A STUDY ON THE SANITARY QUALITY OF PACIFIC OYSTERS, *CRASSOSTREA GIGAS* AND GROWING WATERS IN BURLEY LAGOON, WASHINGTON

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美國 Washington州 Burley Lagoon에 있어서의 참굴, *Crassostrea gigas*과 그 棲息水域에 對한 衛生學的 硏究

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참굴(*Crassostrea gigas*) 垂下式養殖에 있어 上層과 底層 그리고 潮汐에 따른 海水 및 굴의 衛生學的 性狀에 관한 變化를 調査하였으며 本 硏究는 美國國際開發處(AID) 練修計劃에 의하여 Washington州 Burley Lagoon 멧목 垂下式 養殖場에 對하여 實施되었다.

海水의 水溫, 鹽分 및 濁度는 底層이 上層보다 다소 높았고 그 差異는 水溫 0.3℃, 鹽分 0.5‰, 濁度 0.1 JTU 였다. 潮汐에 따른 水溫 및 鹽分의 變化는 대체로 潮汐 週期에 一致하는 起伏狀을 보였다.

海水의 衛生指標微生物 含量은 上層이 低層보다 대체로 높았고 一般的으로 Coliform MPN 및 Fecal coliform MPN의 變化는 潮高에 逆比例하고 있어 이들 微生物群의 出處는 이 海域으로 流入하는 河川임을 알수 있다. 35℃ Plate count는 潮汐 變動에 대하여 보다 安定하였다.

굴에 있어 Coliform 및 Fecal coliform MPN의 平均値는 上層굴이 底層보다 一般的으로 높은 값을 示現하였으나 35℃ Plate count는 이와 같은 傾向을 나타내지 않았다. 海水에서와 같이 굴에 있어서도 Coliform 및 Fecal coliform MPN의 平均値는 兩者 모두 潮高에 逆相關關係를 나타내었고 調査期間中 굴의 細菌蓄積比는 Coliform MPN은 8.6~19.7, Fecal coliform MPN은 16.9~44.3이였다.

以上의 結果로서 굴 垂下式 養殖에 있어 貝類衛生管理上 重要한 것은 漲潮時에 굴을 採取하는 것이 衛生 指標 細菌의 含量이 보다 낮다는 것을 들수 있다.

INTRODUCTION

This study was conducted to obtain basic data on the sanitary quality of Pacific oysters, *Crassostrea gigas*, and growing waters associated with hanging culture(raft culture). Sanitary quality changes between top and bottom of both water and oysters and sanitary quality of oysters and growing waters depends upon tidal changes in an estuarine area are investigated.

The study was carried out on the raft cultivation of Pacific oysters in Burley Lagoon, Washinton during period of January 20 to January 21, 1969 under the support of the U.S. Public Health Service Pacific Northwest Marine Health Sciences Laboratory, Gig Harbor, Washington as a part of study of sanitary control of shellfish in the United States of America.

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MATERIALS AND METHODS

Description of Area

The area map is presented in Fig. 1. Burley Lagoon is a partially enclosed narrow and flat-bottomed tidal basin located at the north end of Henderson Bay in the southern part of Puget Sound, Washington. It is approximately 3.2 kilometers long and from 0.5 kilometer to 0.8 kilometer in width. The southern end of the lagoon is protected by the spit stretching from the west to east approximately 0.8 kilometer long, and the channel approximately 100 meters wide connects the lagoon with the outer estuary.

The marine area is approximately 1.5 square kilometers. Practically all of the lagoon becomes

