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The distribution of *Vibrio parahaemolyticus* in British coastal waters: report of a collaborative study 1975–6*

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SUMMARY

A collaborative survey has shown that *V. parahaemolyticus* is widely distributed in British coastal waters, sediments and shellfish, especially in southern and western areas. The relatively small numbers in the environment do not present significant health hazards from marine products, provided that processing, storage and distribution are adequate. The presence of this organism in small numbers in British coastal waters or in shellfish should not in itself be regarded as cause for concern.

INTRODUCTION

It is more than 20 years since the organism described as *Pasteurella parahaemolytica* was first isolated in Japan from an outbreak of gastroenteritis following the consumption of seafoods (Fujino *et al.* 1953). Later a similar, salt tolerant organism called *Pseudomonas enteritis* was isolated by Takikawa (1958) who confirmed its enteropathogenicity in human volunteers. Miyamota, Nakamura & Takizawa (1961) suggested a new genus, *Oceanomonas*, for this organism. Extensive taxonomic studies by Sakazaki, Iwanami & Fukumi (1963), however, indicated

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that it was in fact a vibrio, and the term *Vibrio parahaemolyticus* is now generally accepted.

In Japan, *Vibrio parahaemolyticus* is regarded as the causative agent of 50–70% of all cases of diarrhoea associated with the consumption of fishery products in summer (Zen-Yoji, Le Clair, Ohta & Montague, 1973). With increasing awareness of the potential hazards to public health associated with this organism, a number of investigations have been carried out elsewhere. As a result, *Vibrio parahaemolyticus* has now been isolated from marine sources in many parts of the world, including Denmark (Kristensen, 1974), England (Barrow & Miller, 1972), the United States (Baross & Liston, 1968; Bartley & Slanetz, 1971), South Africa (Bubb, 1975), Canada (Varga & Hirthe, 1975) and Korea (Chun, Chung, Seol & Tak, 1974). It has also been confirmed as the cause of a number of food poisoning incidents associated with the consumption of seafoods in many parts of the world (Battey, Wallace, Allan & Keeffe 1970; Bockemühl, Amédomé & Triemer, 1972; Barker, 1974) and on several occasions in Britain (Barrow & Miller, 1976). Although the majority of cases in Britain were attributed to imported crustacea, an outbreak involving shellfish from British waters occurred in 1973 (Hooper, Barrow & McNab, 1974).

To assess the potential hazards to health posed by *V. parahaemolyticus* in sea water, a survey was undertaken to determine the distribution of this organism in British coastal waters. For this purpose, an *ad hoc* working group was formed in 1974 to combine the expertise and facilities at laboratories of the Public Health Laboratory Service, the Ministry of Agriculture, Fisheries and Food and other bodies in different areas of Britain. In the survey, the distribution of *Vibrio parahaemolyticus* in marine waters, shellfish and sediments was examined during a two year period from January 1975 to December 1976.

This paper presents the results of the study and attempts to assess the potential health hazards of *Vibrio parahaemolyticus* in British coastal waters and shellfish.

MATERIAL AND METHODS

Samples

In order to ascertain whether there was any variation in the seasonal or geographical distribution of *Vibrio parahaemolyticus* in the sea, samples of water, sediments and shellfish were taken from selected sites in estuarine and coastal areas of the British Isles. Several molluscan bivalve shellfish (oysters, cockles and mussels) were examined as it proved impracticable to select a single species common to all sampling sites. In some areas, other marine animals, particularly shore crabs (*Cancer maenas*), were more readily available. Each laboratory participating in the survey was responsible for its own sampling programme and as far as possible the selection of sites within its area. Some inland laboratories without ready access to the coast were supplied with samples by staff of the Ministry of Agriculture, Fisheries and Food, the Sea Fisheries Committee or other bodies. When practicable, sampling on a routine basis was carried out from the pre-selected sites, with a minimum frequency of at least once per month. To extend the coverage of the coastline, additional samples were taken by MAFF and other workers from areas not included in the survey programme.

