SHELLFISHERIES SESSION

THURSDAY—NOVEMBER 20

Chairman—William W. Anderson, Fish & Wildlife Service, Brunswick, Ga.

Bacteriological Standards for Oysters Grown in a Semi-Tropical Climate

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A number of careful epidemiological studies have established that oysters which have become contaminated by intestinal or urinary discharges present a danger from typhoid fever and other enteric diseases to the consumers of these raw oysters. Since the Wesleyan University epidemic, investigated in 1894, definitely proved the possibility of a widespread outbreak of typhoid fever resulting from the eating of polluted oysters, a good deal of thought has been given to the problem of shellfish sanitation. The well-known epidemic typhoid fever that occurred during the winter of 1924-1925 affecting oyster eaters in major eastern cities was studied. While it cannot be determined exactly how many of the 1500 cases and 150 deaths from typhoid fever were caused by eating infected oysters, the data presented show that practically all these cases may have been caused by the consumption of polluted oysters.

For fifteen years the Florida State Board of Health has carried on an inspection program with the oyster industry, and during this time no outbreaks of enteric disease caused by eating oysters have been reported from any of the oyster houses under the State inspection program. In the winter of 1949-50 a shellfish laboratory was established by the Florida State Board of Health in Apalachicola, seat of the major oyster-producing area in the State. In 1951 the laboratory became a joint State Board of Health and Franklin County project. The purpose of the laboratory has been to protect the public through laboratory control activities with oysters and oyster growing waters. Approximately 100 stations have been established in Apalachicola Bay for the purpose of obtaining water samples of the oyster-growing areas to determine whether the area is safe for the growing of commercial oysters. Sporadic sampling was done in the Bay between the years 1937 and 1947, at which time a major bacteriological survey was made of Apalachicola Bay and the estuary of Apalachicola River, which flows into the Bay. Due to the shallowness of the Bay there is little stratification of water in the oyster-growing areas, eliminating the possibility that samples showed varying bacteriological populations are due to stratification of the water. This extreme shallowness of Apalachicola Bay is one of its outstanding features. The average depth of water is approximately five feet. There are many shallow flats, which at low tide have only inches of water covering them. Salinity within the Bay varies considerably. Ingle and Dawson (1950) pointed out that annual variations in salinity from fresh water to 42.5 parts per thousand of salinity were noted in the Bay and that tidal variations of 8.1 parts per thousand are not uncommon. These salinity conditions are tolerated by the oysters, and commercial production is in operation where these conditions exist.

Studies indicate a rapid growth in the oysters of the Bay. The size of an inch may be achieved in five weeks and 2.6 inches of growth has been in 16 weeks. These growth rates suggest a high metabolic activity of the oysters, which may pump as much as 100 gallons per day through their gills. This may account for the high numbers of bacteria found in the bodies of the oysters.

In an attempt to aid the oyster industry to improve the quality of their product, samples of the oysters processed and sold by the industry were bacteriologically tested in the laboratory between September 1951 and May 1952. Laboratory procedures followed the routines as set forth in "Recommended Methods of Procedure for Bacteriological Examination of Shellfish and Shellfish Waters", published by the American Public Health Association (1943) except that a Waring Blender was used to marinate the oyster meats, instead of the use of glass beads.

Samples were taken from shucking houses. Shellstock was placed in clean paper bags, which were iced until delivered to the laboratory. Samples of shucked oysters were also obtained. These were shucked into sterile 150 ml ground glass-stoppered bottles. A third sample was obtained by choosing a random can of refrigerated shucked oysters from the oyster house cooler. Approximately 50 ml of shucked oysters were removed with sterile forceps, from the top of the, can, another 50 ml were obtained from the center of the can after pouring the top half of oysters onto a skimmer, and a third 50 ml portion was taken from the bottom of the can after pouring the remaining oysters out of the can onto the skimmer. Laboratory results were recorded as 'Most Probable Number" of coliform organisms per 100 ml of sample. Total counts were determined by plating on Tryptone Glucose Extract Agar, and recorded as counts per ml of sample. Table 1 shows the bacteriological findings of the samples collected in the oyster houses. There is no correlation between shellstock results, shuckers results and random can sample results. No correlation can be found between any combination of these three sampling points. The correlation coefficient for the series of Most Probable Numbers is as follows: Shellstock and shuckers 0.65, shellstock and random can samples 0.0483, shuckers and random can samples 0.620.

