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Indicator bacteria in freshwater and marine molluscs

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Abstract

The freshwater mussel *Anodonta cygnea* and four marine shellfish (mussels, *Mytilus edulis*; cockles, *Cerastoderma edule*; clams, *Mya arenaria*; *Scrobicularia plana*) from a total of six sites were surveyed for *Escherichia coli, Clostridium perfringens*, faecal streptococci, 25 and 37 ° C coliforms, 25 ° C and 37 ° C total viable numbers and fluorescent pseudomonads. The *A. cygnea* from an urban lake contained greater numbers of the faecal indicator bacteria than animals from a rural lake. There were also differences in the other bacterial counts and these were discussed with respect to bacterial parameter and animal characteristics. When freshwater mussels were transferred from the city site to the rural site for 24 h the load of faecal indicator bacteria was eliminated or significantly reduced. Other bacterial types took longer to become stabilised. Loss of indicator bacteria from *Anodonta* was also demonstrated using cleansing in the laboratory. Very high bacterial numbers were found in some marine molluscs notably *Scrobicularia plana* and most shellfish contained significant numbers of the three faecal indicator bacteria at every sampling occasion. The relationship between bacterial types was discussed and it was concluded that in both freshwater and marine animals the bacterial numbers were determined more by sampling site than by species of shellfish.

Introduction

Most bacteriological studies on marine molluscs have been concerned with the public health problems often associated with eating shellfish, for example the incidence and survival of *Salmonella* and *Shigella* (Kelly & Arcisz, 1954; Colwell & Liston, 1961). These workers also reported on the presence of *Escherichia coli* and other indicator organisms as did Thomas & Jones (1971) who concluded that the incidence of salmonellae in their unpurified mussels was related to the incidence of salmonellosis in the Conwy population. Ayres (1975) compared the numbers of *E. coli*, total coliforms at 37 ° C, faecal streptococci and *Clostridium perfringens* (*welchii*) from oysters, mussels and hard clams and considered that the latter three bacterial tests have a complementary value but do not replace *E. coli* as an index of faecal pollution in assessing shellfish entering British markets.

Several investigators have determined the numbers and types of indicator and other bacteria in various species of freshwater fin fish caught in relatively clean to moderately polluted waters (Thjotta & Somme, 1942; Venkataraman & Steenivasan, 1953). We are not aware of any similar studies on freshwater molluscs except that of Cooke (1976b) who found coliform bacteria including *E. coli* in the mussel *Hydridella menziesii* collected from New Zealand lakes. Therefore we have determined various bacterial numbers in the freshwater mussel *Anodonta cygnea* from two different sites and compared these values with those from four species of marine shellfish. We also studied the changes in bacterial populations which followed the transfer of freshwater mussels between sample sites. Our

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results show how bacteriological analysis of indigenous or transferred molluscs can be used to reveal sewage contamination of freshwater bodies in a way previously reported for marine waters (Webber & Trollope, 1976; Trollope & Webber, 1977; Ayres *et al.*, 1978). Some observations were also made of the application to *Anodonta* of laboratory cleansing (depuration).

Materials and methods

The collection of molluscs

Marine shellfish were collected at random from the shores as soon as they were uncovered by the tide. The freshwater mussels (*Anodonta cygnea*) were collected by hand from the bottom of the two lakes using a long handled sampling net. Each site was sampled on three different occasions at intervals of between one and thirteen days. All shellfish were transferred to the laboratory without water in dry sterile plastic bags and analysed within 1–2 h. All the animals, except those analysed in Table 4, were collected during May, June and July 1980. On each sample occasion, ten marine shellfish or two to five *Anodonta* were analysed.

Sampling sites

Anodonta cygnea was collected from an urban site, Brynmill Lake, Swansea (OS Reference SS 634925) and from Fairwood Lake, Gower (SS 583917) which is a rural site 5.2 km from Brynmill Lake. *Mytilus edulis* was collected from Mumbles, Swansea (SS 632876), West Pier, Swansea Docks (SS 660919) and near Loughor Railway Bridge (Loughor Bridge M; SS 563978). *Scrobicularia plana* was obtained near the latter (Loughor Bridge S; SS 563977); the remaining animals were from two nearby regions of the Burry Estuary situated approx. opposite Salthouse Point: cockles *Cerastoderma edule* (Salthouse Point C; SS 530961) and clams, *Mya arenaria* (Salthouse Point M; SS 521961).