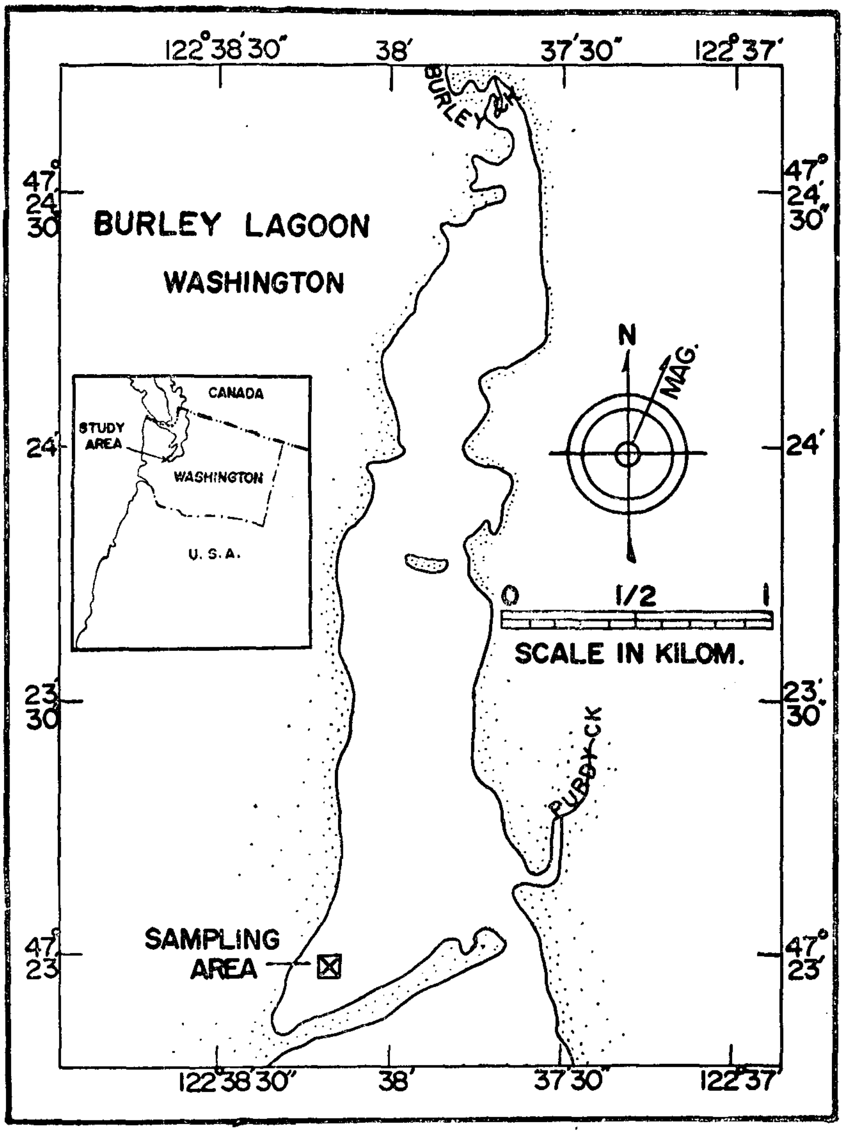

Fig. 1. Map of Burley Lagoon and sampling area.

dry at extreme low tides, except in the courses of tributary streams. The area north of the island is covered with soft mud, whereas south of the island the bottom is fairly firm, being sandy or gravelly in places. A hydrographical study of the lagoon by Kelly[1] showed that the depth ranged from 2.7 to 9.4 meters at mean higher high water.

Practically all of the lower two-thirds of the lagoon is utilized for cultivation of Pacific oysters. Hanging oyster culture is being practiced in the curved water area at the southwest end of the lagoon. The average water depth here is about 6.7 meters at mean higher high water. Total water area utilized for hanging cultures of Pacific oysters is estimated at 17,000 square meters, 140 meters long and 120 meters wide. Each raft is about 9.1 meters long and 5 meters wide or approximately 45 square meters in area. On each raft 196 hanging strings, each 1.8 to 2.4 meters long, are suspended.

The main streams carrying surface runoff into the lagoon are two creeks, Burley and Purdy. Burley Creek has a length of approximately 10.5 kilometers and drains into the northern end of the lagoon. The Burley Creek watershed covers approximately 25.9 square kilometers. The average annual streamflow recorded by U.S. Department of Interior[2] was 27.9 *feet/sec* per day. Average flowduring January 1965 was 44.0 *feet/sec* per day. Purdy Creek, which is 5.6 kilometers long, flows in a southwesterly direction and discharges into the southeast corner of Burley Lagoon. The watershed of Purdy Creek is approximately 1.6 kilometers wide and 4.8 kilometers long.

