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Yaquina Bay Laboratory
Department of Fish and Game Management
Oregon State University

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Report on Japanese oyster-Spent Sulfite Liquor Bioassays

R.E. Dimick and W.P. Breese
~~W. P. Breese and R. E. Dimick~~

Following a series of preliminary oyster-spent sulfite liquor (SSL) bioassays in 1958, three separate continuous flow tests were conducted with the two varieties of Crassostrea gigas grown commercially in Oregon waters; namely, the Pacific and Kumamoto oysters. One bioassay (No. I) covered a period of 266 days within the SSL range of 50 to 200 ppm; another (No. II) of one year duration used effluent concentrations between 10 and 50 ppm; and the third test (No. III) employed high SSL concentrations ranging from 500 to 2000 ppm.

All SSL samples were blow-pit effluents sometimes referred to as "hot-blown liquor." These were received weekly from an Oregon mill employing a calcium base process and which uses approximately 95 percent hemlock and 5 percent white fir. Stock solutions were prepared at two day intervals from liquor freshly adjusted to 10 percent total solids. Stock solutions were continually delivered by chemical pumps to test trays (aquaria) where thorough mixing with salt water took place immediately before passing over the oysters. The dilutent water was pumped daily from the bay at particular tidal stages so as to maintain salinities at about 25 ppt and above. Water temperatures varied with the daily and seasonal changes occurring in the bay. Dissolved oxygen in test trays was for the most part above 5.0 mg/l, except occasionally in the high SSL concentrations, 1000 to 2000 ppm. When such low DO readings were obtained the iodine

modification of the Winkler method (Ohle, 1953) gave results of 5.0 mg/l or above; and the corrections for SSL interferences compared favorably with those reported as obtained by the use of other methods described in Technical Bulletin 77, National Council for Stream Improvement and the Research Bulletin I, Washington State Department of Fisheries (G.A. Holland, editor).

The Pacific and Kumamoto oysters employed were about 1½ to 2 years old when each bioassay was begun. Condition factors (CF) and shell-volume increment (SVI) were obtained from individual test oysters alive at the termination of the bioassays except for those that had been exposed to the high SSL concentrations (500 to 2000 ppm). The formula for $CF = \frac{\text{Dried meat weights}}{\text{Volume of shell cavity}} \times 100$ and the procedures followed closely those described by Westley (1959). Shell-volume increments were obtained by weighing each oyster in freshwater at the beginning and the termination of the bioassay, and expressing the differences in weights as millimeters displacement.

Bioassay I

Pacific and Kumamoto oysters were exposed to 50 ppm SSL for 266 days (April, 1959 to January, 1960) and in addition Kumamotos were subjected to 100 and 200 ppm concentrations. There were 100 oysters per test tray and dead oysters were replaced in exact tray locations in which deaths occurred. Solution flows were two liters per minute or 20 ml/min per test oyster.

Table I summarizes the main results; namely, percentage mortalities, mean CF's and mean SVI.

Since there were only 2 percent mortality increases for both Pacifics and Kumamotos in 50 ppm concentrations as compared to the control groups and since the percentage mortalities for Kumamoto in both 100 and 200 ppm SSI were identical to those of the control group (7 percent), it was thought that SSL concentrations

within the range of 50 to 200 were not the cause of the deaths to Crassostrea gigas during the continuous flow exposures of 266 days.

Although there were no significant differences in the mean CF's of the 50 ppm SSL groups for Pacifics and Kumamotos, compared to control groups, there were indications that Kumamoto CF's might have been significantly less in the 100 and 200 SSL concentrations. Dr. Jerome Lee, formerly of the Statistical Department at Oregon State University, made analyses of variance calculations for condition factors in relation to test tray location (first third, middle third, and last third), and for SSL concentrations. Tables 2A and B present his tables for the Pacific and Kumamoto oysters. According to Dr. Lee the analyses of variance showed that in both experiments the tray position affected the CF means significantly. The mean CF's decreased at a constant linear rate from front to the rear. Also the analyses supported the position that 50 ppm SSL did not affect the CF's of the Pacific and Kumamoto but did do so significantly for the Kumamotos in the 100 and 200 ppm SSL concentrations.