Transfer experiments

Some freshwater mussels were collected from Brynmill Lake where they were relatively plentiful and transported dry to Fairwood Lake where they were placed on the lake bottom in a small basket constructed from plastic mesh and the top and base of a plastic bottle (Trollope, in press). In this way the mussels were able to function normally but were held captive thus enabling individuals to be withdrawn and examined bacteriologically after periods of immersion in the new freshwater location.

Preparation of tissue suspensions

The shells were cleansed with a small scrubbing brush under running water, dried with absorbant paper, and the animals opened using a sterile scalpel and appropriate twisting pressure (Thomas & Jones, 1971). The shell water was discarded (Trollope, 1982). For the *Anodonta*, individual animals were examined, all the flesh being cut free using sterile fine scissors and the volume determined using a sterile measuring cylinder. With the marine shellfish the flesh from 5 animals was pooled in a sterile measuring cylinder. For all the shellfish, two volumes of sterile double-distilled water were added to the cylinder, the contents transferred to a sterile polyethylene bag and the mixture homogenised for 1 minute using a Colworth Stomacher (A. J. Seward, Bury St. Edmunds, Suffolk).

Bacterial counting methods

Appropriate volumes (0.1 ml for coliforms and for total viable numbers; all others 0.2 ml) of the tissue suspensions were spread on the surface of dried agar plates in duplicate. Coliforms were determined using MacConkey agar No. 3 (Oxoid CM 115) at 37 °C and at 25 °C; lactose-fermenting colonies were enumerated after 24 h and 48 h respectively. The same medium incubated in special vented brass canisters (Astell Laboratory Service Co. Ltd.) completely immersed in a water bath controlled at 44 ± 0.25 °C yielded the *E. coli* counts after 24 h (Thomas & Jones, 1971). Total viable numbers (TVC) were obtained from nutrient agar (Oxoid CM 3) with separate incubation at 37 °C for 24 h and at 25 °C for 48 h. *Clostridium perfringens* was enumerated using blood agar (horse blood, 7%) containing neomycin sulphate (75 $\mu g\ ml^{-1}$) incubated anaerobically at 37 °C for 24 h. Faecal streptococci were counted on Slanetz and Bartley agar (Oxoid CM 377) with incubation at 37 °C for 48 h.

Pseudomonads were determined on Bacto-Pseudomonas agar F (Difco) using u.v. (254 nm) to locate fluorescent colonies following 36–48 h incubation at 25 °C.

Results

Bacterial types in the freshwater mussels

The *A. cygnea* were collected during May and June on three separate occasions over 12 and 18 days from Brynmill and Fairwood Lakes respectively. The animal lengths ranged from 10 to 16 cm with the Fairwood animals having the greater mean length. The total tissue volume of each of the Fairwood mussels ranged from 20 to 95 ml whereas those from Brynmill varied between 28 and 60 ml. These two freshwater lakes have markedly different environments. Brynmill lake is situated in the city, towards the edge of a heavily populated urban area and downhill from other heavily populated regions. It is set in a public park; dogs and birds (especially ducks) are much in evidence. In contrast, Fairwood Lake is lined by trees in a rural location on a private estate; human access is minimal but there are some ducks. The mean numbers of bacteria indicative of faecal pollution (*E. coli, C. perfringens,* faecal streptococci) were considerably greater in the molluscs from Brynmill than in those from Fairwood (Table 1). *E. coli* and *C. perfringens* were isolated from all Brynmill samples whereas *C. perfringens* was never isolated from the Fairwood samples and *E. coli* occurred only in two animals. The presence of faecal streptococci was variable but they were detected twice as often in the Brynmill animals as compared to the Fairwood ones. High numbers of *E. coli* appeared to be correlated with high numbers of faecal streptococci when both bacterial types were detected. Differences in the *E. coli* numbers per ml of flesh between animals collected from one site on the same occasion were not related to animal length or flesh volume.

