EFFECTS OF PULP MILL POLLUTION ON OYSTERS[1]

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INTRODUCTION

The purpose of the present investigation, which was undertaken at the request of the oystermen of Shelton, Wash., was to throw some light on the difficulties faced by the oyster industry in Oakland Bay, where oyster culture has suffered a setback amounting to almost complete cessation. Oysters are adapted to life in inshore waters which are considerably diluted by land drainage and where the conditions of their natural habitat may be subjected to changes caused by the addition of waste matter of wide variety. Suspicion had been directed toward the waste liquor poured into the bay from a pulp mill established on Oakland Bay in 1927, for in this industry there is necessarily a large amount of commercially worthless material remaining after the pulp is removed from the wood. Inasmuch as little experimental work has been done which can serve as a basis for predicting the effect on the oyster industry of waste matter discarded from the factories, it is important to determine just what possible effect certain specific waste substances may have upon the life of the oyster. It was expected that the present investigation would show what effect pulp-mill wastes may have on the oyster and would serve as a foundation for general

[1] Approved for publication Mar. 11, 1931.

recommendations concerning the location of pulp mills in the vicinity of oyster grounds.

A laboratory was established on Oyster Bay, near Olympia, Wash., and equipped with running seawater.[2] At this place the water was probably not contaminated with the waste liquor to any great extent, for Oyster Bay is a considerable distance from Oakland Bay.

SULPHITE WASTE LIQUOR

Although in certain cases sulphite pulp mills have established plants for the recovery of chemicals and the manufacture of by-products from the waste liquor, this is the exception rather than the rule in the United States. Mills are most often located on fresh-water streams into which all waste matter is dumped after little or no treatment to prevent possible damage to aquatic life. This method of disposal is generally inadequate because of the great amounts of waste matter produced.

In the manufacture of pulp by the sulphite process the chips of wood are cooked under pressure in a solution consisting primarily of calcium bisulphite with an excess of sulphurous acid. Something over half of the constituents of the wood go into solution to form the waste liquor while the fiber remains. The liquor contains, in addition to the substances in the original cooking solution, though changed somewhat in the process, nearly all of the noncellulose constituents of the wood. The specific gravity of the liquor from the digester at the end of the cooking process is about 1.05, and may vary from 1.045 to 1.055. It is a dark reddish-brown liquid, rather syrupy in consistency.

The general nature of the liquor is shown by the following results of an analysis of a sample of liquor of specific gravity 1.0425 by Bryant, as stated by Sutermeister (1929, p. 233):

Constituents	Grams per liter	Pounds per ton pulp	Constituents	Grams per liter	Pounds per ton pulp
Total solids	115.00	2,999	Total sulphur	7.83	204
Loss on ignition	105.36	2,748	Sulphur as SO_3	.76	20
Ash	9.64	251			

The actual solids in solution amount to more than the pulp produced. The most of these substances are organic, and there is no limit to the possible number of compounds present. The variety of components is indicated in the figures given by Sutermeister (1929, p. 234), after Klason, for the waste liquor remaining from production of a ton (2,202 pounds) of dry pulp: 600 kilograms lignin, 200 kilograms sulphur dioxide combined with lignin, 90 kilograms CaO combined with lignin sulphonic acid, 325 kilograms carbohydrates, 15 kilograms proteins, and 30 kilograms rosin and fat.

Complete analysis of such a highly complex mixture of organic compounds is extremely difficult. While the lignin-containing compounds make up the bulk of the solids, other compounds of a complex nature may be present in such minute amounts as to be overlooked. Most of the analytical work which has been done refers to the question of manufacture of by-products. The sugars and related

[2] The Bureau of Fisheries is indebted to State Senator J. H. Post of Thurston County, Wash., for allowing the use of his culling house as a laboratory and for furnishing a Kohler electric plant and various pumps, motors, and other electrical equipment. We also wish to express our appreciation to C. R. Maybury, director of the Washington State Department of Fisheries and Game, and to C. R. Pollock, supervisor of fisheries of the same department, for furnishing certain laboratory equipment and otherwise cooperating in this investigation.

substances in the liquor have been investigated from the standpoint of manufacture of commercial alcohol, which is often carried on in Europe. According to Sutermeister (1929, p. 234), Krause made the following determinations for liquor from autumn-cut wood treated by the Ritter-Kellner process; the results are expressed as percentage: Furfural, 0.02; pentosans, 0.29; hexosans, 0.49; total sugars, 1.47; pentoses, 0.41; mannose, 0.48; levulose, 0.25; galactose, 0.21; and dextrose, trace.