The two creeks run through mostly agricultural area. A sanitary survey of Burley Lagoon by the State of Washington[3] reported 305 premises with a total of 854 residents and 468 large animals on the watersheds of Burley and Purdy Creeks.

January is in the rainy season in this area. Average precipitation for January during the past five years (1965~1969) obtained from laboratory rain gauge records was 20.2*mm* or 0.65*mm*. Total precipitation for January 1969 obtained from the laboratory gauge was somewhat lower than normal January rainfall, or 0.55*mm*. Total precipitation during the three days prior to the study and the two days during which the survey was conducted, was 1.47*mm* or 0.3*mm* per day. Therefore, the precipitation during the study period was about half the average normal daily rainfall in January.

Sampling

As shown in Fig. 2, three sampling stations were established in the the upper, middle and lower part of the study area according to the direction of stream flow. Top and bottom samples of both water and oyster samples were collected from the top and bottom ends of the strings at each sampling station. Bottom water samples were collected at 3 meters using 1 liter florence flasks. To collect the sample, a flask, sterilized and stoppered with a rubber plug, was lowered to the proper water depth. The stopper was then pulled, using a string attached to it. The flask was then brought to the surface. Prior to replugging the flask, a small portion of water was poured out to allow room for mixing.

The bottom oyster samples were collected from the lowest portion of the string. At certain sampling intervals, oysters from the bed were also collected. These oysters had been placed in aluminium baskets measuring 91.4*cm* long×43.2*cm* wide×17.8*cm* deep. Two baskets, each having sixty oysters removed from strings, were placed on the bottom at sampling station 2 three days prior to sampling to allow time for acclimation.

Sampling of water and oyster was conducted at low, mid and high tides through one complete tidal cycle (24 *hrs*). The time interval of each sampling tour was two hours and thirty minutes to three

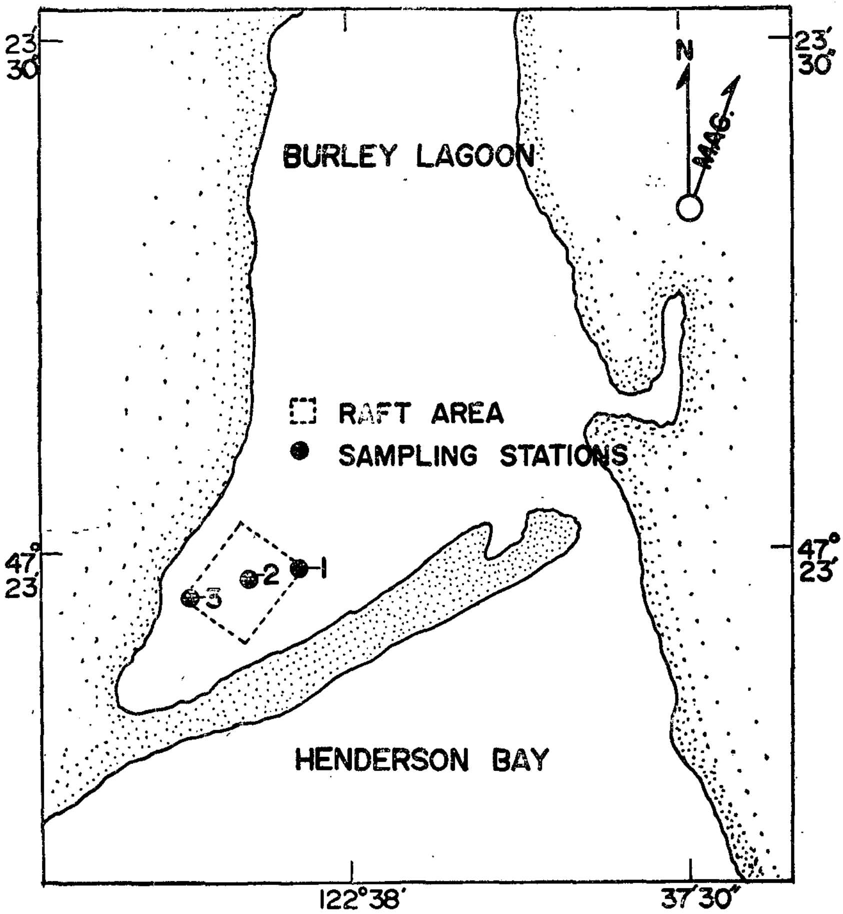

Fig. 2. Location of sampling stations.

