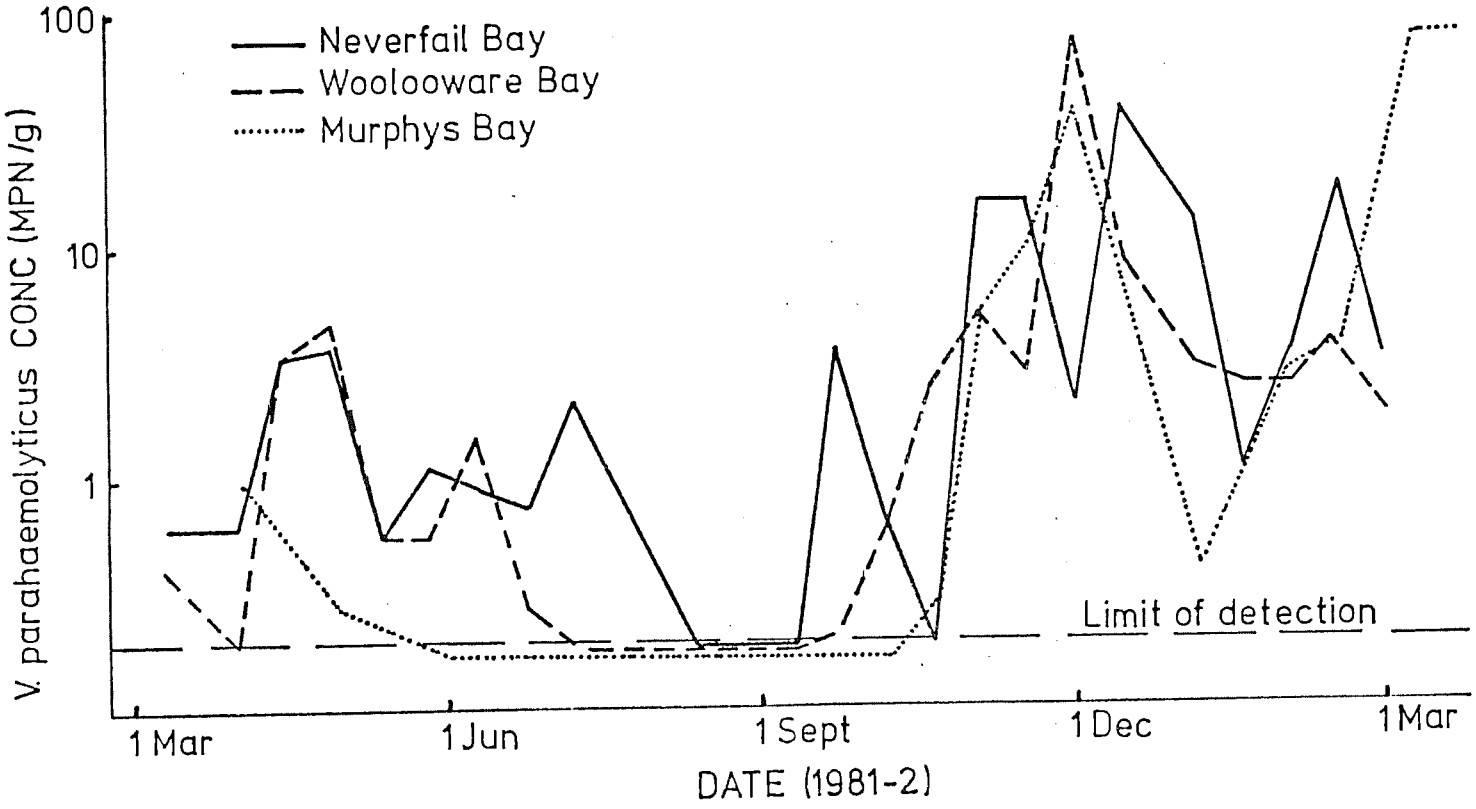

Figure 3
Mean V. parahaemolyticus counts at the three permanent estuarine sampling stations

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Detection of *Vibrio cholerae* in oysters, water and sediment from the Georges River

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Vibrio cholerae biotype *eltor* serotype Ogawa was detected in purified oysters which had originally been harvested from Woolooware Bay, a major oyster-producing area in the Georges River estuary, and in water in Woolooware Bay. Neither of these isolates was toxigenic. Non-01 strains of *V. cholerae* were detected in sediment and unpurified oysters collected from the same area. The average salinity in the area from which the isolates were obtained is 32.5°/∞. *Vibrio cholerae* has been considered to be an inhabitant of waters with a much lower salinity. *Vibrio parahaemolyticus* was also detected in the same series of samples of unpurified and purified oysters.