Sutermeister (1929, p. 233) summarized a work by Walker as follows:

"Among the constituents present he mentions sulphur dioxide, sulphur trioxide, free sulphur, calcium and magnesium lignin sulphonates, pentoses and pentosans, mannose, dextrose, galactose, free furfural, traces of vanillin or vanillinlike body and small quantities of terpenelike substances." Citing another work, Sutermeister wrote: "Hoenig claims that no organic acids except formic and acetic are present and that the ratio of these is 1:1.56. He finds 2.15 to 9.08 grams of volatile acid per liter." Further (Sutermeister, p. 235); "The waste liquor, according to Walker, yields brominated and chlorinated products; it contains active carbonyl and methyl groups and is a strong reducing agent." Methyl alcohol, acetone, aldehyde, acetic and formic acids, and a brown oil, part of which is cymene, were found in condensed digester vapors by Bergström, according to Sutermeister. The same author stated that 8 to 10 kilograms of methyl alcohol are produced per ton of pulp. Sutermeister (p. 241) stated: "Other substances which it has been proposed to recover from the waste liquor are antiseptic materials, calcium sulphate, calcium sulphite, coniferin, cymol, acetic acid, furfural, levulinic acid, oxalic acid, sulphur, turpentine, lignorosin, vanillin, etc."

In addition to the wide variety of substances mentioned above, the liquor contains all of the inorganic components of the wood. These would be in relatively small amounts, but might be of some significance with regard to aquatic animals. These analyses appear not to indicate definitely any particularly toxic substances which might be expected to exert an unfavorable influence upon aquatic life. However, the wide variety of substances present suggests the possibility that many other compounds may be in the liquor in small amounts which would be difficult to detect chemically. It has been stated that workmen in sulphite mills often drink the liquor for its laxative effect, which indicates that it certainly is not a violent poison to man.

Usually pure digester liquor is not dumped into the bodies of water on which mills are located. In "blowing" a digester and separating the pulp from the waste liquor enough water is used to reduce the specific gravity of the liquor from 1.05 to between 1.01 and 1.02. Just after a "blow" the liquor is likely to be of high specific gravity, which is reduced in the liquor which follows by dilution. Such heavy liquor on entering a turbulent stream is thoroughly diluted, but if it enters a relatively still body of water would be expected to sink to the bottom.

Because of its great excess of sulphurous acid, the liquor is highly acid, a characteristic which is not conducive to favorable aquatic conditions. However, in the case of salt water in particular, the acidity does not last long, partly because of direct neutralization and partly due to loss of sulphur dioxide into the air.

In addition to the liquor, there is a considerable quantity of pulp fiber too small to be held in the separators, which must be disposed of as waste. Typically, this settles to the bottom of the body of water into which it is thrown and is very slowly decomposed. This is an important polluting material in some places, especially in streams, where it may interfere with the feeding and breeding of fish. The bleach fluid, containing chlorine compounds primarily, is also a constituent of the wastes from sulphite mills.

In some cases the waste liquor is treated to neutralize acidity by passing it over limestone before allowing it to go into the water. Running the liquor into a pond or settling basin from which it flows into the river has been found advantageous.

Experiments of this nature by the Wisconsin State Board of Health in 1927 succeeded in markedly reducing the oxygen demand of the liquor.

Experimental observations on the effect of sulphite liquor on aquatic organisms have been made in certain cases, but most of the reports on the subject consist of surveys made primarily from the standpoint of dissolved oxygen. Also these works have been concerned with fresh-water streams and lakes and not with salt-water bays and estuaries. It has been well established that fish avoid the waters which are polluted with sulphite wastes, but it appears that this is largely due to depletion of oxygen in the water rather than to any great toxic effect.

That sulphite liquor exerts a germicidal action was shown by Levy (1905), according to Phelps (1909). He found that a 5 per cent solution of sulphite liquor in water highly polluted with sewage reduced the number of bacteria by 86 per cent in 6 hours, while a 10 per cent solution killed all bacteria within the same length of time. Presumably this effect is primarily directly toxic, for the highly polluted water was probably very deficient in oxygen even without the liquor. Levy suggested that the harmful effect of the liquor on fish might be due to removal of free oxygen from the water.

In a series of experiments on the effect of waste liquor on perch, bass, and brook-trout fry, March (1907) found that in solutions up to 1:200, without aeration, the specimens died, but that neither perch nor bass were killed after 27 days exposure to an aerated solution of 1:50 (specific gravity of stock liquor 1.028 at 11° C.). This would appear to indicate that death had been produced by insufficient dissolved oxygen. Later, however, March (1908, p. 896) stated, "A sample experimented with by the writer had little or no reducing action on the dissolved oxygen in the water, and it is likely that it kills by its direct action alone."