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Laboratory examination

In a collaborative study of this kind it would have been desirable to select 'standard' methods; many laboratories, however, had already established their own procedures and consequently these variations of similar methods were accepted. For culture of *V. parahaemolyticus* from raw seafoods and other marine samples, several enrichment techniques, with subculture to solid selective media were used. Combinations of the basic media used for enrichment and isolation were as follows:

(1) Double strength salt colistin broth: Yeast extract (Oxoid) 6 g; Tryptone (Oxoid) 20 g; sodium chloride 40 g; distilled water to 1000 ml; autoclaved at 121 °C for 15 min; final pH 7·4. Before use, 'Colomycin' (Colistin sulphomethate sodium) was added aseptically to give a final concentration of 10^3 i.u./ml.

(2) Single strength alkaline salt peptone water: Peptone 10 g; sodium chloride 30 g; distilled water 1000 ml; autoclaved at 115 °C for 10 min; final pH 8·4.

(3) Double strength alkaline salt tryptone water: Tryptone (Oxoid) 20 g; sodium chloride 60 g; distilled water 1000 ml; autoclaved at 115 °C for 10 min; final pH 8·5.

(4) TCBS – Thiosulphate-citrate bile-salt sucrose agar (Oxoid TCBS Cholera medium).

Preparation of samples for culture

Shellfish

If possible 100 g wet weight of macerated or homogenized shellfish tissues were added to 100 ml of double strength salt colistin broth; with smaller amounts of tissue, equivalent volumes of broth were used. These were incubated at 37 °C for 18 h and then subcultured heavily to TCBS agar plates for incubation at 37 °C for 18 h. Some laboratories cultured the shells, either separately, or together with the tissues.

Sediments

The cultural procedures adopted for shellfish were also used for sediment samples.

Water

Standard volumes of water were examined by one or more of three methods:

Membrane filtration. 100 ml volumes were filtered through 47 mm membranes (pore size 0·45 μm), which were placed on TCBS plates and incubated at 37° C for 18 h.

Sample dilution. Ten ml volumes of samples were added to each of 10 tubes containing 10 ml quantities of either double strength salt colistin broth or double strength alkaline salt peptone/tryptone water and incubated at 37 °C overnight. All tubes, whether or not they showed visible growth, were then subcultured on TCBS plates for overnight incubation at 37 °C.

Clarifying filtration. Three litre samples of seawater were passed through sterile Carlson-Ford 15 cm clarifying filters. Each filter was then cut into four pieces:

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two were placed in 100 ml of single strength alkaline salt peptone water, and two in 100 ml of double strength salt colistin broth. These were incubated at 37 °C for 18 h and subcultured on TCBS plates for incubation overnight at 37 °C.

Isolation and identification of Vibrio parahaemolyticus

After overnight incubation at 37 °C on TCBS medium, the characteristic large, green, non-sucrose fermenting, dome shaped colonies of *Vibrio parahaemolyticus* were usually readily distinguishable from the flat, yellow, sucrose fermenting colonies of the common marine organism, *V. alginolyticus*. However, occasional strains of *V. parahaemolyticus* ferment sucrose and in order to avoid missing isolations solely on grounds of colour, a random selection of both yellow and green colonies were subcultured for further investigation. It was observed that unless plates were examined soon after removal from the incubator, reversion of colony colour might occur, especially if left on the bench for some hours. In general, subcultures from green colonies were referred to Dr G. I. Barrow (PHLS Truro) and those from yellow colonies to Dr A. L. Furniss (PHLS Maidstone) for identification and confirmation. Subcultures from yellow colonies which grew well in 10% salt tryptone broth, showed spreading either on marine agar or on Kanagawa test medium, and which gave a positive reaction in the Voges-Proskauer test were regarded as strains of *V. alginolyticus* and were not further examined (Donovan, Lee & Furniss, 1976). Non-spreading yellow colonies with characteristic morphology and which gave the correct biochemical reactions were regarded as sucrose-positive strains of *V. parahaemolyticus*. Certain laboratories, particularly on the south-west, south andeast, subcultured multiple colonies from TCBS plates, thus isolating a total of 1484 strains of *V. parahaemolyticus* from the 606 positive samples. The tests cited by Barrow & Miller (1976) were used to confirm all strains of *V. parahaemolyticus* before serological typing.