hours and thirtyfive minutes. Bed oyster samples were collected only at low and high tide. The first sampling tour was initiated at higher low tide: A total of nine sampling tours were conducted during the study period. The sampling schedule conducted in this study was presented in Table 1.

Table 1. Sampling schedule conducted

Date	Time	Hour	Tide	Tide height (*feet*)	No. of samples (1)		
					Water	Oyster	Total
1—20—69	1325	0	Low	7.9	6	7	13
	1555	2:30	Mid	10.1	6	6	12
	1825	5:00	High	12.2	6	7	13
	2145	8:20	Mid	6.5	6	6	12
1—21—69	0105	11:40	Low	0.8	6	7	13
	0440	15:15	Mid	7.9	6	6	12
	0815	18:50	High	15.0	6	7	13
	1115	21:50	Mid	10.9	6	6	12
	1410	24:45	Low	6.8	6	7	13

(1) Water samples consisted in top and bottom at three stations. Oyster samples consisted in top and bottom, and bed samples at low and high tide only.

Test Procedures

Coliform, fecal coliform and 35℃ plate count determinations on oysters and sea water were made according to the Bacteriological Examination of Seawater and Shellfish[4]. Salinity and temperature determinations were made using a portable conductive salinometer. Turbidity of the sea water was measured in Jackson Candle Turbidity Units(JTU) using a Hach Laboratory Turbidimeter. Current velocity was measured by a drogue method and direction of current was determined using a hand compass. Because of short time interval between sampling tour and difficulty of using drogues at night, the current survey was not conducted during study period. Current measurements were obtained during daylight hours on the two days following the experiment.

Data Analysis

Water temperature, salinity and turbidity ranges and averages were compared by station and by top and bottom. Water and oyster bacterial data were also compared by station, by top and bottom and by tidal level. Mean MPN values of coliform and fecal coliform were calculated as geometric mean and mean levels of plate count were as arithmetic mean. Geometric means were computed by dividing the sum of the logarithms of each MPN value by the number of samples involved. The range of results represent the extreme in each index organism. Relationship between the oyster and their surrounding water was presented by the mean accumulation ratio for each index. Each ratio was computed by dividing the mean level of each index microorganism in oysters by the mean density in water.

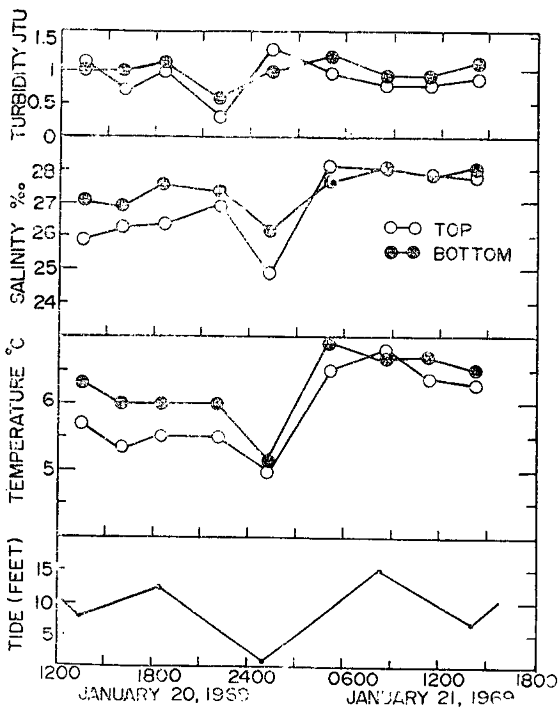

Fig. 3. Changes in temperature, salinity and turbidity of water.