Vibrio cholerae may be subdivided into many serotypes on the basis of its 0 antigens, with the aetiological agent of pandemic cholera belonging to 0 group 1. Serotypes other than 01, once called non-agglutinable (NAG) vibrios or non-cholera vibrios (NCV), are generally considered to be much less significant to public health, although they can cause cholera-like diarrhoea. The pathogenicity of *V. cholerae* is not fully understood. Typical cholera is due to the production by *V. cholerae* of an enterotoxin, cholera toxin. Strains of *V. cholerae* which do not produce cholera toxin cannot cause typical cholera, but some appear able to cause a milder diarrhoea by some other mechanism. Water has long been accepted as the major vehicle for transmission of cholera. However, it was usually assumed that the cholera vibrios did not persist for long periods in natural waters and that they were present in water only as a result of relatively recent contamination by infected humans. Reports from several countries have indicated that *V. cholerae* serotypes other than 01 are widespread in aquatic environments and are probably often present in shellfish (Desmarchelier 1978, Kaper *et al.* 1979, De Paola 1981). It has also been suggested that *V. cholerae* 01 might be much more widely distributed in the environment than was believed previously, having a natural habitat in estuarine and brackish waters (Colwell *et al.* 1981).

This report deseribes the detection of *V. cholerae*, including 01 strains, in oysters *(Crassostrea commercialis)* from the Georges River estuary in New South Wales (NSW), Australia, and in other samples from the Georges River. The Georges River estuary is the most important of the many estuarine systems used for cultivation of *C. commercialis* in NSW. The Georges River catchment includes large areas of bushland, rural areas and expanding urban areas. The estuary, approximately 50 km long and including several large bays, lies within the southern metropolitan area of Sydney. Like many estuaries close to large cities it suffers from various types of pollution from time to time. Sources of microbial pollution and geographical features in the area from which samples were taken during the present study have been described by Qadri, Buckle & Edwards (1975).

Vibrio cholerae 01 has not been isolated from environmental samples collected in NSW nor has the presence of *V. cholerae* in shellfish from Australian waters been reported previously. The results presented were obtained during a study of the microbial ecology of oyster-growing areas and oyster purification processes in NSW. Thus, the samples were also examined for the presence of other pathogenic and indicator bacteria, including *V. parahaemolyticus*, another potentially pathogenic *Vibrio* species indigenous to estuarine environments.

Materials and methods

Sampling

Samples were collected from two points in late April - early May 1981. The first of these sampling points was a tray of oysters on a commercial oyster lease in Woolooware Bay (a major oyster-producing area approximately 10 km from the mouth of the Georges River estuary). The second was a commercial oyster purification plant in which oysters from Woolooware Bay are purified. The purification plant is of the recirculating type and employs ultraviolet light for sterilisation of the water. The following samples were collected from the oyster lease: 60 oysters divided into 5 sub-samples of 12 oysters each, 200 g of sediment, 1 L of water and 2 Moore swabs. The Moore swabs (Anon. 1976) had been suspended in the water beneath the tray for 24 h before the samples were collected. A second sample of 60 oysters, subdivided as above, was collected from the purification plant. The latter oysters had been harvested from Woolooware Bay two days after the first samples were collected from the lease and had subsequently been purified for 48 h.

The temperature, salinity, turbidity and pH of the water at the time the samples were collected from the oyster lease were measured. Turbidity was determined by the nephelometric method (Anon. 1976) using a Hach Laboratory Turbidimeter, Model 2100A. Salinity was measured by determining the density of a 100 mL sample of water and referring to density/salinity conversion tables (Wolf & Collins 1979).

Bacteriological analyses

A homogenate of each sub-sample of oysters was prepared as follows. The oysters were scrubbed thoroughly under running potable water to remove mud, barnacles and other material attached to the shell. The shell surface was flooded with 70% ethanol and excess ethanol was allowed to drain from the shell

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surface before the oysters were opened. The flesh of the oysters was removed from the shell, placed in a stainless steel blender jar with an equal weight of nutrient broth (Oxoid) and homogenised for 30 sec at setting 7 on a Sorvall Omni-Mixer. An aerobic plate count and an *Escherichia coli* count were performed on each homogenate. Three of the sub-samples from each sample were each tested for the presence of *V. cholerae*, *V. parahaemolyticus* and salmonellae.