Serological typing and Kanagawa reaction

After confirmation at PHLS Truro, all strains of *V. parahaemolyticus* were examined for slide agglutination with specific O and K antisera. Strains which failed to agglutinate with any antisera or which showed auto-agglutination in 3% saline were regarded as untypable. Irrespective of the serological results, all strains were tested for their Kanagawa reaction precisely as described by Barrow & Miller (1976).

RESULTS

The sites sampled during the survey are shown in Figure 1, together with the arbitrary boundaries dividing the coastline into areas to assist collation and interpretation of the results. It should be noted that in Area IV the River Humber has been considered separately in assessing the results; for reasons explained later, it is regarded as a special area. The sites where *Vibrio parahaemolyticus* was identified are also shown.

During the survey, 2816 samples were taken from 105 sites; *V. parahaemolyticus* was isolated from a total of 606 samples from 67 of the sites. Because of the distribution of participating laboratories and remoteness or inaccessibility of some

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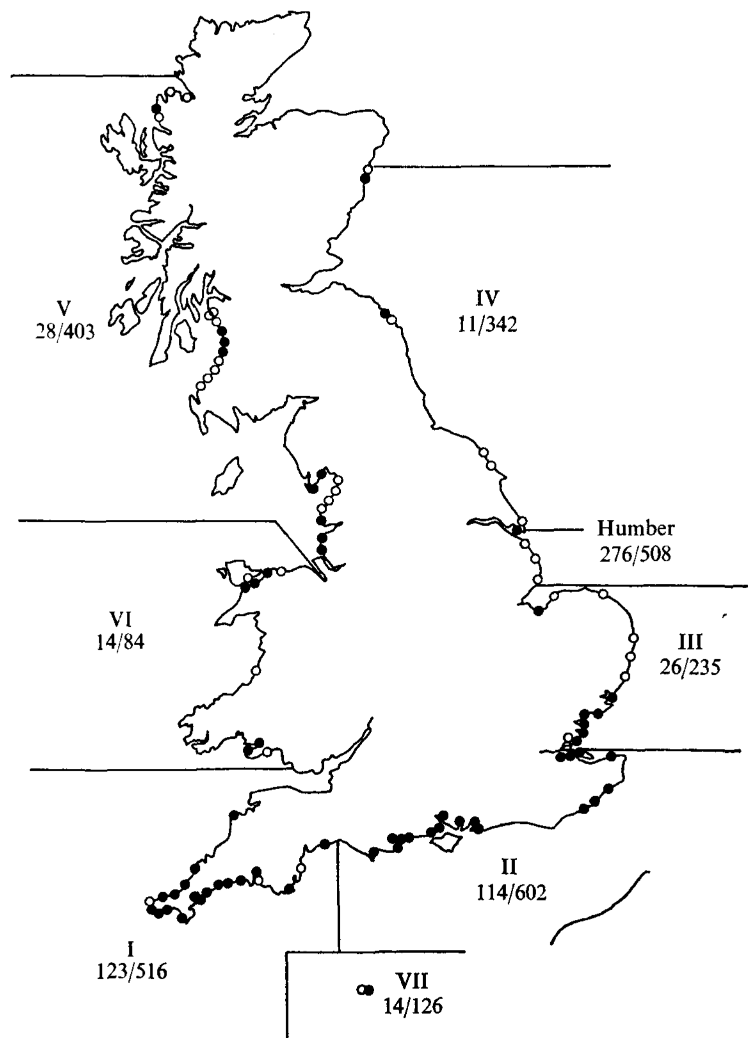

Fig. 1. Distribution of samples by areas (I–VII) and incidence of *Vibrio parahaemolyticus*. ●, At least one sample positive. ○, All samples negative. Numerator shows number of samples positive, and the denominator, the number of samples taken in each area.

areas, relatively more samples were taken from the southern areas I, II and III than from the other areas. However, this was perhaps fortunate as the main areas of sampling activity correspond broadly to many of the main commercial shellfish areas, particularly molluscan shellfish, which are usually more estuarine or coastal in nature than the crustacean fisheries.

Analysis of the results from each of the three types of samples (water, sediment and shellfish) in areas I to VII are summarized in Tables 1, 2 and 3 respectively; the data from the River Humber are also shown in Table 3. The combined results obtained during 1975 and 1976 have been summarized on a quarterly basis (Table 4) to show the total number and types of samples examined and the numbers yielding *V. parahaemolyticus*.

