

ACCUMULATION OF BACTERIA BY TWO SPECIES OF WEST COAST SHELLFISH
MAINTAINED IN AN ESTUARINE ENVIRONMENT

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INTRODUCTION

Several investigations on the accumulation of coliform bacteria by shellfish under controlled laboratory conditions have been made. Species used in these experiments have included the Eastern oyster (Crassostrea virginica) (Kelly, et al, 1960), the Pacific oyster (Crassostrea gigas) and Olympia oyster (Ostrea lurida) (Kelly, 1961) and Native Littleneck clams (Protothaca staminea) and Manila clams (Tapes philippinarum) (Beck, et al, 1966). In addition, some observations on accumulation of coliform organisms by the Eastern oyster under natural conditions were made by Presnell and Kelly, (1961).

Studies on wet storage of oyster and clam shellstock are being conducted at this laboratory. These studies are providing information on the immediate or short term response of shellfish to changes in bacteriological and physical conditions during the individual experiments (Jakubowski and Vasconcelos, 1966). Comparison of the overall results of the experiments conducted under various hydrographic and climatological conditions provided additional information. The latter type of comparison is the subject of this paper.

MATERIALS AND METHODS

Experimental Design:

The apparatus, shellfish sampling procedures and methods of examination used in the wet storage studies were described in the preceding presentation (Jakubowski and Vasconcelos, 1966).

Data Analysis:

The data presented in this report are based on geometric means calculated from all results for each index in each experiment. The number of sampling intervals during each experiment were as follows: Experiment one 14, Experiment two 12, Experiment three 13, Experiment four 13, and Experiment five 17. Since duplicate samples were taken at each interval the number of samples represented is twice the number shown above for each experiment.

The values calculated represented the mean level or concentration of each index organism in the water and the two shellfish species during the 48 hr duration of each experiment. Accumulation ratios for each index were also calculated by dividing the mean level in the shellfish by the mean water concentration.

each of the five experiments are shown in Fig. 1. Variations in mean coliform levels in the water were fairly large. The difference between levels in experiments 2 and 5 was nearly 100 fold. The mean coliform levels accumulated by the shellfish during the first four experiments were very similar although the mean levels in the water varied considerably. These results also show that mean coliform levels in the clams were higher than those in the oysters in all five experiments.

Mean fecal coliform levels are shown in Fig. 2. The fecal coliform MPN's of the water followed the same general pattern as was shown by the coliform MPN's and the difference in fecal coliform level between experiments 2 and 5 was of the same magnitude as the coliform difference. In this case also mean levels accumulated by the shellfish were more uniform than the levels in the water. Fecal coliform levels in the clams were consistently higher than in the oysters.

The results using mean 20 C plate count levels are shown in Fig. 3. Levels of this index in the water were much more uniform than the coliform and fecal coliform levels. In addition, the highest plate count levels were shown in experiment 5 when coliform and fecal coliform levels were lowest. The plate count levels in both shellfish species were also much more uniform than were the coliform and fecal coliform levels. In all except one experiment, oysters showed higher levels of organisms enumerated by the 20 C plate count than did clams.

The accumulation ratios of the means shown by the two species are shown in Table 1. The lowest coliform and fecal coliform accumulation ratios in both species occurred in experiment 2. During this experiment, water temperatures were lower than in any of the other four experiments. Nearly 4 inches of rain with a corresponding large variation in salinity also occurred during this experiment. However, in experiment 4, during which water temperatures attained only slightly higher levels and a similar wide range in salinity occurred, much higher mean coliform and fecal coliform ratios occurred.

When coliform and fecal coliform ratios are ranked in increasing order they follow similar patterns in both species with the exception of experiment 5. In this experiment accumulation ratios of the clams were very high while those of the oysters were much lower. It is also interesting to note that the clams showed the lowest coliform and fecal coliform ratios when the levels of these indices in the water were highest (Exp. 2) and the highest ratios when the levels of these groups in the water were the lowest (Exp. 5).

An entirely different pattern is shown by the 20 C plate count ratios. The accumulation ratios are more uniform throughout the series of experiments. However, the highest ratios again occurred in both species when 20 C plate count levels in the water were lowest (Exp. 4) and the lowest ratios occurred when levels in the water were highest (Exp. 5).