Whipple (1922) called attention to the filamentous fungi which thrive in sulphite polluted water which may in a secondary manner make the water unfit for fish life.

The review of experimental data by Suter and Moore (1922) pointed out that the harmful effect of sulphite liquor on fishes is limited to fairly concentrated solutions, in general within 1:200. More recent work by Nightingale and Loosanoff (1928) on the effect of liquor on early stages of salmon indicated that although lower concentrations may be fatal, the effect primarily is due to low oxygen content. Low concentrations produced an apparently chemical effect on the scales of fry.

Recent investigations of the fish life in the Ausable River by Carpenter (1930) showed that fish avoid sulphite polluted water even though the most contaminated water of the river was about 35 per cent saturated with oxygen (Faigenbaum, 1930).

The results of Knight (1901) differ from those of other workers in concentrations required to kill fish. The sulphite "waste water" which he used was of specific gravity 1.00005, which presumably represents digester liquor diluted with 1,000 parts water. Yet a solution made up of 1 part of this to 9 parts water was fatal to trout, white perch, sunfish, and rock bass. The effect may have been due to the very high acidity of the waste water. It is also not impossible that the potency of the waste liquor depends upon the kind of wood employed in the mill.

Because of the usual location of pulp mills on streams, there has been no pressing need of determining the effect of sulphite liquor on oysters. Most of the observations indicated in the foregoing account are probably not applicable to marine organisms and to oysters in particular. In the first place oysters are immobile and can not avoid unfavorable water by changing their position, as can fish. Also, it

is probable that the sensitivity of oysters to foreign substances may be decidedly different from that of fishes.

The problem of the effect of factory wastes on the aquatic life in streams is very different from the problem which grows out of the dumping of such wastes into bays and estuaries where oysters occur. While the flowing water of a stream constantly carries away fluid wastes, the more sluggish waters of a tidal basin are liable to absorb such substances and retain them. In the latter case, the tidal currents do not readily eliminate pollution. In a body of water of this kind, oysters would be subjected to the foreign substance more continuously and for longer periods of time.

Sulphite liquor contains a large quantity of calcium sulphate in solution. When the liquor is neutralized with sodium hydroxide this appears as a precipitate, relatively insoluble in neutral fresh water but quite soluble in sea water. That the calcium sulphate may be partly responsible for the toxicity of the liquor is suggested by the observations of Oku, Ito, and Fujita (1901). They found that Japanese oysters died in aerated solutions of calcium sulphate in sea water in concentrations of 0.633 grams per liter and above. The lowest concentration which would produce death was not determined. Oysters 2 and 3 years old were found to be more susceptible than 1-year-old specimens. In a later publication by Oku (1904), it was stated that oysters died as a result of the presence of calcium sulphate only in relatively warm water, for no deaths occurred in a similar series of tests made in winter. A further statement in this publication was to the effect that meats of oysters in ordinary sea water contained less copper than those kept for some time in solutions of calcium sulphate in sea water. This is an interesting suggestion but further evidence would be required to demonstrate the significance of the observation.

MATERIAL AND METHODS

For the experimental work Olympia oysters (*Ostrea lurida*) were taken fresh from the dikes of the Blass Oyster Co. in Oyster Bay. These specimens were selected because of their excellent condition, showing that they were relatively normal oysters and not obviously suffering from any cause as is the case with Oakland Bay oysters. The oysters used were all of approximately the same size, about 4.5 to 5.5 centimeters long by about 3.5 to 4 centimeters wide, and the same age, 4 to 5 years. Relatively large specimens were employed to facilitate the recording of shell movements on the kymograph. That they were in good condition is shown by the fact that no control specimens died during experimentation.

For laboratory use, liquor, through the courtesy of D. B. Davies, manager of the Rainier Pulp and Paper Co. at Shelton, Wash., was drawn directly from the digester at the end of a cook into a keg, in which it was transported and kept in the laboratory.

The apparatus employed for the experiments is described in detail below. Most of the tanks, aquaria, etc., used were made of clear, transparent celluloid plates, one-eighth inch thick. Parts of any such piece of equipment were sealed together with a solution of the same celluloid in acetone. The material is well suited to such work and oysters live well in equipment made of it.