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Table 1. *Frequency distribution of* Vibrio parahaemolyticus *in shellfish samples from the seven areas*

Area	I	II	III	IV	V	VI	VII	Total	%
1975									
Jan./Mar.	—	0/4	0/22	1/4	—	—	—	1/30	3
Apr./Jun.	0/1	0/15	5/28	0/7	0/2	1/3	—	6/56	11
July/Sept.	4/39	12/20	2/21	1/8	—	—	—	19/88	21
Oct./Dec.	0/14	13/28	3/7	1/6	2/5	0/11	—	19/71	27
1976									
Jan./Mar.	2/20	4/25	1/16	1/6	0/4	0/18	0/26	8/115	7
Apr./June	0/3	4/13	0/5	0/4	0/2	0/8	2/36	6/71	8
July/Sept.	—	1/8	0/4	0/2	—	7/13	6/40	14/67	21
Oct./Dec.	—	—	—	—	—	—	0/12	0/12	0
Total	6/77	34/113	11/103	4/37	2/13	8/53	8/114	73/510	—
%	8	30	11	11	15	15	7	14	—

Numerator: number of positive samples. Denominator: number of samples examined.

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Table 2. *Frequency distribution of* Vibrio parahaemolyticus *from sediments in samples from the seven areas*

Area	I	II	III	IV	V	VI	VII	Total	%
1975									
Jan./Mar.	—	4/18	1/9	0/3	—	—	—	5/30	17
Apr./June	0/4	7/38	3/21	0/14	0/19	—	—	10/96	10
July/Sept.	34/119	14/41	5/21	0/21	6/15	—	—	59/217	27
Oct./Dec.	7/31	13/57	1/4	1/4	6/15	2/7	—	30/118	25
1976									
Jan./Mar.	13/53	7/37	3/18	0/13	0/22	0/7	—	23/150	15
Apr./June	0/3	1/28	1/1	0/11	0/44	0/5	6/6	8/98	8
July/Sept.	—	2/15	—	0/7	2/60	4/9	—	8/91	9
Oct./Dec.	—	—	—	—	—	—	—	—	—
Total	54/210	48/234	14/74	1/73	14/175	6/28	6/6	143/800	—
%	26	20	19	1	8	21	100	18	—

Numerator: number of positive samples. Denominator: number of negative samples.

Table 3. *Frequency distribution of* Vibrio parahaemolyticus *in water samples from the seven areas and the Humber*

Area	I	II	III	IV	V	VI	VII	Total	%	Humber	%
1975											
Jan./Mar.	—	1/22	0/9	0/3	—	—	—	1/34	3	14/22	64
Apr./June	0/2	1/37	0/19	0/17	1/29	—	—	2/104	2	45/76	59
July/Sept.	31/124	11/42	1/18	0/18	6/30	—	—	49/232	21	50/76	66
Oct./Dec.	5/35	8/69	0/4	0/3	0/16	—	—	13/127	10	42/78	54
1976											
Jan./Mar.	27/65	0/38	0/8	0/7	0/32	0/1	—	27/151	18	37/78	47
Apr./June	0/3	6/30	—	0/12	0/48	0/1	0/6	6/100	6	26/74	35
July/Sept.	—	5/17	—	5/128	5/60	0/1	—	15/206	7	60/78	77
Oct./Dec.	—	—	—	1/44	—	—	—	1/44	2	2/26	8
Total	63/229	32/255	1/58	6/232	12/215	0/3	0/6	114/998	—	276/508	54
%	28	12	2	3	6	0	0	11	—	54	—

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Table 4. *Frequency distribution of* Vibrio parahaemolyticus *in all samples*