Bacterial counts made at 25 °C were always greater than counts from the same samples made at 37 °C (Table 1); this difference was more noticeable in the Fairwood samples. All counts made at 37 °C were more variable than those made at 25 °C.

Fluorescent pseudomonads were isolated consistently from the Brynmill samples but the Fairwood animals showed a wider range of numbers (Table 1). The presence and quantity of these bacteria did not correlate with the presence and quantity of *E. coli, C. perfringens* or faecal streptococci. This lack of correlation was best demonstrated by relating the mean number of fluorescent pseudomonads to mean numbers of the other bacteria isolated from any one site, fluorescent pseudomonads being expressed as 1.0 (Table 2).

In general, for each sample site and for each bacterial type isolated, the variation in bacterial load between animals on different sampling occasions was no greater than the variation in bacterial load observed between different animals collected on the same occasion. High bacterial loads and lower bacterial numbers in individual *Anodonta*

Table 1. Numbers of bacteria per ml of tissue of the freshwater mussel *Anodonta cygnea* collected from two lakes and percentage of sampled mussels in which the bacteria were detected.

Bacteria	Brynmill Lake			Fairwood Lake		
	Mean	Range	%[a]	Mean	Range	%[b]
37 °C coliforms	8208	525–30000	(100)	4834	360– 19500	(100)
25 °C coliforms	21891	1200–48000	(100)	31256	9900– 76800	(100)
37 °C TVC[c]	16851	3300–45000	(100)	13485	2700– 36000	(100)
25 °C TVC	36138	12300–96000	(100)	52901	15600–132000	(100)
Escherichia coli	138	40– 315	(100)	3	0– 15	(25)
Clostridium perfringens	73	30– 150	(100)	0	0	(0)
Faecal streptococci	46	0– 135	(80)	6	0– 23	(50)
Fluorescent pseudomonads	309	90– 450	(100)	420	0– 1200	(75)

[a] % of 10 animals
[b] % of 8 animals
[c] Total viable count.

Table 2. Mean bacterial numbers per ml of mollusc tissue expressed as ratios to the mean number of fluorescent pseudomonads from each type of mollusc and site.

	Ratios of bacterial types*							
	1	2	3	4	5	6	7	8
Freshwater mollusc								
Anodonta cygnea								
Fairwood lake	1.0	.01		.01	12	74	32	126
Brynmill lake	1.0	.15	.24	.45	26	71	55	117
Marine molluscs								
Mytilus edulis								
West Pier	1.0	.63	1.23	1.23	32	78	62	152
Mumbles	1.0	1.77	1.22	1.99	15	57	56	245
Loughor Bridge M.	1.0	.1	.07	.31	7	20	17	36
Scrobicularia plana	1.0	.33	.18	.64	10	21	22	37
Mya arenaria	1.0	4.6	.32	2.6	10	87	78	195
Cerastoderma edule	1.0	7	.3	1.7	11	109	121	561

* 1: Fluorescent pseudomonads; 2: Faecal streptococci; 3: *Clostridium perfringens*; 4: *Escherichia coli*; 5: 37 °C coliforms; 6: 25 °C coliforms; 7: 37 °C total viable count; 8: 25 °C total viable count.

were not related in any obvious way to shell length or total tissue volume of the animal after dissection.

Transfer of freshwater mussels to a new site

The bacterial analysis of the changes that accompanied the transfer of the *Anodonta* from Brynmill to Fairwood (Table 3) was performed on two different animals at each transfer time except at 48 h when only one mussel was available. The faecal indicator bacteria that were more typical on Brynmill (*E. coli, C. perfringens* and faecal streptococci) were present initially and at 6 h but absent at 24 h and 48 h. *E. coli* appeared to be reduced more rapidly than *C. perfringens*; this could be due to a differential elimination rate of process for vegetative cells (*E. coli*) compared with endospores (*C. perfringens*) which could be influenced by the smaller size of the latter. The counts of fluorescent pseudomonads in transferred animals were variable as were those in indigenous animals. Counts made at 6 h were not typical of indigenous animals from either site. Counts made following immersion for 48 h showed a greater resemblance to Fairwood than to Brynmill and it seemed that immersion periods less than this were insufficient to effect a stable picture for bacteria other than those of faecal origin.