A diagram of the apparatus used in the laboratory to produce a running mixture of liquor and sea water is presented in Figure 1. It is essentially an arrangement of constant levels whereby the rate of flow due to gravity is kept constant. In the 5-gallon carboy (*A*) was a mixture of liquor and sea water in proportions 1:4, 1:9, or 1:19, depending upon the final concentration desired. The tube in the mouth of the bottle projected into the jar (*B*) in such a manner as to cause liquor to flow down when the

level of the fluid in the jar fell low enough to expose the tube. The distributing chamber (*D*) was on a lower level and the fluid was admitted to it at a nearly constant rate through the tube (*C*) which was fitted with a stopcock for regulating the rate of flow. The overflow space (*E*) at the proximal end of the distributing chamber was sufficiently large to prevent the level of the fluid from rising any higher. Variation of the level of fluid in this chamber might therefore be downward, due to stoppage of tube *C*, but not upward. Attached by adjustable clamps on vertical bars to the wall of chamber *D* were four dripping chambers (*F*), only one of which is shown in the diagram.

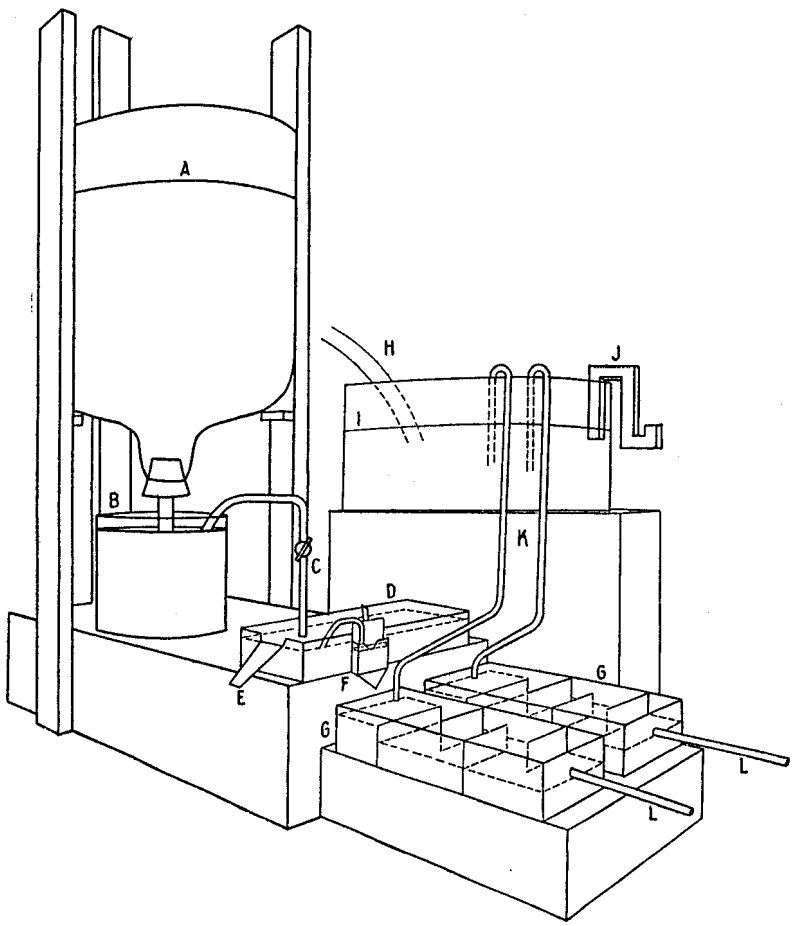

FIGURE 1.—Diagram of the apparatus used to deliver running mixtures of sea water and sulphite liquor in constant proportions. See text for full description

In Figure 2 the dripping chamber is shown in detail. The inside dimensions were: Height, 4.5 centimeters; width, 2.5 centimeters; and thickness, 1.5 centimeters. In the top of the front wall a V-shaped opening (*C*) allowed the fluid to spill over and run down, where it dripped from the extending tip (*D*). The back wall (*B*) was extended upward to allow attachment to the fixed upright (*E*). Fluid from the distributing chamber (*F*) passed through the glass tube (*A*) into the dripper, from which it overflowed through *C* and dripped into the mixing chamber (fig. 1, *G*).

In Figure 3 is a detailed diagram of the mixing chamber. This was 15 centimeters long, 5 centimeters wide, and 6 centimeters deep, and consisted of a small

chamber (*A*) separated from the larger one by a wall (*B*), 4.5 centimeters high. Baffle plates (*C* and *C'*), approximately equally spaced, divided the chamber incompletely into several parts. Both sea water and liquor solution flowed into chamber *A*, where they were well mixed. The resulting solution flowed over plate *B* and back and forth among the baffle plates, becoming thoroughly mixed, until it flowed out through the tube (*D*) into the aqarium containing the experimental specimens.