Samples	Shellfish	Sediment	Water	Total	Humber Estuary
1975					
Jan./Mar.	1/30 (3)	5/30 (17)	1/34 (3)	7/94 (7)	14/22 (64)
Apr./June	6/56 (11)	10/96 (10)	2/104 (2)	18/256 (7)	45/76 (59)
July/Sept.	19/88 (22)	59/217 (27)	49/232 (21)	127/537 (24)	50/76 (66)
Oct./Dec.	19/71 (27)	30/118 (25)	13/127 (10)	62/316 (20)	42/78 (54)
1976					
Jan./Mar.	8/115 (7)	23/150 (15)	27/151 (18)	58/416 (14)	37/78 (47)
Apr./June	6/71 (8)	8/98 (8)	6/100 (6)	20/269 (7)	26/74 (35)
July/Sept.	14/67 (21)	8/91 (9)	15/206 (7)	37/364 (10)	60/78 (77)
Oct./Dec.	0/12 (0)	(—)	1/44 (2)	1/56 (2)	2/26 (8)
Total	73/510 (14)	143/800 (18)	114/998 (11)	330/2308 (14)	276/508 (54)

Figures in ( ) indicate percentage of all serotypes found in area.
Numerator: number of positive samples.
Denominator: number of samples examined.

Tables 1–3 indicate that *V. parahaemolyticus* was isolated from each area. Table 4 shows that 14 % of the 510 shellfish samples, 18 % of the 800 sediment samples and 11 % of the 998 water samples taken from all areas were positive, as were 54 % of the 508 samples taken from the Humber estuary. From these results there does not appear to be any overall correlation between the frequency of the organism in the three types of samples. Thus, only 7 % of 114 samples of shellfish from Area VII were positive for *V. parahaemolyticus*, whereas the 6 samples of sediment examined were all positive. In contrast, in Area I, the highest isolation rate for water and sediment (28 and 26 % respectively) was accompanied by one of the lowest isolation rates for shellfish (8 %). The highest isolation rate from shellfish occurred in samples from Area II (30%); from sediments, isolation rates of 20–26 % were obtained from Areas I, II and IV; and from samples of seawater, the highest isolation rate of 28 % was from Area I.

In all areas except the Humber estuary, there was suggestive evidence of seasonal variations in the incidence of *V. parahaemolyticus*, higher isolation rates usually occurring in summer and autumn – 21–27 % in shellfish, 25–27 % in sediments, and 21 % in water samples. In winter, the isolation rates were 3–11, 9–17 and 3–18 % respectively for shellfish, sediments and water samples. In the Humber estuary, apart from the last quarter of 1976, isolation rates ranged from 35 to 77 % and showed no evident seasonal change. This was attributed to the discharge of effluents from the processing of imported crustacea.

By means of agglutination tests with specific O and K antisera, different serological types of *V. parahaemolyticus* may be recognized (Sakazaki, Iwanami & Tamura, 1968; Sakazaki, 1973). The serological classification is based on isolations from cases of human infection, and consequently the high proportion (31 %) of strains isolated in the survey which were not typable was to be expected (Table 5). The remainder all belonged to one or other of 47 different O:K serotype combinations recognized in the current antigenic scheme (Miwatani & Takeda,

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Table 5. *Incidence and distribution of serotypes among* 1479 *strains of* Vibrio parahaemolyticus *isolated from* 606 *of* 2816 *samples*