RESULTS AND DISCUSSION

The hydrographical data obtained during the study period are summarized in Table 2. Their changes by tide, over all the stations, are presented in Fig. 3. Differences in water temperature, salinity and turbidity among stations and also between top and bottom samples were slight. However, the averages of all three of the parameters showed slightly higher values in bottom samples than in the top. The differences were about 0.3℃ in temperature, 0.5‰ in salinity and 0.1 JTU in turbidity. As shown in Fig. 3., changes of tempertaure and salinity by tide were apparent with a tendency to vary with the tide cycle, showing a notable decrease at lower low tide.

Current velocity in the study area ranged from 0.28 knot to 0.18 knot per hour. The highest velocity occurred at approximate high tide and low at about low tide. The results also showed that the current velocity at Station 1 was faster than at Station 3 during all tidal

Table 2. Temperature, salinity and turbidity of water in Burley Lagoon (1/20~1/21—69)

Station	No. of samples	Temperature(℃) Top Range	Top Average	Bottom Range	Bottom Average	Salinity (‰) Top Range	Top Average	Bottom Range	Bottom Average	Turbidity (JTU) Top Range	Top Average	Bottom Range	Bottom Average
1	9	4.6—6.7	5.9	4.6—7.0	6.2	25.2—28.2	26.9	25.8—28.5	27.4	0.1—1.2	0.8	0.6—1.3	0.9
2	9	4.9—6.8	5.8	6.0—6.8	6.3	23.4—28.0	26.8	26.0—28.1	27.4	0.1—1.3	0.8	0.3—1.5	1.0
3	9	5.3—7.0	6.0	4.6—7.0	6.2	25.9—28.1	27.1	25.4—28.7	27.5	0.8—1.6	1.1	0.8—1.7	1.1

Table 3. Bacteriological results of water and oysters by different water levels

Sample	Station	No. of samples	MPN per 100 *ml* or *g.* (1) Coliform Top Range	Coliform Top Mean	Coliform Bottom Range	Coliform Bottom Mean	Fecal coliform Top Range	Fecal coliform Top Mean	Fecal coliform Bottom Range	Fecal coliform Bottom Mean	35℃ Plate count (2) per *ml* or *g.* Top Range	Top Mean	Bottom Range	Bottom Mean
Water	1	9	17—920	100	11—110	43	0.5—24	3.9	0.2—4.9	1.2	7—52	27	8—27	17
	2	9	13—240	66	12—110	40	1.1—49	9.1	1.1—9.5	3.3	14—48	27	8—36	18
	3	9	22—240	100	11—240	52	0.5—24	3.5	0.5—13	3.9	7—55	30	2—140	33
Oysters	1	9	220—13,000	940	170—13,000	970	45—790	110	45—1,100	140	8,900—39,000	20,000	4,700—82,000	35,000
	2	9	220—3,300	870	210—790	510	45—220	92	20—490	93	8,500—110,000	32,000	12,000—53,000	24,000
	3	9	330—4,900	1,300	130—3,300	630	78—330	210	20—270	71	4,800—150,000	51,000	15,000—230,000	59,000
	Bed	5			78—1,300	490			45—170	66			9,000 94,000	37,000

(1) Geometric mean
(2) Arithmetic mean

stages. The direction of current at the stations where the measurements were made indicated clockwise movement throughout all tide stages.

A comparison by range and geometric mean of index microorganisms in oysters and their surrounding waters collected during the study period is shown in Table 3. Mean values of water and oyster bacteria density at three tide levels are presented in Table 4. No remarkable differences in bacteria density in water by stations were demonstrated. However bacteria concentrations in top water were higher than those in bottom in both ranges and mean values. Similar results were found in the estuaries on the southern coast of Korea.[5] Differences of bacteriological water quality by tide are shown in Table 4. At low tide, mean coliform and fecal coliform MPN's of both top and bottom samples were higher than at mid and high tides. The levels in top samples generally were more variable than those in bottom samples. The differences of water coliform concentration by tide were relatively great in contrast with those of the fecal coliform and 35℃ plate counts. The higher coliform and fecal coliform concentrations which occurred at low tide were probably due to influence by surface runoff. The lower salinities encountered in those samples also indicate this (Fig. 3).