It should be pointed out that the mean CF's for all Kumamoto groups, 12.0 to 13.7, would have rated excellent (Westley, 1959) and only fair for the Pacific, 6.2 and 6.7.

Although no statistical analysis was made of the SVI data, inspection of the results (Table 3) indicated that mean values decreased in each instance as position moved from front to rear of the testing trays.

Bioassay II

Kumamoto oysters were tested in continuous flows of 10, 15, and 25 ppm SSL for 365 days, April, 1960 to April, 1961. In each test tray there were 30 Kumamotos interspaced with 75 Native oysters. Solution flows to each test tray were four

liters per minute or approximately 40 ml/min for each oyster. Simultaneously 25 Pacifics were tested in 0.0 and 50 ppm SSL concentrations in which the flows were five liters per minute or approximately 200 ml/min per test oyster.

Summarized results are presented in Table 4. One death occurred in each of the 15 and 25 ppm SSL-Kumamoto groups and in the Pacific 50 ppm SSL concentration. There were two deaths in the Pacific control tray. No Kumamoto mortalities occurred in control and 10 ppm SSL groups.

The mean condition factors and shell-volume increment values are arranged by SSL concentrations and by tray locations, Tables 4 and 5 respectively. Although the mean condition factors in Pacifics were low, 5.5 and 6.1, and only fairly good in the Kumamotos, 8.2 to 8.4, there were no indications that SSL concentrations, 10 to 50 ppm, caused any adverse CF effects. There were, as previously noted in bioassay I, reductions in mean condition factors as positions moved from front to rear sections of all the testing trays.

The mean shell-volume increment value, in both Pacifics and Kumamotos, greatly increased over those recorded for bioassay I, particularly the Pacifics. There were no indications that SSL concentrations, 10 to 50 ppm, had any adverse SVI effects. There was, however, reduction in mean SVI values as position moved from front to rear in the testing trays.

Bioassay III

Kumamoto oysters were subjected to abnormally high SSL concentrations of 500, 1000, 1500 and 2000 ppm for a period of one year or until complete mortality occurred in a particular SSL concentration. There were 25 test oysters alternately arranged with 25 market-size Native oysters per SSL concentrations and the solution flows were one liter per minute per test tray.

The number of deaths occurring in consecutive ten day intervals are presented in Table 6. Inspection of the data shows that Kumamoto specimens experienced marked numbers of mortalities in all of the SSL concentrations. There were no deaths in the control group. Although the mortalities were 32 percent in the 500 ppm concentrations and 92 percent in 1000 ppm SSL, total deaths occurred in 1500 and 2000 ppm SSL in 255 and 59 days respectively. The cumulative mortality rates increased with increases in SSL concentrations.

References

Westley, Ronald E. 1959. Olympia and Pacific oyster condition factor data, State of Washington, 1954–1958. Wash. Dept. of Fish., pp. 1–8.

Holland, G. A. (editor). 1953. Toxic effects of sulfite waste liquor on young salmon. Res. Bull. No. 1, Wash. Dept. Fish., pp. 1–111.

Ohle, Waldemar. 1953. The chemical and electro-chemical determination of dissolved molecular oxygen in fresh waters. Internat. Assoc. of Theoretical and Applied Limnology, Com. No. 3, pp. 1–44.

Table 1.

Bioassay data for Crassostrea gigas - Spent Sulfite Liquor, 50 to 200 ppm concentrations; 100 test oysters each kind per test solution; 266 day exposure and 20 ml/min solution flow per test oyster.

	SSL concentrations ppm			
	0.0	50	100	200
Pacifics:				
Percent mortality	1.0	3.0		
CF means	6.2	6.5		
SVI means	7.1	7.0		
Kumamotos:				
Percent mortality	7.0	9.0	7.0	7.0
CF means	13.3	13.7	12.0	12.7
SVI means	1.9	2.1	1.8	1.7

~~Table 2*~~
Table 2

(A) Mean condition factor values for Pacific oysters; bioassay I.

SSL concentrations	Tray position			Means
	First	Middle	Last	
Control	7.21	6.29	5.17	6.22
50 ppm	8.14	6.26	4.97	6.45
Means	7.67	6.28	5.07	6.34

(B) Mean condition factor values for Kumamoto oysters; bioassay I.