All of the results in Table 1 represent production from one oyster house, and many of the samples included in the table are those from oysters from one particular bar in Apalachicola Bay. The presence of high densities of coliform organisms in the oysters showed no pattern or correlation with external factors such as air temperature, water temperature, tidal action or coliform organism densities.

Since all of these oyster samples were taken from a certified shucking house, all of the oysters represented in Table 1 are from waters which have been declared safe for the commercial production of oysters and which therefore show a median Most Probable Number (MPN) of coliform organisms per 100 ml of sample of well below 70.

Parallel samples on the chart are those taken at the same time in the oyster house. Table 2 shows results that are typical of the work done in connection with oyster sanitation in Apalachicola Bay. The results indicate quite clearly that this is no close correlation between corresponding Most Probable Num-

TABLE 1

Bacteriological Results of Samples Taken from Shucking Houses Expressed as MPN (most probable number of coliform organisms per 100 m.l. of sample and TC (total bacterial count))

SHELLSTOCK		SHUCKERS		RANDOM CAN	
MPN	TC	MPN	TC	MPN	TC
		3,500	8,000		
		1,700	4,000		
16,000	>3,000				
1.8					
45	>3,000				
340	>3,000				
330					
490					
790					
1,100					
1,700					
1,300					
1,700	3,000				
3,500	5,700				
1,100	>300				
790	>300				
330					
330					
490					
1,300					
		11,000	20,000	5,400	5,000
		1,700	70,000	160,000	<300,000
790		1,100		2,400	
110	>3,000	3,500	12,000	16,000+	20,000
		490	30,000		
				3,300	800
				1,700	12,000
1,300	>300				
9,200					
		230	50,000		
		49	30,000		
		330	63,000	3,500	NO
		9,200	<300,000		
		1,300	3,500	1,100	5,000
		2,400	4,500		
33	>3,000	3,500	27,000	3,500	22,000
		11,000	900	3,500	ND
1,400	>3,000				
		700	>7,000		
3,500	>3,000	2,400	>3,000	3,500	>3,000
		11,000	16,000	17,000	7,000
		950		16,000	
		110	ND	4,600	<30,000
		13,000			
1,700	>3,000	220	>3,000	130	1,200
				330	
				700	
		5,400	9,000	5,400	60,000

SHELLSTOCK		SHUCKERS		RANDOM CAN	
MPN	TC	MPN	TC	MPN	TC
				3.500	300,000
				9,200	<300,000
				16,000	<300,000
				210	36,000
		13	>3,000	68	4,000
460	>3,000	490	5,000		
2,300					
		350	3,000	310	5,000
		16,000	12,000	16,000	20,000
9,200	10,000				
		170	4,000	700	5,000
480	5,000	3,500	12,000	5,400	ND
		950	3,100	170	13,000
78	>3,000	230	5,000		
				17,000	<3,000
		490	3,600		
490				1,300	
		16,000	80,000	1,700	140,000
				1,300	270,000
				9,200	22,000
		78	50,000	2,200	280,000
		16.000	10,000	16,000	20,000
5,400	>3,000				
		1,700	10,000	1,700	7,000
490	>3,000	2,400	>3,000	4,300	ND
		6,400	20,000	54,000	9,000
				7,000	>30,000
		13,000		9,200	
		16,000	20,000	16,000	8,000
		2,400			
		92	>3,000		
260	3,000	3,500	15,000	790	ND
		78	3,500	68	>3,000
140	>3,000	340	20,000	790	30,000
				5,400	3,000
				1,700	20,000
790		790		2,200	
		2,400	7,000	1,300	7,200
		1,300	20,000		
1,700	5,000	330	12,000	9,200	
		9,200	20,000		
		3,500	ND	3,500	ND
2,200	>3,000				
		330	4,300	3,500	3,600
1,300	ND	490	4,000	490	3,000
				2,400	7,000
16,000+	20,000	3,500	10,000	3,500	10,000
		16,000	24,000		
				1,400	6,000
		16,000	<30,000	16,000	30,000
		16,000+	25,000	16,000	30,000
		7,000	ND		
		4,900	5,000	9,200	3,500