DISCUSSION

Coliform and fecal coliform levels in the water followed a pattern to be expected in the Pacific Northwest. Levels were much higher during the winter and spring months when considerable runoff occurred and much lower in midsummer when little precipitation and consequent runoff occurred. In contrast the 20 C plate count levels in the water remained fairly constant. Although the level was constant the composition of the species making up the flora undoubtedly varied with changing temperatures, nutrients available, and other factors.

As indicated in the previous paper (Jakubowski and Vasconcelos, 1966) changes in bacterial concentration in both water and shellfish occurred rapidly during each experiment. In view of the lag and lesser degree of change shown by the shellfish the validity of the use of mean ratios derived from all the results in each experiment may be questioned. However, the sampling intervals and the number of tide cycles were similar in each experiment. Also, the lag in shellfish response occurred during both increases and decreases in bacteriological levels in the water. Therefore, the low and high ratios observed at these extremes would tend to offset each other.

The accumulation ratios shown in these experiments were considerably higher than those observed previously in laboratory experiments using these same species. In a series of 11 such experiments conducted by Kelly (1961) the highest average accumulation ratio obtained with Pacific oysters was less than 10 fold. In another series of 12 experiments, the highest average ratio attained by Manila clams was 38 fold (Beck *et al*, 1965). Under laboratory conditions, Eastern oysters showed accumulation ratios as high as 16 fold (Kelly, Arcisz, and Presnell, 1960). Later studies by Presnell and Kelly (1961) indicated that Eastern oysters sampled under natural conditions showed accumulation ratios similar to those attained under laboratory conditions.

Several factors may have contributed to the high accumulation ratios observed in these experiments. As indicated above, bacterial levels in the shellfish usually did not decline as rapidly as the levels in the water which would tend to make the shellfish mean levels higher than those of the water. Holding the shellfish suspended in the water rather than on the bottom may have had some effect but during two of the experiments, oyster samples were taken from the bed at low tide in the vicinity of the float. Since these oysters showed bacterial levels similar to those of oysters in the float sampled at the same time it appears that this may not have been a factor. Concentration of microorganisms present in the water during these experiments may have been a factor. Mean coliform MPN's ranged from 4.5 to 310 and fecal coliform MPN's from 2.5 to 150, while the laboratory experiments referred to above were usually conducted at MPN levels of about 1,000. The inverse relationship between bacterial levels in the water and levels in the oysters was pointed out above. However, it is difficult to perceive how differences in bacterial concentration in this range could influence accumulation.

It should be pointed out that this investigation is still in progress. The five experiments conducted to date have revealed some significant information relative to accumulation and elimination of bacteria by shellfish. However, more experiments and further analysis of the data accumulated will be necessary to identify the causes of the variations observed.

COMMENTS BY PARTICIPANTS

Mr. McMillin inquired about the relationship of the bacterial content of shellfish to that of the overlying water. Dr. Sparks indicated that the rates of uptake should be lower in the winter than in the summer due to slower physiological activity of the shellfish. However, the bacterial values of the overlying water were much higher in the winter and this may counteract the reduced physiological activity.

Miss Campbell asked if 35 C plate counts were included. Dr. Hoff replied that only 20 C plate counts were made. The plate counts were more stable throughout the study than the MPN's for coliform or fecal coliform organisms.

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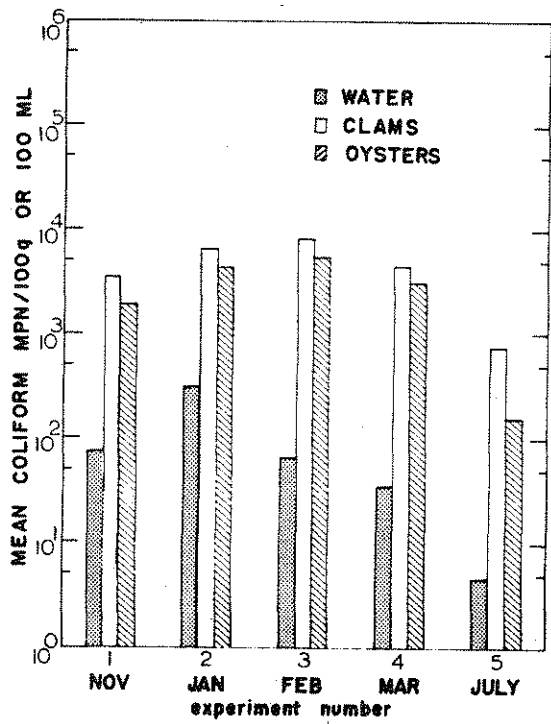