SSL concentrations	Tray position			Means
	First	Middle	Last	
Control	15.24	12.64	12.05	13.31
50 ppm	13.96	15.07	12.17	13.73
100 ppm	14.34	11.66	9.92	11.97
200 ppm	15.26	12.51	10.44	12.74
Means	14.70	12.97	11.14	12.94

* Prepared by Dr. Jerome Lee.

Table 3.

~~Table~~

Mean shell volume increments for Pacific and Kumamoto oysters, bioassay I; solution flows 20 ml/min per ~~test~~ oyster.

	Conc. SSL ppm	Tray position Mean milliliter displacement by tray location First portion	Middle portion	Last portion	Means
Pacifics:	0.0	17.1	4.6	0.3	7.1
	50.0	19.4	1.7	1.1	7.0
Kumamotos:	0.0	3.8	1.6	0.4	1.9
	50.0	4.0	1.4	0.8	2.1
	100.0	3.3	1.6	0.4	1.8
	200.0	2.8	1.3	0.7	1.7

II

Table 4

Bioassay II data for Crassostrea gigas – Spent Sulfite Liquor, 10 to 50 ppm concentrations; 25 Pacifics and 30 Kumamotos per test solution; one year exposure.

	SSL concentrations ppm				
	0.0	10	15	25	50
Pacifics:					
Percent mortality	8.0				4.0
CF-means	5.5				6.1
SVI-means	20.2				24.9
Kumamotos:					
Percent mortality	0.0	0.0	3.3	3.3	
CF-means	8.2	8.3	8.3	8.4	
SVI-means	4.4	4.5	4.6	4.7	

Table 5

~~Table 4~~

Mean condition factor values for Pacifics in bioassay II; solution flows 200 ml/min per test oyster.

SSL ppm	Tray position			Means
	First	Middle	Last	
0.0	6.8	5.4	4.2	5.5
50	7.5	6.2	4.7	6.1

Mean condition factor values for Kumamotos in bioassay II; solution flows approximately 40 ml/min per test oyster.

SSL ppm	Tray position			Means
	First	Middle	Last	
0.0	9.5	8.4	6.8	8.2
10	9.9	7.7	7.3	8.3
15	9.5	8.1	7.3	8.3
25	9.5	7.9	7.7	8.4

Table 6

~~Table 5~~

Mean shell-volume increment values for Pacifics in bioassay II; solution flows 200 ml/min per test oyster.

SSL ppm	Tray position			Means
	First	Middle	Last	
0.0	21.7	25.5	12.6	20.2
50	28.1	25.1	25.5	24.9

Mean shell-volume increment values for Kumamotos in bioassay II; solution flows approximately 40 ml/min per test oyster.

SSL ppm	Tray position			Means
	First	Middle	Last	
0.0	5.4	3.9	3.9	4.4
10	5.2	4.3	3.9	4.5
15	4.8	5.3	3.6	4.6
25	5.7	4.6	3.8	4.7

Table 6

Mortalities of Kumamoto oysters exposed to high SSL concentrations; tabulated by 10-day periods; 25 test oysters per concentration.

Period	Concentration of SSL in parts per million				
	0.0	500.0	1000.0	1500.0	2000.0
1	0	0	0	0	0
2	0	0	0	1	1
3	0	1	0	0	4
4	0	0	0	10	14
5	0	0	0	4	4
6	0	0	2	0	2
7	0	0	0	1	
8	0	1	3	1	
9	0	0	1	0	
10	0	0	1	0	
11	0	0	1	0	
12	0	1	1	1	
13	0	0	2	2	
14	0	0	2	2	
15	0	0	0	1	
16	0	1	0	0	
17	0	0	0	0	
18	0	0	0	0	
19	0	0	1	1	
20	0	0	1	0	
21	0	1	0	0	
22	0	0	0	0	
23	0	0	0	0	
24	0	0	1	0	
25	0	0	0	1	
26	0	0	3		
27	0	0	1		
28	0	0	0		
29	0	0	0		
30	0	1	1		
31	0	0	1		
32	0	1	0		
33	0	0	1		
34	0	1			
35	0	0			
36	0	0			
36½	0	0			
Total	0	8	23	25	25
Percent mortality	0	32	92	100	100