SHELLSTOCK		SHUCKERS		RANDOM CAN	
MPN	TC	MPN	TC	MPN	TC
2,200	3,000	1,700	3,000	3,500	6,000
		1,800	2,000	3,500	3,000
		5,400		16,000	
790	>3,000	9,200	20,000		
5,400	>3,000				
16,000+	24,000	16,000+	ND		
4,100	>3,000				
790	>3,000	2,800	>3,000	5,400	>3,000
				11,000	9,000
				3,500	ND
		16,000		490	
				1,300	1,000
		2,400	8,000	16,000	10,000
				790	15,000
		490	>3,000	1,300	7,500
400	>3,000	790	3,200	3,500	16,000
		1,700		2,200	
9,200	10,000	16,000+	20,000	16,000	10,000
		3,500		1,300	
3,500		16,000+	28,000	16,000	12,000
16,000	8,000	9,200	20,000	16,000	
5,400	10,000	9,200	28,000	9,200	16,000
2,400	3,500	9,200	20,000	2,200	8,000
2,400		9,200	10,000	9,200	
490	3,000	1,700	4,000	1,400	4,000
170	3,000	460	3,000	490	4,000
330	3,000	2,400	5,000	1,300	8,000
1,800	3,000	790	3,000	460	3,000
330	3,000	330	4,000	490	9,000
16,000	32,000	16,000	30,000	16,000	30,000
16,000	30,000	5,400	30,000	16,000	30,000
MAXIMUM					
16,000	32,000	16,000	300,000	160,000	300,000
MEAN					
3,244	5,700	5,052	19,351	8,244	40,590
MEDIAN					
1,200	3,000	2,400	10,000	3,500	11,000
MINIMUM					
1.8—	300	13	900	68	800

ber of coliform organisms and Total Count. The correlation coefficient between the Most Probable Number and Total Count is 0.035.

Table 3 shows the Most Probable Number frequency of occurrence in the samples taken from the shucking houses. As can be noted, between 5 per cent and 10 per cent of the samples had an MPN of less than 230. Between 41 per cent and 60 per cent showed an MPN less than 2,300, and between 40 per cent and 60 per cent showed an MPN higher than 2,300. Whatever the point of sampling and whether or not the oysters were shucked from the shell aseptically in the laboratory or in the oyster shucking house, these percentages are representative.

TABLE 2
CORRESPONDING MPN AND TOTAL COUNT OF SAMPLES TAKEN FROM SHUCKING HOUSES

MPN	Total Count—Expressed as Thousands														
100		1		8							1				1
200		1	1	3										1	
300			1	1	1						2				1
400			3	3	2							1			
500			4	6	3										
600															
700					(1)			1			1	1			
800	1		1	3											
900															
1,000				1						1	3				3
2,000	(3)	1	(4)	(5)	2	1	1	3							1
3,000			3	2	2		(3)	(3)		1	(4)	2			(1)
4,000	1		1	3		(2)		1		1		2			
5,000				1	2				1	1					1
6,000			1	2								1			1
7,000											1		1		
8,000															
9,000			1							1	3	1			1
10,000										(2)	2	1			1
15,000	1								(1)		2				
20,000			1	1						3	8	(12)	1		2
20,000									1						1

It can be noted from Table 3 that MPN values above 2300 occur in greater frequency when there was more handling of the oysters. Conversely, MPN frequencies less than 2300 seem to decrease with handlings of oyster meats.

In an effort to determine the relationship to the Most Probable Number of coliform organisms found in oysters and water were run in the laboratory. The results of these samples are shown in Table 4. Unfortunately, procedures of sampling the water on an oyster bed at the same terms that oyster samples are being taken from the bed had not progressed to a degree where ample results are available for statistical analysis. The correlation coefficient between the water MPN and oyster MPN is 0.0110. From such samples there is no apparent correlation. No fixed ratio is evident so that it would be possible to compute the MPN of an oyster sample from its corresponding water score. Perhaps the oyster reflects past quality of its growing water and further study may reveal the time element relationship between water and oyster MPN's.