Mean levels of oyster bacterial density were somewhat variable from station to station. However, the differences were not significant. Mean values of top oyster coliform and fecal coliform MPN's were generally slightly higher than those of bottom oysters. Similar results were reported in a bacteriological study of shellfish on commercial wet storage by Vasconcelos, *et al*[6]. In addition, coliform and fecal coliform levels in bed oysters were lower than in suspended oysters. This phenomenon seemed to be correlated with the results from water samples which show that coliform and fecal coliform densities in top water were higher than those at the bottom in this study. On the other hand, mean values of 35℃ plate count in bottom oysters were higher than top except at Station 2 (Table 3). Differences of bacterial density in oysters by tide levels were less definitive than shown in water samples. However, mean levels of both coliform and fecal coliform MPN's were slightly higher at low tides and lower at high tides. The lack of good correlation of levels of the sanitary indicator organisms in oysters with those in the water by tide levels, was probably due to delayed response of oysters to changes in bacteriological quality of the overlying water. This in turn may have been due to less feeding activity because of the low water temperatures. During the period of the study, water temperature ranged from 4.6 to 7.0℃ and salinity from 23.4 to 28.7‰.

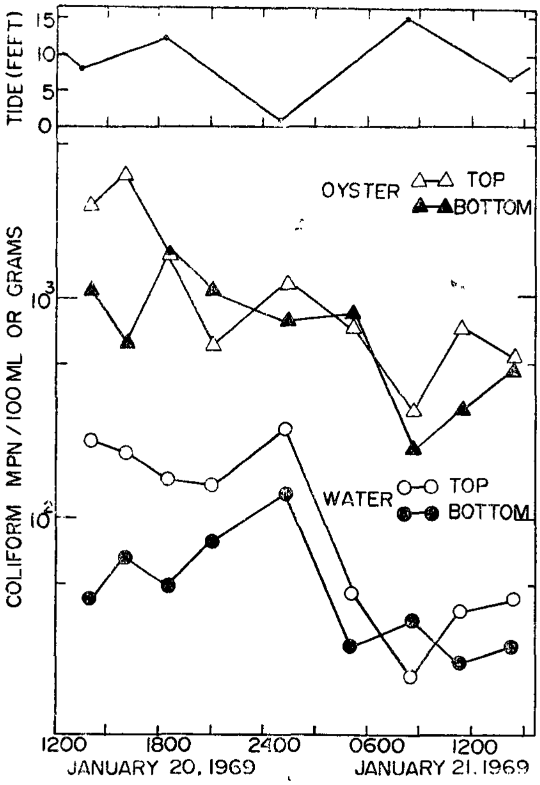

Fig. 4. Changes in coliform MPN of water and oysters.

Comparisons of change in coliform, fecal coliform and 35℃ plate count of water and oysters by tide are shown in Fig. 4, 5 and 6,

Table 4. Mean levels of bacteria density in water and oysters by tide stages

		Water						Oysters					
		Mean MPN per 100 *ml* (1)				Mean 35℃ plate count per *ml* (2)		Mean MPN per 100 *g.* (1)				Mean 35℃ plate count per *g.* (2)	
		coliform		Fecal coliform				Coliform		Fecal coliform			
Tide stage	Station	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Low	1	170	39	4.6	1.7	30	16	830	680	78	130	20,000	47,000
	2	90	50	12	2.9	29	18	1,600	430	86	120	39,000	28,000
	3	170	71	6.3	9.2	38	27	1,400	1,400	250	140	71,000	26,000
	Bed								270		45		13,000
Mid	1	110	27	3.6	1.0	23	15	1,300	1,000	130	150	16,000	25,000
	2	53	32	8.2	3.8	24	16	690	570	110	110	26,000	22,000
	3	97	45	2.6	2.5	21	44	1,300	520	160	47	30,000	48,000
High	1	47	39	3.5	1.0	32	25	560	1,500	120	150	27,000	39,000
	2	64	44	7.3	3.0	31	22	540	510	73	45	64,000	42,000
	3	51	41	2.6	2.7	36	22	1,300	260	250	59	61,000	130,000
	Bed								950		120		74,000

(1) Geometric mean computed for 3 at low, 4 at mid, and 2 samples at high tide from both top and bottom.
(2) Arithmetic mean computed for 3 at low, 4 at mid and 2 samples athigh tide from both top and bottom.