Serotypes		Areas								
O	K	I	II	III	IV	Humber	V	VI	VII	Total
1	1	2	—	—	—	—	—	—	—	2
1	25	10	3	—	—	2	—	—	—	15
1	26	—	—	—	—	1	—	—	—	1
1	32	1	1	—	—	2	—	—	—	4
1	33	—	—	—	—	2	—	—	—	2
1	38	1	—	—	—	—	—	—	—	1
1	41	1	—	—	—	2	—	—	—	3
1	56	8	8	—	—	2	—	—	1	19
2	3	44	55	2	—	8	1	—	—	110
2	28	18	5	—	—	47	8	1	—	79
3	4	3	—	—	—	1	1	—	—	5
3	5	—	—	—	—	2	—	—	—	2
3	6	5	6	—	—	—	—	—	—	11
3	7	—	1	—	—	—	2	—	—	3
3	29	1	21	—	1	2	—	3	—	28
3	30	39	1	1	—	—	—	—	—	41
3	31	1	1	—	—	1	—	—	—	3
3	33	12	2	1	—	1	—	—	—	16
3	37	—	—	1	—	3	—	1	—	5
3	45	—	1	—	—	1	—	—	—	2
3	57	—	—	1	1	19	—	1	—	22
4	4	—	3	—	—	1	1	—	—	5
4	8	—	2	—	—	—	—	—	—	2
4	10	—	1	—	—	1	—	1	—	3
4	12	4	8	2	—	3	1	—	—	18
4	13	—	1	—	—	—	—	—	—	1
4	34	—	2	—	—	1	—	—	—	3
4	42	8	—	3	—	1	1	1	—	14
4	49	—	—	—	—	2	—	—	—	2
4	53	—	—	—	—	6	—	—	—	6
4	55	—	2	—	—	7	—	—	—	9
5	15	7	1	—	—	—	—	—	—	8
5	17	55	11	6	—	50	3	—	—	125
5	30	9	36	—	5	294	2	—	—	346
6	18	—	2	—	—	1	—	—	—	3
6	46	—	—	—	—	25	1	—	—	26
7	19	—	—	—	1	2	—	—	—	3
8	20	7	8	3	—	18	3	—	—	39
8	21	—	1	—	—	—	—	—	—	1
8	22	2	2	—	—	1	—	—	—	5
8	39	2	3	1	—	1	—	—	—	7
9	44	—	1	—	2	—	—	—	—	3
10	24	—	1	—	—	1	—	—	—	2
11	36	1	3	2	—	4	—	—	—	10
11	40	2	1	—	—	—	—	—	—	3
11	50	—	1	—	—	—	—	—	—	1
11	51	—	2	—	—	2	—	—	—	4
Untypable		21	89	25	8	293	6	6	13	461
Totals		264	286	48	18	810	30	14	14	1484
No. of samples		516	602	235	342	508	403	84	126	2816
No. positive		123	114	26	11	276	28	14	14	606

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Table 6. *The frequency distribution of the main serotypes of* Vibrio parahaemolyticus *identified*

Serotypes						
O	K	I	II	Humber	III, IV, V, VI, VII	Total
1	56	8 (3)	8 (2·8)	2 (0·2)	1 (0·8)	19 (1·3)
2	3	44 (16·7)	55 (19·2)	8 (1·0)	3 (2·5)	110 (7·4)
2	28	18 (6·8)	5 (1·7)	47 (5·8)	9 (8)	79 (5·3)
3	29	1 (0·4)	21 (7·3)	2 (0·2)	4 (3·4)	28 (1·9)
3	30	39 (14·8)	1 (0·3)	—	1 (0·8)	41 (2·8)
3	33	12 (4·5)	2 (0·7)	1 (0·1)	1 (0·8)	16 (1·1)
3	57	—	—	19 (2·3)	3 (2·5)	22 (1·5)
4	12	4 (1·5)	8 (2·8)	3 (0·4)	3 (2·5)	18 (1·2)
5	17	55 (20·8)	11 (3·8)	50 (6·2)	9 (7·6)	125 (8·5)
5	30	9 (3·4)	36 (12·6)	294 (36·3)	7 (5·9)	346 (25·4)
6	46	—	—	25 (3·1)	1 (0·8)	26 (1·8)
8	20	7 (2·6)	8 (2·8)	18 (2·2)	6 (5·0)	39 (2·6)
Untypable		21 (7·9)	89 (31·1)	293 (36·2)	58 (44·5)	461 (30·8)

Figures in ( ) indicate percentage of all serotypes found in area.

1976); only 8 serotypes were not represented. Although 3 new K antisera (58, 59 and 60) were used during the last few months of the study, no such strains were found.