Fig. 1 Comparison of mean coliform MPN's of shellfish and seawater from five float runs

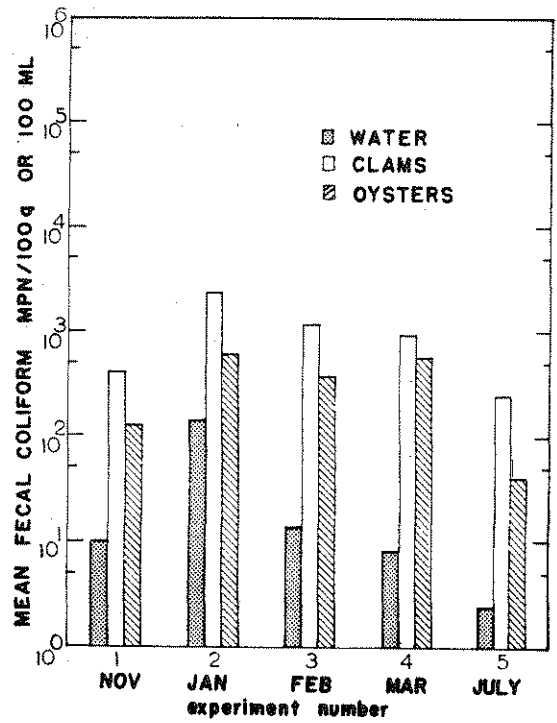


Fig. 2 Comparison of mean fecal coliform MPN's of shellfish and seawater from five float runs

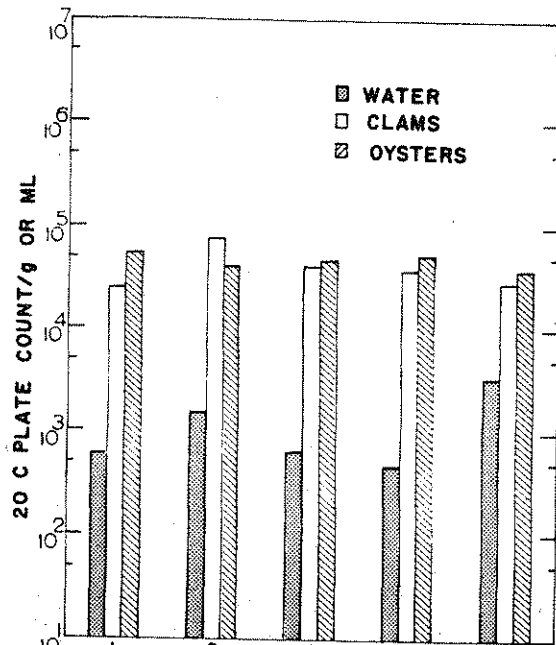


Table 1. Coliform, fecal coliform and 20 C plate count accumulation ratios of Pacific oysters and Manila clams

Experi- ment No.	Temp. Range (C)	Salinity Range o/oo	Total Rainfall (inches)	Accumulation Ratio:				Concentration in shellfish	
				Coliform MPN		Fecal Coliform MPN		Concentration in water	
				clams	oysters	clams	oysters	clams	oysters
1	8.7-10.6	23.5-30.8	0.28	50	26	41	13	43	90
2	7.3- 7.9	18.2-27.6	3.80	22	14	16	4	45	24
3	7.0- 8.0	26.5-29.3	0.44	120	85	86	27	63	72
4	4.0- 9.0	18.7-28.5	1.00	130	83	120	72	78	100
5	15.0-23.0	25.7-27.2	0.00	160	38	100	16	9	12