Table 3. Mean numbers of bacteria per ml of tissue of the freshwater mussel *Anodonta cygnea* collected from Brynmill lake and held captive in Fairwood lake.

Bacteria	Immersion period after transfer			
	0 h	6 h	24 h	48 h
37 °C coliforms	29610	23025	1155	5970
25 °C coliforms	55815	26160	5070	16590
37 °C TVC*	43785	24870	2490	7170
25 °C TVC	69900	31395	6570	37980
Escherichia coli	23	4	0	0
Clostridium perfringens	46	34	0	0
Faecal streptococci	15	8	0	0
Fluorescent pseudomonads	660	255	45	300

* Total viable count.

A second transfer experiment and the laboratory cleansing of freshwater mussels

A repeat of the transfer experiment during September 1982 met with changes at Fairwood lake; only three indigenous *Anodonta* specimens were found (at 48 h) and the lake had dense macrophyte growth and debris. Furthermore, the *E. coli* content of the indigenous *Anodonta* and following transfer indicated a change in the pollution regime. However, the bacteriological analyses did confirm several of the previous observations and some additional experiments were possible. *Anodonta* taken from Brynmill at the same time as the transferred animals

were placed in tap water in the u.v. cleansing apparatus used previously for marine molluscs (Trollope & Webber, 1977; Webber, 1982; Trollope, in press). The bacteriological values from transferred and cleansed mussels were means from two animals at each occasion and those data from Fairwood mussels were the means from three animals (Table 4).

Of the three faecal indicator bacteria sought in the Brynmill mussels, *C. perfringens* was absent and this organism was not evident also in any of the transferred and cleansed animals. The faecal streptococci were markedly reduced by 4 h cleansing and were absent after 24 h cleansing or transfer whereas *E. coli* was eliminated only by the cleansing apparatus and after 24 h. At 48 h the transferred mussels contained reduced numbers of *E. coli* that were comparable to those in the indigenous animals. As in the first transfer experiment fluorescent pseudomonads were present on all occasions and the numbers were variable. A trend was present suggesting that 37 ° C counts decreased with increasing immersion and 25 ° C counts concurrently increased, but was not so marked as previously. The shorther immersion periods produced some counts that were at variance with other trends, e.g. 4 h cleansing yielded numbers that were the lowest or second lowest (with the exception of *E. coli* and faecal streptococci). At 72 h, transferred mussels revealed several changes in bacterial counts including a rise in *E. coli* numbers and it is concluded that these were due to the pollution flux of Fairwood Lake.

Bacterial types in the marine molluscs

With the marine shellfish, the highest mean number for each of the eight bacterial types and the highest total bacterial load per ml was evident in the *S. plana* from the Loughor Bridge S site (Table 5). Mussels (*M. edulis*) from Loughor Bridge M (Table 6) also contained high bacterial numbers and for four of the eight bacterial types they yielded the second highest mean count in this marine comparison. Based on mean counts these Loughor M mussels contained just over half the total bacterial load of that isolated from *S. plana* at Loughor Bridge S, whereas all other shellfish contained between 30 and 39% of the *S. plana* loading. When ratios with fluorescent pseudomonads were compared, the Loughor Bridge mussels showed greater similarity to the *Scrobicularia plana* from Loughor Bridge than to the mussels from West Pier and Mumbles. (Table 2).

Animals from four of the six comparisons (*M. edulis* and *S. plana* from Loughor Bridge; *M. edulis* from Mumbles and *M. arenaria* from Salthouse Point M (Tables 5 & 6)) contained significant numbers of the three faecal indicator bacteria at every sampling occasion. Of the two remaining sites, the *M. edulis* from West Pier lacked both *E. coli* and faecal streptococci for one of the six counts made whilst the cockles from Salthouse Point C lacked *C. perfringens* with one count of the six made. Both these latter shellfish, on one other different sampling occasion, showed zero counts for

Table 4. Mean numbers of bacteria per ml of tissue of the freshwater mussel *Anodonta cygnea* collected from Brynmill lake on 13th September 1982 and either held captive in Fairwood lake or placed in a laboratory cleansing apparatus.