Table 5. Accumulation ratios of oysters at different tides

	Temperature Range ℃		Salinity Range ‰		Turbidity Range (JTU)		Ratio: Mean concentration in oysters / Mean concentration in water						No. of samples
							Coliform MPN		Fecal coliform MPN		35℃ plate count		
Tide	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	
Low	4.6—6.6 (5.6)(1)	4.6—6.7 (5.9)	23.4—28.0 (26.2)	25.4—28.0 (27.1)	0.7—1.6 (1.1)	0.5—1.7 (1.0)	8.6	14.4	16.9	37.1	1,300	1,700	9
Mid	5.1—7.0 (6.0)	5.6—7.0 (6.4)	26.2—28.2 (27.4)	26.0—28.7 (27.5)	0.1—1.4 (0.7)	0.3—1.5 (1.0)	11.5	19.7	30.2	44.3	1,000	1,300	12
High	5.2—6.8 (6.2)	6.0—6.8 (6.4)	25.8—28.1 (27.3)	27.4—28.2 (27.9)	0.7—1.2 (0.9)	0.8—1.2 (1.0)	13.5	14.1	31.7	37.0	1,500	3,000	6

(1) Average of the range.

The values plotted in the figures are presented as geometric mean computed from the data obtained at the same sampling tour from three stations. As shown in Fig. 4 and 5, changes of mean coliform and fecal coliform MPN's in both top and bottom water showed generally negative correlation with the tide cycle. Top samples were consistently higher than bottom samples. In the oysters, there were more fluctuations between top and bottom, especially in coliform MPN. However, the changes both in coliform and fecal coliform by tide generally changed in similar pattern to those of the water. Fecal coliform levels in top oysters were very stable. The response of the plate count in oysters to changes in water were about the same in both top and bottom. However, plate count changes both in oysters and water by tide appeared to be the opposite of those shown in the coliform and fecal coliform groups (Fig. 6).

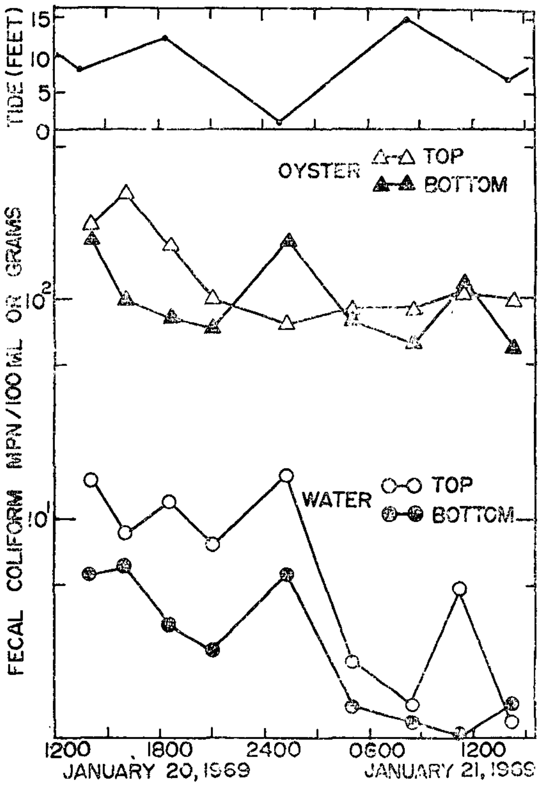

Fig. 5. Changes in fecal coliform MPN of water and oysters.