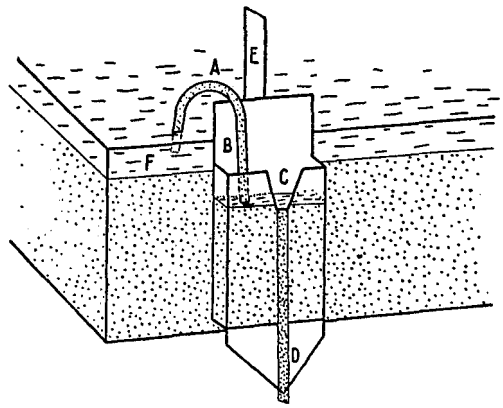

FIGURE 2.—Diagram of the dripping chamber from which liquor dripped at a constant rate into the mixing chamber. See text for full description

The rate of flow of sea water was fixed as shown in Figure 1. Through the tube (*H*) water continuously flowed more rapidly than was necessary into the 10-gallon aquarium (*I*). A large constant-level siphon (*J*) of celluloid, having a cross-section area of about 5 square centimeters, maintained a constant level in the aquarium jar in spite of minor variations in the rate of flow of entering water. Glass and rubber tubes of 3 to 5 millimeters inside diameter led from the aquarium to the mixing chamber (fig. 1, *G;* fig 3, *A*) into which the liquor was dripping.

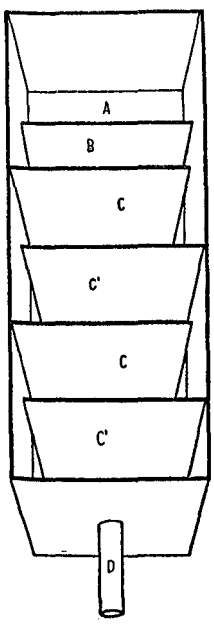

FIGURE 3.—Diagram of the mixing chamber (fig. 1, *G*) in which water and sulphite liquor were thoroughly mixed before entering the aquaria. See decription in text

The experimental and control specimens were contained in a tank (fig. 4) consisting of three separate but adjoining compartments (*A, B, C*), each having a capacity of about three liters. In the front wall of each compartment an overflow space (*D*) was cut to allow continuous overflow without passing of the fluids from one chamber into another. A tube from a mixing chamber led to each of the two end compartments, while another tube led from the aquarium jar (fig. 1, *I*) into the middle compartment. The two end chambers contained running mixtures of sea water and sulphite liquor in known proportions, while the middle chamber contained pure, running sea water as a control.

Fresh oysters were mounted on a plaster of Paris base to hold them in a fixed position and at the same time not interfere with their natural functions. Two such specimens were placed in each of the three experimental compartments. (Fig. 4, *A, B, C.*)

Records of the shell movements were kept continuously by means of a kymograph. A slender strip of celluloid (fig. 4, *E*) rested upon the shell of each specimen and was movably attached at its upper end to a horizontal celluloid lever (*F*). A short wire at the distal end of the lever came into contact with the smoked, slowly moving kymograph paper. All six specimens of a series were arranged to write their records on the same paper.

The kymograph carried a paper about 2 meters long and moved at a constant rate of about 31 millimeters per hour. Usually after the paper had made a complete circuit the drum was slightly lifted and another circuit made, the levers writing in between the lines made the first time.

Changing a paper occupied only 15 minutes, during which time the loss of records is insignificant. After fixing a completed paper in shellac it was marked off into hourly and daily periods and the records analyzed.

During most of the experiments thermograph records were kept of the temperature of the water in which the specimens were immersed. The bulb was inserted into the middle chamber with the control specimens, for the metal might be attacked by the liquor in the other chambers and so introduce a source of error. In the three experimental compartments the temperature was so nearly the same that the maximum difference observed was hardly over 0.2° C.