Bacteria	Immersion period after transfer to Fairwood					Immersion period in cleansing apparatus		
	0 h	24 h	48 h	F[a]	72 h	4 h	24 h	48 h
37 ° C coliforms	300	66	1040	2633	170	170	55	350
25 ° C coliforms	170	560	2130	5053	990	100	3100	300
37 ° C TVC[b]	9380	8780	3870	9280	4480	3980	8700	11730
25 ° C TVC	18500	10000	11000	16667	5500	1300	12250	9240
Escherichia coli	225	25	6	8	29	125	0	0
Clostridium perfringens	0	0	0	0	0	0	0	0
Faecal streptococci	180	0	0	0	0	1	0	0
Fluorescent pseudomonads	85	485	33	30	185	50	180	35

[a] The mean numbers from 3 mussels indigenous to Fairwood lake sampled on 15th September 1982.
[b] Total viable count.

Table 5. Numbers of bacteria per ml of tissue of three marine molluscs from the Burry estuary.

Bacteria	*Scrobicularia plana* (Loughor Bridge S)		*Mya arenaria* (Salthouse Point M)		*Cerastoderma edule* (Salthouse Point C)	
	Mean	Range	Mean	Range	Mean	Range
37 °C coliforms	9980	4890–21690	795	360– 1260	435	300– 840
25 °C coliforms	20960	6660–35760	6775	1560– 8490	4355	1290– 6330
37 °C TVC*	22025	12420–38460	6105	2160– 8460	4840	2010– 8460
25 °C TVC	37010	19560–60000	15205	10050–22650	22425	6810–54300
Escherichia coli	635	300– 1380	204	165– 270	69	30– 165
Clostridium perfringens	180	53– 345	25	8– 38	12	0– 23
Faecal streptococci	330	75– 810	356	240– 525	276	98– 585
Fluorescent pseudomonads	1000	450– 1500	78	30– 180	40	0– 60

* Total viable count.

Table 6. Numbers of bacteria per ml of tissue of the marine mussel *Mytilus edulis* from three locations.

Bacteria	West Pier		Mumbles		Loughor Bridge M	
	Mean	Range	Mean	Range	Mean	Range
37 °C coliforms	3485	240– 8280	1105	15–2070	2445	2220– 8970
25 °C coliforms	8545	750–17130	4185	180–7800	12155	7260–14790
37 °C TVC*	6765	1530–12000	4060	840–7290	10345	7080–15000
25 °C TVC	16715	3000–39630	17905	810–4080	22065	15000–26490
Escherichia coli	135	0– 300	145	8– 225	188	75– 375
Clostridium perfringens	135	45– 240	89	23– 210	40	15– 113
Faecal streptococci	69	0– 128	129	8– 255	63	23– 143
Fluorescent pseudomonads	110	0– 300	73	0– 120	607	240– 1200

* Total viable count.

fluorescent pseudomonads. This event also occurred once with *M. edulis* from Mumbles. In contrast, *M. edulis* from Worms Head, Gower (situated 14.4 miles west of Mumbles, Swansea) has shown zero counts for *E. coli* and faecal streptococci on all sample occasions (unpubl. observ.).

For nine of the 12 comparisons in Tables 5 and 6, the mean counts of *E. coli* were greater than the mean counts of *C. perfringens* and faecal streptococci. Two of the exceptions reflect individual counts from Salthouse Point clams and cockles which contained higher numbers of faecal streptococci than *E. coli* in all sample occasions. In the case of West Pier mussels, these contained more *C. perfringens* than *E. coli* with five of the six samples. The two different shellfish from the Salthouse Point region contained relative bacterial numbers (Table 2) that often differed to those of all the other animals. It is considered likely that this reflects the different location of the shellfish from Salthouse Point, in particular the greater distance from the shore line. In addition, this region was largely sand whilst all the others were varying mixtures of mud, sand and stones.

A further comparison produced data that were site rather than animal dependant. Computation of the ratios between *E. coli* and faecal streptococci for individual animals and pooled tissue (Table 7) yielded wide ranging values for any one mollusc, e.g. with *M. edulis*: 0.26–3.25 for Mumbles, but 1.67–8.0 for Loughor Bridge M. The values for the freshwater mussels were not dissimilar to the *M. edulis* values whereas cockles and clams were mostly lower. Using these individual results, and using ratios derived from mean numbers, the tendency revealed was for higher ratios to be obtained from sites nearest to known or suspected sources of faecal pollution. Consideration of representative bacterial numbers from individual animals and tissue pools shown as scatter diagrams for each marine

Table 7. Ratios of *Escherichia coli* ml^{-1} to faecal streptococci ml^{-1} in mollusc tissue.