The results of serological typing of the 1484 strains of *V. parahaemolyticus* isolated from the 606 positive samples in the survey are given in Table 5. Although there were differences in the incidence of different serotypes from different areas, this was perhaps to some extent a reflexion of the number of samples taken and the different isolation techniques used as well as the number of colonies subcultured for examination. Thus, samples from the Humber and Areas I and II yielded a wide range of serotypes. Untypable strains formed about a third of all the cultures examined and they were again dominant in the Humber and in Area II. In Area I, 24 different serotypes were identified among 264 isolations, with only 21 strains (8 %) untypable; in contrast, in Area II, 33 different serotypes were recognized among 286 isolations, but 89 strains (31 %) were untypable. A total of 47 different serotypes were identified among the 1484 strains examined. The incidence and area distribution of the more common serotypes found are summarized in Table 6. Serotype O5.K30 accounted for 23·5 % of the strains found in all samples, followed by O5.K17 (8·5 %) and O2.K3 (7·5 %). The commonest serotypes in Area I were O5.K17 (20·8 %), O2.K44 (16·7 %) and O3.K30 (14·8 %), and in Area II, O2.K3 (19·2 %) and O5.K30 (12·6 %). Serotype O5.K30 accounted for 36·3 % of the strains isolated from the Humber estuary. In the remaining areas, no particular serotype was predominant. All 1484 strains of *V. parahaemolyticus* gave negative reactions in the Kanagawa test for potential pathogenicity. Sucrose-positive variants accounted for 103 (6·9 %) strains; of these, 72 were untypable and the remainder represented 15 different serotypes. During this investigation, 985 halophilic strains, as yet unclassified, were also isolated on TCBS medium; these resembled *V. parahaemolyticus* colonially, but they varied in their biochemical characters.

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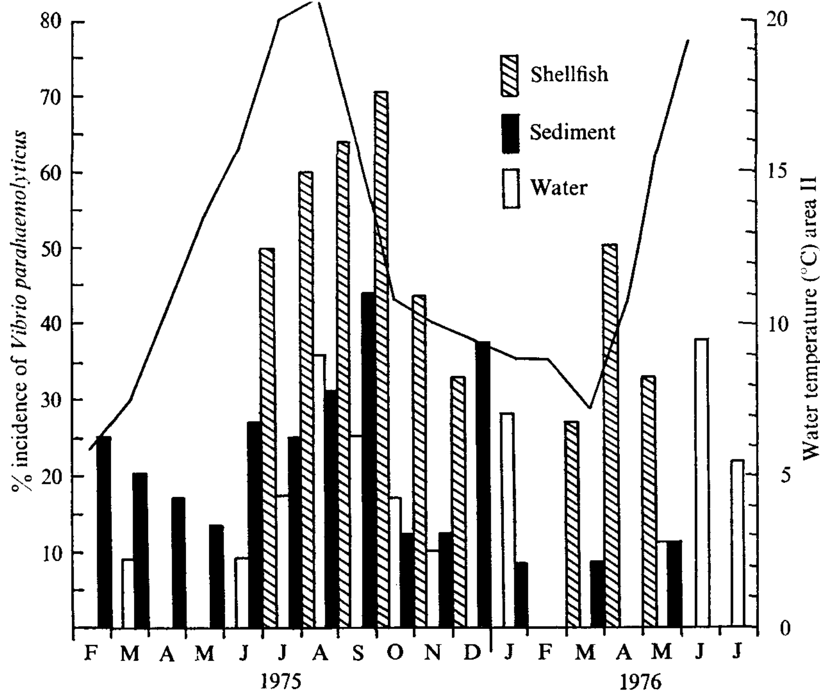

Fig. 2. Percentage incidence of *Vibrio parahaemolyticus* from samples of shellfish, water and sediment in Area II in relation to water temperature shown by continuous line.

DISCUSSION

In attempting an extensive survey of the nature described in this paper there are inevitable shortcomings which require consideration before drawing any conclusions from the results. More extensive coverage of the coastline, both in number of sites and the frequency of sampling would have been desirable for detailed assessment of the distribution of *V. parahaemolyticus* and its seasonal variations. Remoteness of much of the coastline and distance from laboratories concerned inevitably led to great variation in the sampling rates, with sporadic sampling or none at all in some areas. The methods used for sampling as well as for laboratory examination also need consideration. Since many laboratories had already developed their own techniques, it was not considered necessary to adopt 'standard' methods for this survey. However, the use of both membrane and clarifying filters permitted the examination of larger volumes of water and they were notably successful in detecting *V. parahaemolyticus* in certain laboratories. Similarly, the examination, by some laboratories, of oyster shells and shellfish tissues separately often yielded *V. parahaemolyticus* from the shells but not from the tissues alone; the crevices in the rough shells presumably retained sediment containing the organism. Despite these limitations, however, the results of this collaborative study have yielded useful data on the distribution of *V. parahaemolyticus* in British coastal waters. The results suggest that the organism is usually present in southern and western areas, but is less common in the north and east (Fig. 1). During 1975, a seasonal trend in incidence was observed in the south and west

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with greater isolation rates during the summer and autumn, although the maximum water temperature rarely exceeded 20 °C (Fig. 2).