TABLE 3

OYSTER

MOST PROBABLE NUMBER FREQUENCY OCCURRENCE

MPN Less Than	Shellstock	Shuckers	RC
100	4	4	2
230	3	5	3
300	1		
400	6	6	2
500	8	7	5
600			
700		1	1
800	6	2	5
900			
1,000		2	
2,000	14	10	14
3,000	7	8	7
4,000	3	9	13
5,000	1	1	2
6,000	4	3	7
7,000		2	
8,000			1
9,000			
10,000	6	11	7
15,000		4	1
20,000	17	16	23
25,000			
30,000			
40,000			
50,000			
50,000+			
Total Samples	80	91	95

TABLE 4

Oyster Samples and Corresponding Water

	DEPTH SAMPLE		MOST PROBABLE NUMBER	
	Water (In.)	Oyster (Ft.)	Water	Oyster
Cat Pt.	6	2	17	78
”	6	6	2	130
”	6	3	7.8	40
”	6	3	4.5	37
”	6	2	1.8	78
11 Mi. Flat	6	unknown	2	490
Paradise	6	”	1.8—	130
”	”	”	11	110
E. Bay	6	4	49	17,000
”	6	4	70	35,000
”	6	4	33	9,500
”	6	3½	7.8	3,500
”	6	3½	2	3,300
”	6	3½	1.8	28,000
”	6	4	17	4,600
”	6	4	33	24,000
”	6	3½	2	7,000
”	6	7	170	1,300
”	6	7	540	3,500
”	6	7	4.5	490
”	6	4	2	3,500
”	6	3½	2	2,400
”	6	4	1.8—	9,200
”	6	4	1.8—	790
”	6	3	1.8—	790
”	6	6	1.8—	130
”	6	4	1.8—	220
”	6	8	110	45
”	6	5	79	1,700
”	6	6	79	1,700
”	6	4	240	330

Summary

1. Comparison of the Most Probable Number of coliform organisms and total bacterial count of oysters taken from the oyster houses reveals that the number of bacteria tends to increase with each successive handling of the oysters in the shucking house.

2. There appears to be no correlation between the Most Probable Number of coliform organisms with the total bacterial count of samples taken in the oyster houses.

3. The recommended scores listed by the Federal Security Agency, United States Public Health Service, should be reviewed relative to application to oysters from semi-tropical waters such as the Gulf of Mexico.

4. Studies should be made to determine the effects of salinity in brackish water on the death rate of coliform organisms.

5. Studies should be made to determine the effects of salinity in brackish water on the death rate of coliform organisms.

6. Studies should be made to determine the effects of temperature on the bacterial population of oyster-growing waters and oysters from these waters.

7. Studies should be made to determine the effects of temperature on the death rates of coliform organisms in oyster-growing waters.

8. Studies and investigations should be made relative to setting standards by which the sanitary quality of oysters may be judged.

9. Studies should be made to determine the relationships between bacterial counts obtained when a Waring Blendor is used and those obtained as outlined in Standard Methods where glass beads are used.

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Importance of Local Environment in Oyster Growth

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Cultivation of the oyster, *Crassostrea virginica*, Gmelin, has always been dependent to some degree on the transplanting of young oysters from so-called seed areas to growing grounds. In recent decades, however, the planter has had to go progressively farther afield for his source of seed and the scarcity of seed oysters in many producing areas is a problem of increasing concern. The transportation costs involved in these operations make it imperative that the imported stock survive and grow at better than average rates. Oysters are readily transplanted many thousands of miles from the area in which they set, but stocks from some areas have been found to grow better than others. It has been shown too, as in the case of the eastern oyster transplanted to the Pacific coast, that oysters, while growing well in a new environment may fail to reproduce normally. For these and other reasons, it has become desirable, if not imperative, that the biologist study growth and mortality rates in as many different imported stocks as possible so that the best of these may be recommended to the commercial planter.

Stocks of oysters from the Pensacola area, where there is an abundant set, have been sent to several different geographical regions to develop information on this problem. In cooperation with Mr. Francis Beaven of the Chesapeake Biological Laboratory at Solomons Island, Maryland, reciprocal plantings of native oysters have been made. This paper summarizes observations on Chesa-