	Range	Mean
Freshwater		
Anodonta cygnea		
Fairwood lake	1.7 -4	2.9
Brynmill lake	0.94-1.88	1.41
Brynmill transferred for		
0 h	1.53	
6 h	0.5	
Marine		
Mytilus edulis		
West Pier	1.17-2.53	1.98
Mumbles	0.26-3.25	1.29
Loughor Bridge M.	1.67-8	3.91
Scrobicularia plana	1.48-3.25	2.36
Mya arenararia	0.34-1.06	0.63
Cerastoderma edule	0.08-0.52	0.29

site was also used (Fig. 1). The totals of the three faecal indicator bacteria (*E. coli, C. perfringens* and faecal streptococci) (Fig. 1a) were compared with fluorescent pseudomonads (Fig. 1b), 37 ° C coliforms (Fig. 1c) and 37 ° C total viable count (TVC; Fig. 1d). The 25 ° C coliforms (not shown) at each site showed considerable similarity to the 37 ° C TVC and the 25 ° C TVC (not shown) was a spread out version of the 37 ° C TVC. It was assumed that each mollusc behaved in a consistent manner towards bacterial levels in the water (and sediment where applicable), regardless of bacterial type. The diagrams indicated that the mussels, for each bacterial type except faecal indicator totals exhibited slightly different ranges that were consistent for the three different locations (Fig. 1b, c, d). The *Scrobicularia* from Loughor Bridge S consistently showed the widest range with all bacterial types but there was also overlap into the higher counts from mussels at Loughor Bridge M. The clams and cockles from the Salthouse Point region portrayed counts that were always overlapping with each other to a considerable extent. However, comparisons with other sites and other molluscs did not show consistent differences. In the case of the faecal indicator totals (Fig. 1a) there was similarity with mussels at Loughor Bridge M but in 37 ° C TVC (Fig. 1d), 25 ° C TVC and 25 ° C coliforms (not shown), they compared more closely to West Pier mussels. For fluorescent pseudomonads (Fig. 1b) they compared more closely to mussels from Mumbles and West Pier, but with 37 ° C coliforms (Fig. 1c) the similarity was only with Mumbles mussels.

Discussion

When the relative populations from the freshwater *Anodonta* at both sample sites were compared with the bacterial populations isolated from the four varieties of marine molluscs, no obvious correlations were evident. Differences in the bacterial numbers isolated from the freshwater animals were likely to be a function of the two sites examined. This conclusion was substantiated by the transfer experiments. The counts of *E. coli* and 37 ° C coliforms obtained with indigenous *Anodonta* from the rural site (Fairwood), were considerably greater than comparable values reported by Cooke (1976b) from *Hydridella menziesii* sampled in three New Zealand freshwater lakes. Furthermore, our counts of these two bacteria from the marine shellfish were about 100-fold greater than comparable values from unspecified marine shellfish from New Zealand (Cooke, 1976a). With both freshwater and marine samples Cooke found that the majority of the coliforms isolated were resistant to one or more antibiotics but only the marine isolates had a capacity for resistance transfer (Cooke, 1976a, b).

Numbers of faecal bacteria *E. coli*, coliforms and faecal streptococci are reduced but not eliminated by conventional sewage treatment processes, and pseudomonads are amongst the most frequently isolated bacteria from such treatments (Lynch & Poole, 1979). However, pseudomonads are common in all aquatic environments, their numbers increasing with increasing nutrients (Lynch & Poole, 1979). In the sea water samples analysed by Merchelano *et al.* (1975) pseudomonads were always the numerically dominant bacteria comprising 65–73% of the isolates. They dominated (17% of isolates) the Gram-negative flora of fresh North Sea cockles but were the second most numerous group in scampi (*Nephrops norvegicus*) and queens (*Chlamys opercularis*) comprising 11% and 16% of the isolates respectively (Cann 1977). The same situation was reported with fresh oysters (*Ostrea edulis*) where pseudomonads comprised 32% of isolates (Ayres *et al.*, 1978). *Pseudomonas aeruginosa* was present in 48% of the oysters and 74% of the mussels examined by Denis (1975).