The results from the Humber area stress the importance of non-indigenous sources of *V. parahaemolyticus*. Imported crustacea from the Far East and elsewhere are processed and repackaged in this area, and only on such occasions was *V. parahaemolyticus* isolated from the Humber estuary. The serotypes present in the crustacea were similar to those isolated from the estuary, suggesting that the origin of the strains was effluent from the processing plants. The incidence of *V. parahaemolyticus* was high throughout the year and was related more to the degree of processing activity than to any other factor. It also is interesting that low water temperature (< 8 °C) during winter did not affect the frequency of isolation in the Humber as it did in other areas. However, despite some seasonal variation, it was still possible to detect *V. parahaemolyticus* in other coastal waters – even during winter when the maximum water temperature ranged from 5–10 °C. This is in contrast to the results of other workers (Kaneko & Colwell, 1973) who suggested retardation of growth in seawater below 15 °C with a minimum temperature of about 10 °C for isolation. Later, Kaneko & Colwell (1975*a*, *b*) concluded that in Chesapeake Bay *V. parahaemolyticus* over-winters on chitinous material from plankton which sinks to the bottom, the organism reappearing in the water column as temperatures rise in the following year – in effect an annual cycle of sedimentation and resuspension. In the present study, the organism was isolated throughout the year from sediments, water and shellfish, although sampling was not adequate to make any direct comparison with the American studies.

During a small offshore survey carried out in Cornwall in 1975 by MAFF Torry Laboratory *V. parahaemolyticus* was isolated from one of 140 edible crabs (*Cancer pagurus*) examined (Cann, 1976). However, none of the relatively few samples of shellfish, comprising lobster (*Homarus gammarus*), edible crab (*Cancer pagurus*), 'scampi' (*Nephrops norvegicus*) and shrimps (*Crangon vulgaris* and *Pandalus montagui*), collected offshore elsewhere on other occasions yielded *V. parahaemolyticus*. This confirms other observations (Hori *et al*, 1964; Hechelman, Asakawa & Leistner, 1969, Varga & Hirthe, 1975) which suggest that it is largely confined to coastal areas. Although Golten & Scheffers (1975) failed to isolate *V. parahaemolyticus* from samples of water from the Dutch coast during mid-summer, Kampelmacher, van Noorle Jansen, Mossel & Groen (1972) isolated the organism from larger volumes of sediment-rich estuarine water early in the year. In the present survey, more isolations of *V. parahaemolyticus* were obtained from muddy sediments than from sand or gravel, even when the different samples were collected very near to each other. This was possibly due to the presence of organic matter in the muddy sediments.

The survey has shown that many different serotypes of *V. parahaemolyticus* are widely distributed in coastal waters, sediments and shellfish around Britain – the more colonies examined per plate, the more serotypes were identified. All cultures isolated gave negative reactions in the Kanagawa test for potential pathogenicity; this accords with the findings of other workers, although Barrow & Miller (1974)

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and Wagatsuma (1974) detected occasional Kanagawa positive among numerous negative strains from marine sources when large numbers of colonies from primary plates were examined. The number of sucrose-positive variants of *V. parahaemolyticus* identified in this survey seems high (6·9 %), presumably because numerous yellow colonies from TCBS plates were examined. No urea-positive strains were found.

Despite the presence of *V. parahaemolyticus* in British coastal waters, only one confirmed incident attributed to home-produced seafood has been reported (Hooper, Barrow & McNab, 1974). This outbreak, caused by processed crab meat – probably contaminated by raw materials, kept and displayed at ambient temperature, thus allowing rapid bacterial multiplication – illustrates the importance of suitable treatment, hygienic processing and correct distribution and storage. Given these, it is clear that the small numbers of *V. parahaemolyticus* present in home produced seafoods, including purified shellfish eaten raw, are unlikely to pose any serious public health hazards.

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