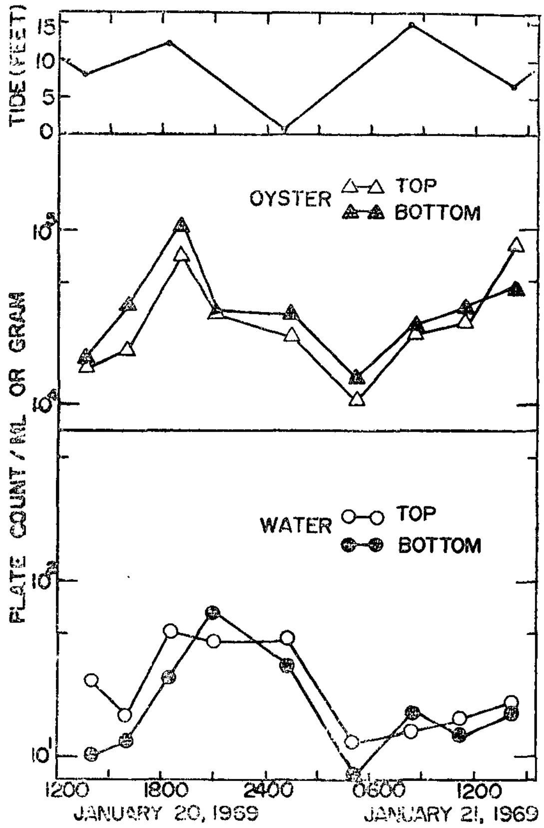

Fig. 6. Changes in 35℃ plate count of water and oysters.

The accumulation ratios of oyster counts to water are shown by tide in Table 5. The mean coliform and fecal coliform ratios for top samples showed a pattern of increase correlated with increasing tidal level. However, the ratios of both groups for bottom oysters were the highest at mid tide. Some of the differences in accumulation ratios are quite large. However, the reason for the differences cannot be explained on the basis of data collected during this study. The ratios of coliform MPN including top and bottom through three different tides ranged from 8.6 to 19.7. This is similar to the mean ratio of 13 obtained from a study conducted on Pacific oysters maintained in suspended baskets in Burley Lagoon during January by Vasconcelos, *et al*[7]. However, the ratios of fecal coliform MPN were about ten times greater than that observed by Vasconcelos. The accumulation ratios of the 35℃ plate count group were much higher than the coliform and fecal coliform at every tide level in this study. The reason for this is not known.

According to the results obtained from the present study, one suggestion could be of considerable importance for the sanitary operation in hanging culture of Pacific oysters. That is, that oysters harvested at high tide may be of better sanitary quality than those harvested at low tide in this estuary.

SUMMARY

A study of the sanitary quality of Pacific oysters (*Crassostrea gigas*) and growing waters associated with raft culture in Burley Lagoon, Washington was conducted. The study was sponsored by the Agency for International Development of the U. S. Department of State.

The results obtained in this study are as follows:

The average values of temperature, salinity and turbidity in the water showed that the values of bottom sample were slightly higher than top samples. The difference was about 0.3℃ for temperature, 0.5‰ for salinity and 0.1 JTU for turbidity. The changes of temperature and salinity by tide generally followed the tide cycle pattern.

Sanitary indicator microorganism concentrations in top water were generally higher than those in bottom water. In general, the levels of mean coliform and fecal coliform MPN's varied inversely with tide level indicating that the sources of these groups of microorganisms are the fresh water streams flowing into the estuary. The 35℃ plate counts were more stable at different tide levels.

Mean values of coliform and fecal coliform MPN's in oysters demonstrated that levels in top oysters were generally higher than those in bottom oysters. However, mean values of 35℃ plate count in oysters did not show this pattern. The mean levels of both coliform and fecal coliform MPN's in oysters also correlated inversely with tidal level.

The accumulation ratios of oysters obtained during the study period ranged from 8.6 to 19.7 for mean coliform MPN and 16.9 to 44.3 for fecal coliform MPN.

According to the results obtained from present study, one suggestion could be of considerable importance for the sanitary operation in hanging culture of Pacific oysters. The results indicate that harvest of the oysters at high tide would result in lower levels of indicator organisms in the shellfish.

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