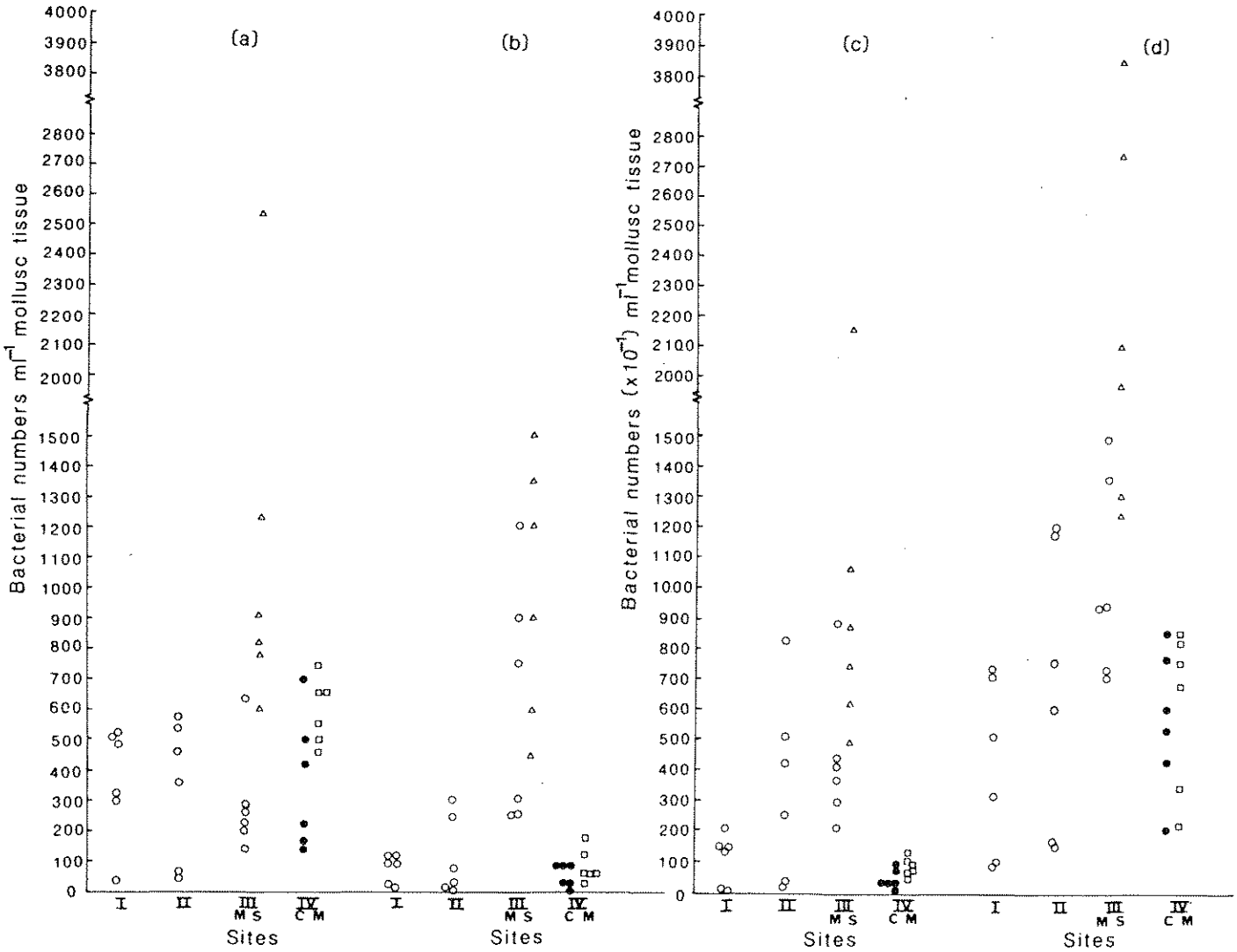

Fig. 1. Numbers of bacteria in marine mollusc tissue (a) faecal indicator bacteria (*Escherichia coli* + *Clostridium perfringens* + *faecal streptococci*) ml $^{-1}$; (b) fluorescent pseudomonads ml $^{-1}$; (c) 37 °C coliforms (× 10 $^{-1}$) ml $^{-1}$; (d) 37 °C total viable count (× 10 $^{-1}$) ml $^{-1}$; Site I: Mumbles, Swansea; Site II: West Pier, Swansea Docks; Site III M: Loughor Bridge M; Site III S: Loughor Bridge S; Site IV C: Salthouse Point C; Site IV M: Salthouse Point M. ○: *Mytilus edulis*; △: *Scrobicularia plana*; □: *Mya arenaria*; ●: *Cerastoderma edule*.

Fluorescent pseudomonads were predominant among strains from the skin, gills, stomach and intestine of four different species of freshwater fish (Thjotta & Somme, 1972). The alimentary tract of freshwater trout and salmon was dominated by *Enterobacter* (36% of isolates) with *Aeromonas* (13%), *Pseudomonas* (9%) and 6% of each of the following: *Acinetobacter, E. coli, Vibrio,* miscellaneous Gram-negative rods (Trust & Sparrow 1974). The faeces of trout from a fish farm contained *E. coli, Enterobacter, Citrobacter, Aeromonas hydrophila* and numbers of faecal streptococci that were elevated when compared with the source material and appeared to be multiplying in fish intestine (Niemi & Taipalinen, 1982).

Several of the foregoing bacterial genera are pathogenic for man and shellfish can be used to reveal the presence of such pathogens. Previous work with mussels from the West Pier and Mumbles sites (Al-Jebouri & Trollope, 1978; Al-Jebouri, 1980) showed the presence in these samples of *Shigella dysenteriae, Yersinia enterocolitica, Klebsiella pneumoniae, Enterobacter cloacae* and *Serratia* sp. There are outfalls discharging sewage effluents near these shore sites and mussel samples from Mumbles which were the subject of a recent report by us on *E. coli* content (Al-Jebouri & Trollope, 1981), concurrently yielded the food poisoning organism *Salmonella hadar* (unpubl. results). These mussels, like the majority of the present data, were obtained from regions suspected or known to be polluted and purification or cleansing treatments were not included.