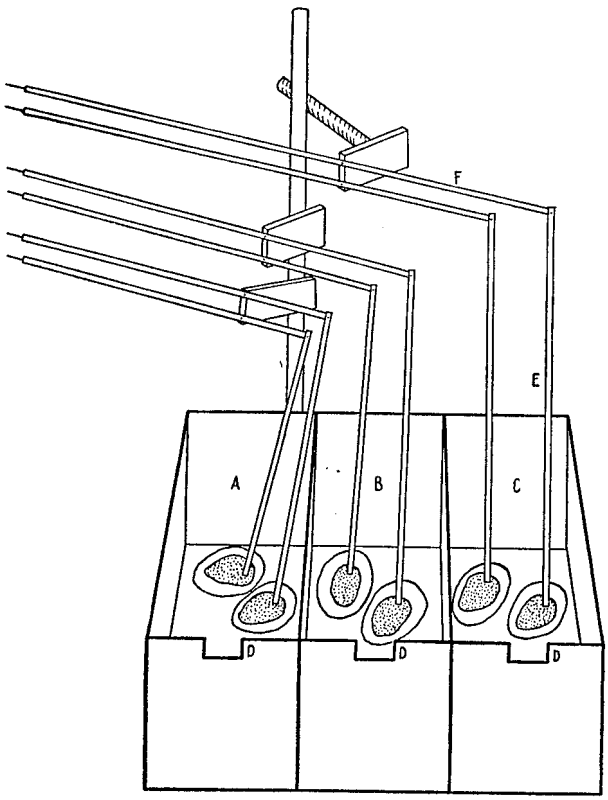

FIGURE 4.—Diagram showing the arrangement of connected aquaria, containing experimental (end chambers) and control (middle chamber) specimens connected to levers which recorded shell movements on the kymograph. Complete description in text

In the winter weather, when the pumped water was around 5° C. or lower, the water was heated slightly before entering the large aquarium jar. It passed through a lead coil which was immersed in a pail of water under which a kerosene flame burned. The resultant water in the experimental chambers was usually of about 15° to 17° C., or high enough to permit the oysters to feed and grow.

A fresh supply of sea water was maintained by means of an automatic pump, which pumped water at intervals of one to two hours into two 50-gallon oak barrels. The laboratory (fig. 5) was in a cove which formed a part of Oyster Bay, but which at low tide was well above the level of bay water. The cove was diked to retain a depth of 2 to 4 feet of water at low tide. At high tide the bay water entered the cove, so that the cove water did not markedly differ from the bay water in salinity.

The supply of liquor and sea water mixture in the 5-gallon carboy was replenished whenever necessary. This amount lasted for from one to three days, depending upon the rate of flow.

Most of the experiments were carried on without first neutralizing the liquor. The pH of the resultant solution was consequently lower than that of the sea water. That the effects observed were not due to acidity was shown by a control series.

GENERAL CONSIDERATIONS

In Oakland Bay four chief abnormal characteristics of the oysters have been observed since spring, 1927. In the first place practically no setting occurred, and consequently no seed oysters were obtained. In addition, many oysters died on

FIGURE 5.—Photograph of culling house used as laboratory

the grounds. (See accompanying report of McMillin.) Further, the oysters remaining were extremely thin and watery, showing none of the rich white meat of normal specimens. Also, new shell growth was seldom to be observed. These four characteristics may be reduced to three for the present purposes by combining lack of reproduction with thinness of meats, for the former is probably largely a consequence of the latter. However, it is probable that liquor may be highly toxic to oyster larvæ. It would be of considerable significance to determine whether these conditions could be reproduced among oysters in the laboratory by adding sulphite liquor to otherwise favorable water. In the following pages are descriptions of experiments designed to determine the potency of the liquor in these respects.

A short description of the feeding activities of oysters is necessary to make the experimental results clear. (See Galtsoff, 1926, 1928.)

The oyster shell consists of two halves, or valves, connected by an elastic hinge which holds the valves several millimeters apart. The adductor muscle of the oyster is attached to both valves and, by contracting, closes them. Feeding can take place only when the muscle is relaxed and the valves open, for only then can the food-bearing water enter. The four gills of the oyster force the water through themselves by ciliary action (see accompanying report of Galtsoff) and filter out the food organisms, which are then swallowed. It is clear that under otherwise identical conditions two oysters may be together, one remaining open most of the time and the other remaining closed most of the time, the former would have opportunity to take in more food than the latter and would consequently be expected to store up a larger reserve food supply, which in the oyster is chiefly glycogen. The so-called fat oyster contains a large supply of glycogen. Relative absence of this reserve causes an oyster to be thin and watery, as has been the case with the Oakland Bay oysters.

Any agent which reduces the number of hours per day that the valves of oysters remain open at the same time reduces the number of possible feeding hours. Such oysters would be expected to store up less reserve food. The present experiments were designed to show whether the presence of sulphite liquor causes oysters to reduce their possible feeding time by remaining closed longer than specimens in uncontaminated water.

Following are detailed descriptions of the behavior of each experimental specimen. In general, a single series consisted of two experimental pairs of oysters, in different concentrations of liquor, and one pair of control specimens in uncontaminated water. Because of large individual variations it is difficult to express the results in any manner other than as separate descriptions. The series are organized according to concentrations of liquor employed, but there is considerable overlapping.

Concentrations of liquor in water are stated as parts per thousand. It must be emphasized that the stated concentrations represent the mixtures which the apparatus was standardized to deliver, and that any variations from this would be due to stoppage of the liquor tubes, causing temporarily a lower concentration. The water system was readily kept constant but, because of suspended matter in the liquor, there was an occasional slowing in its rate due to accumulation of particles in the small opening of the stopcock. This was reduced to a minimum by frequent cleanings, but any error due to this would tend to make the concentration lower, not higher, than that stated.