Escherichia coli counts were performed on the water and sediment samples. The sediment sample was also tested for the presence of *V. cholerae*, *V parahaemolyticus* and salmonellae. One Moore swab was tested for the presence of *V. cholerae*, the other for the presence of salmonellae.

Aerobic plate counts, tests for the presence of salmonellae, and the enumeration of *V. parahaemolyticus* by the most probable number (MPN) method were performed according to the methods described in Australian Standard 1766 (Standards Association of Australia 1976). When testing the Moore swab for the presence of salmonellae the swab was placed in 900 mL of pre-enrichment medium. The MPN test for *V. parahaemolyticus* was modified by a reduction from five to three in the number of tubes inoculated with each dilution. *Escherichia coli* was enumerated in oyster homogenates and sediment using the rapid method of Anderson and Baird-Parker (1975) and in water using a membrane filtration method (Anon. 1969).

To detect *V. cholerae*, 50 g of oyster homogenate, 25 g of sediment or one Moore swab were placed in a glass jar containing 200, 225 or 900 mL, respectively, of pre-warmed alkaline peptone water (Oxoid). The pH was adjusted to 8.6–9.0 and the culture was incubated at 37°C. A loopful of broth was removed from the surface of the culture after 6–8 h incubation and streaked on thiosulphate citrate bile salts sucrose agar (TCBS Oxoid). After 6–8 h incubation 1 mL of this broth was also transferred to 10 mL of fresh alkaline peptone water which was incubated at 37°C for 18–24 h then streaked on TCBS. All TCBS plates were incubated at 37°C for 18–24 h. Typical *V. cholerae* colonies on TCBS were subcultured and checked for purity using CLED medium (Oxoid) then screened for Gram reaction, motility, oxidase reaction, salt tolerance (growth in peptone water containing 0, 3, 5, and 7% NaCl), lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase activity, indole production, and reaction in triple sugar iron agar. The identity of cultures giving results typical of *V. cholerae* was checked using the API 20E system (Analytab Products). Cultures of *V. cholerae* were serotyped by the slide agglutination method using *V. cholerae* polyvalent, Inaba, and Ogawa agglutinating sera (Wellcome).

Isolates of *V. cholerae* 01 were sent to the Cholera Reference Laboratory at the Commonwealth Institute of Health, Sydney for confirmation and biotyping. The cultures were forwarded by the Commonwealth Institute of Health to (a) Dr N. Ryan, Enteric Pathogenicity Laboratory, Latrobe University, Bundoora, Vic. for testing for toxin production using the ligated rabbit ileal loop technique, the Y-1 mouse adrenal tumour cell assay, and an ELISA assay based on that of Back *et al.* (1979); (b) Dr J. Kaper, University of Maryland School of Medicine, Baltimore, MD, USA for examination for the presence of genes homologous to the genes encoding *E. coli* heat labile enterotoxin, a toxin which possesses a high degree of homology with cholera toxin; and (c) Dr J.V. Lee, Public Health Laboratory, Preston Hall Hospital, Maidstone, Kent, England for phage typing.

Results

Vibrio cholerae 01 serotype Ogawa was isolated from one sub-sample of purified oysters and from the Moore swab. These findings were confirmed by the Cholera Reference Laboratory. The biotype of both isolates was *eltor*. Neither of the isolates was shown subsequently to produce cholera toxin by the assays performed by the Enteric Pathogenicity Laboratory. The genetic studies performed by Dr Kaper suggested that the isolates did not possess genes enabling them to produce cholera toxin. The phage types of the isolates from the two sources were not significantly different. The phage type of the isolates reported here is different from the phage types of isolates of *V. cholerae* 01 obtained from some rivers in Queensland from time to time and of isolates obtained early in 1981 from a locally-acquired case of cholera in Lismore, NSW (P.M. Desmarchelier, personal communication).

Non-01 strains of *V. cholerae* were detected in one sub-sample of unpurified oysters and in the sediment sample. *Vibrio parahaemolyticus* was detected in three sub-samples of unpurified oysters at a level of 4.6/g, in one sub-sample of purified oysters at a level of 0.8/g, and in the sediment sample.

Aerobic plate counts and *E. coli* counts of the five sub-samples of unpurified oysters ranged from 1.8×10^3 to 4.0×10^3 and from <1 to 2/g, respectively. Aerobic plate counts and *E. coli* counts of the purified oysters were from 3.2×10^2 to 7.6×10^2 and from <1 to 2/g, respectively. *Escherichia coli* counts on water and sediment samples were 1.1/mL and 30/g, respectively. No salmonellae were detected. At the time the samples were collected the temperature, pH, salinity and turbidity of the water at the sampling station in Woolooware Bay were 19.4°C, 7.6, 32‰ and 3.7 nephelometric turbidity units, respectively.