Before human consumption, shellfish purification (i.e. cleansing; depuration) is usually essential, the recommended purification period for oysters and clams being 48 h although 24 h may sometimes be used and the initial loading is an important consideration (Furfari, 1979). A previous study found that continuous immersion in sea water for 24 h was sufficient to remove *E. coli* from *M. edulis* on some occasions but only 48 h was sufficient to ensure 100% removal (Trollope & Webber, 1977). With *Scrobicularia*, an immersion period of up to 7 days was necessary (Trollope & Webber, 1977) and the longer time was in accord with the low pumping rate and deposit feeding mode (Hughes, 1969). In the present study, complete removal of the three faecal indicator bacteria from *Anodonta* was achieved after 24 h immersion in Fairwood lake during the first transfer experiment (Table 3). During the cleansing experiment, *C. perfringens* was absent but complete elimination of *E. coli* and faecal streptococci was evident after 24 h (Table 4). Twenty four hours is a relatively short immersion period in view of the slow pumping rate reported for *Anodonta* (De Bruin & Davids, 1970). These authors concluded that marine oysters (*Ostrea* and *Crassostrea*) performed a pumping velocity that was 5–10 times as high as that of *Anodonta*. Thus, notwithstanding a low pumping rate, there is a potential role for bacteriological analysis of *Anodonta* to be used following transfer between sites as well as the sampling of indigenous populations.

The present study also demonstrated for the first time that *Anodonta* could be subjected to a laboratory cleansing process which effects removal of faecal indicator bacteria. Therefore cleansed *Anodonta* could be used to monitor waterborne bacterial populations in a manner similar to that demonstrated for marine molluscs (Trollope & Webber, 1977; Trollope, 1983; Webber, 1982). The low values for bacterial numbers noted after 4 h cleansing (Table 4) and 6 h transfer (Table 3) could be caused by the absorption of bacteria already present in the gut contents with any replenishment being initially slow due to some interuption in pumping and feeding brought about by sampling and transfer.

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