The experimental specimens were tested for a short time in water alone before the liquor was run in. When the experimental fluid started this was allowed to run into the chamber already full of pure water, which it slowly replaced.

The kymograph records were fixed in shellac, and then marked off into hourly periods for recording the results. At times one of the levers would not be close enough to the paper to write the record, and several hours of records might thus be lost. In such a case the number of hours per day that the specimen was open was computed for the period of existing records and then calculated on the basis of the 24-hour period.

EXPERIMENTS WITH ACID LIQUOR

Experiment No. 1 (6 parts per thousand, October 15 to 19, 1929).—One specimen was in each of the three experimental tanks. The first and third were controls and the

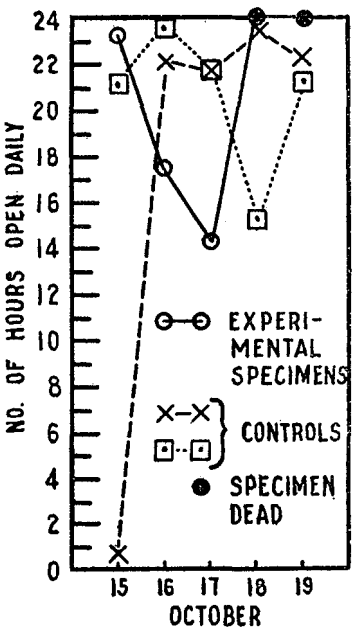

FIGURE 6.—Graph showing the number of hours per day which each specimen employed in experiment No. 1 was open. One specimen was subjected to sulphite liquor solution (6 parts per 1,000), while two were controls in pure water

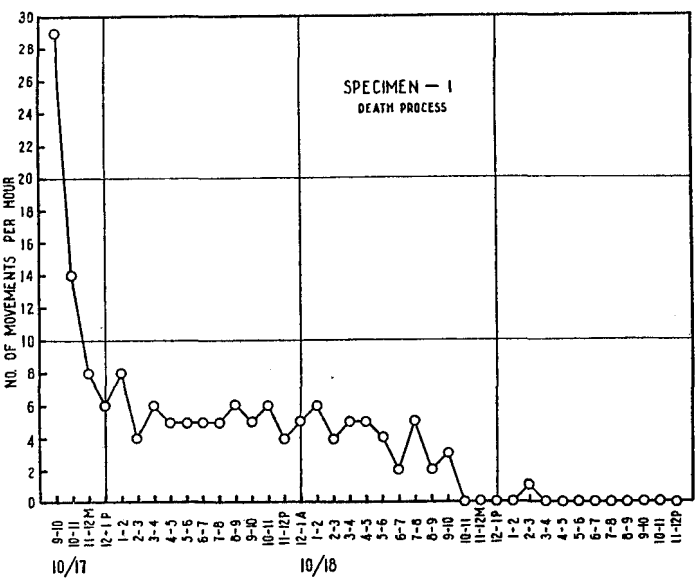

FIGURE 7.—Graph showing the cessation of activity of the liquor-treated specimen in experiment No. 1. The number of closing movements is plotted for each hour from beginning of gaping of the shells until movement had ceased

second, experimental. All specimens were in pure, running sea water on October 15 and up until 7.50 p. m. on the 16th, when liquor was started dripping into the mixing chamber which supplied the experimental oyster. In this case water was flowing at the rate of 134 cubic centimeters per minute and pure liquor was entering at 0.8 cubic centimeters per minute making a final solution of 6 parts per thousand. The pH of the solution in the experimental tank was between 5.8 and 6.1, while that of the control water was 7.7. During the test the temperature of the water in all tanks fluctuated between 10° and 14° C.

In Figure 6 are shown graphs of the number of hours per day that each specimen remained open. There is little difference between the specimens in this respect, but while the two controls remained open and highly active, the experimental specimen

became less and less active until it remained open and entirely motionless on October 18. This is characteristic of a dead or dying oyster. A graph (fig. 7) is presented to show the slow reduction in the number of shell movements, or adductor muscle contractions, per hour. When movement had definitely ceased the specimen was considered dead, and opened. The adductor muscle was flabby and soft, instead of hard as usual when cut. The heart was not beating but reacted slightly to mechanical stimulation. In no other case did death follow so quickly (48 hours) after introduction of the liquor.

It should be pointed out that when a specimen gapes wide open and is unable to close it will quickly be eaten whether dead or alive by small crabs and fish.