Discussion

The original source of the *V. cholerae* strains detected during this study remains unknown. However, the results are consistent with the hypothesis (Colwell *et al.* 1981) that *V. cholerae*, including 01 strains, is a normal part of the microflora of many estuarine systems. Although the Georges River estuary is subjected to pollution with sewage from time to time, especially after prolonged heavy rainfall, faecal contamination could be considered unlikely to have been the source of the *V. cholerae* isolated. We are not aware of any isolations of *V. cholerae* from human sources in the region surrounding the Georges River in recent years, yet a mixture of strains of *V. cholerae* was isolated from a single area over a short period of time. In addition, *E. coli* counts on the oysters indicated that they had not been subjected to serious pollution in the few days before samples were collected.

Although many aspects of the ecology of *V. cholerae* are poorly understood, the available data increasingly indicate that non-toxigenic and perhaps also toxigenic strains of both 01 and non-01 serogroups of *V. cholerae* are indigenous to many waterways. Studies by Kaper *et al.* (1979) suggested strongly that *V. cholerae* was an autochthonous estuarine bacterial species resident in Chesapeake Bay, USA. None of their isolates was agglutinable in 0 group 1 antisera, but most yielded positive results in several toxigenicity tests. *Vibrio cholerae* 01 has been isolated from waterways or shellfish in the UK (Bashford *et al.* 1979) and USA (Colwell *et al.* 1981, Hood, Ness & Rodrick 1981, Twedt *et al* 1981). These isolations have demonstrated the presence of *V. cholerae* 01 in relatively unpolluted waterways in cholera-free areas. Most of these *V. cholerae* 01 isolates were non-toxigenic, as were the 01 strains isolated during the present study. Toxigenic strains of *V. cholerae* 01 have persisted in rivers in south-east Queensland for five years in the absence of evidence of contamination (Rogers *et al.* 1980, Anon. 1981).

It is interesting to note that the salinity of the water in the area in which *V. cholerae* was detected in the present study was 32‰ at the time the samples were collected from the river. The salinity in this area rarely falls below 20‰, with the average being 32.5‰ (Wolf & Collins 1979). During their studies in Chesapeake Bay, Kaper *et al.* (1979) noted a distinct relationship between salinity and the presence of *V. cholerae*, with *V. cholerae* being found only at stations where the salinity was 17‰ or less.

The isolation of *V. cholerae* and *V. parahaemolyticus* from purified oysters is of particular interest and illustrates the need for further studies of the behaviour of potentially pathogenic vibrios during the purification of oysters. Compulsory purification of oysters is presently being introduced in NSW. The purification plant from which oysters were taken during the

present study had been tested and approved for commercial operation by the NSW Government and there were no deficiencies apparent in either the plant or the way it was operated.

Laboratory studies and industrial trials have indicated that purification plants of the type from which oysters were collected during this study are effective in removing a wide range of pathogenic and indicator bacteria from *C. commercialis* (Souness, Bowrey & Fleet 1979, Son & Fleet 1980). In one of the few studies of the elimination of potentially pathogenic vibrios from shellfish, Son and Fleet (1980) found that when *C. commercialis* was contaminated with laboratory cultures of *V. parahaemolyticus* the vibrios were readily cleansed from the oysters during purification. However, cells of *V. parahaemolyticus* which oysters have acquired from an estuarine environment will not necessarily behave in the same way as *V. parahaemolyticus* derived from laboratory cultures. It must be remembered that the primary function of purification processes is to remove pathogenic microorganisms present in oysters as a result of sewage pollution of oyster-producing waterways. Purification processes were not designed to remove microorganisms which are a normal part of estuarine ecosystems, although this might be accomplished in some cases. Thus, it might be unrealistic to expect purified oysters to be free of *V. cholerae* and other potentially pathogenic vibrios.

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The report covers the objectives relating to ecological influences leading to the need for oyster purification comprehensively.

Recommendations are made toward ensuring the maintenance of microbiological standards for oysters and the study supports the view that some standards should be modified.

The problem of oyster bone illness caused by Vibrio cholerae is identified and it is considered essential that it be studied more fully.

Further analysis and discussion will follow and a related study funded by FIRTA is in progress.
Some of the findings have already been published.

G W 13/7/83

A satisfactory and useful conclusion to an important research undertaking.

C G 14/7/83