Experiments Nos. 16 and 17 (February 1 to 20, 1930).—Six specimens were included in these tests: 2 in each of the liquor solutions, and 2 controls in sea water alone. Records were started at 1.10 p. m., February 1. On the 1st, 2d, and 3d the temperature of the water used varied from 5° to 8° C. after which the water was heated and showed a maximum variation of from 12° to 18° C., the usual range being 14° to 17° C.

Experiment No. 17 (10 parts per thousand).—Water entered the mixing chamber at the rate of 90 cubic centimeters per minute, while liquor solution (1 part pure liquor to 4 parts sea water) dripped in at the rate of 4.6 cubic centimeters per minute, producing a solution of close to 10 parts per thousand. The pH of the solution in the experiment chamber varied between 4.7 and 5.6. Liquor was started dripping into the mixing chamber with the water on February 3 at 2.30 p. m. and continued until the end of the experiment. Figures 8 and 9 show the results of both experimental and control specimens in hours per day that they remained open. All four specimens were closed most of the time during the first three days. This was probably due to the low temperature of the water at this time.

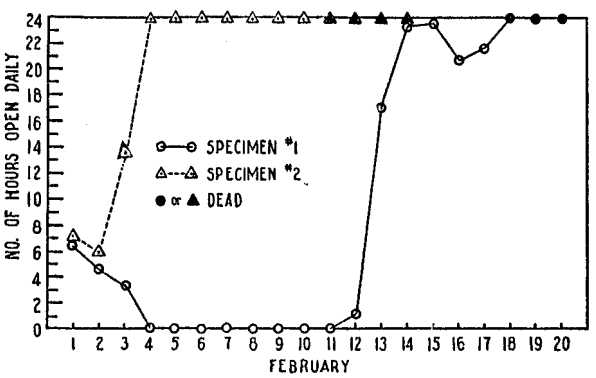

FIGURE 8.—Graph showing the number of hours per day that each specimen in experiment No. 17 (10 parts per 1,000) remained open. The individual difference is striking and shows the longer life of the specimen which remains closed most. Compare with the control specimens shown in Figure 9

From February 4 until death occurred on the 18th, experimental specimen No. 1 (fig. 8) stayed open an average of only 7.7 hours per day. The specimen died after being in the solution for 14 days, during the first eight of which the valves remained tightly closed.

The reaction of specimen No. 2 was decidedly different. It began to gape open (loss of muscle tonus) within two days, and activity became constantly less frequent until the final movement was made on the 13th, nine days after starting of the liquor. In several other cases this individual difference in the reactions to the liquor was observed. Why this should be the case is not clear, but it appears that the specimens which remain closed most of the time live longer than those which remain open in the liquor solutions.

During the period following February 3 the two control specimens (fig. 9) stayed open for a high percentage of the time. From February 4 to 20 specimen No. 3 averaged 23.01 hours per day open, while specimen No. 4 averaged 22.7 hours per

day open. At the end of the experiment the control specimens were as normally active as at the beginning. They both showed a delicate new shell growth at the edge of the valves, which was not the case of the treated oysters. Further, the controls showed that they were feeding for they threw out large quantities of fecal matter, rejected silt, etc., while the experimental oysters did not.

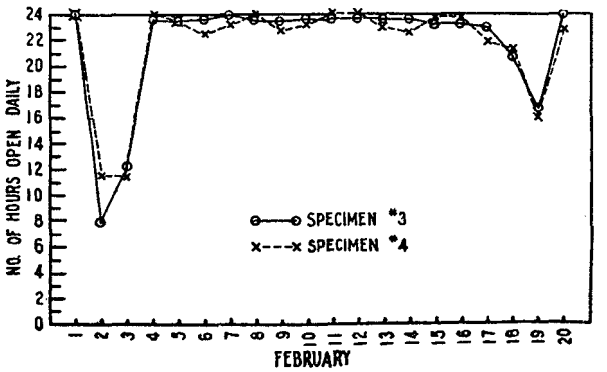

FIGURE 9.—Graph of the daily number of hours open of the control specimens for experiments Nos. 16 and 17. These oysters remained open and active a very high proportion of the time. Compare with Figures 8 and 10

Experiment No. 16 (5 parts per thousand).—Water flowed into the mixing chamber at the rate of 124 cubic centimeters per minute, and liquor (1:4) at the rate of 3 cubic centimeters per minute. The concentration of pure liquor to water was 4.84 parts per thousand, which is stated roughly as 5 parts per thousand. The pH of the solution in the experimental chamber varied from 6.1 to 6.5. Liquor was started February 3, at 2.30 p. m. and continued until the end of the experimental series. Specimens 5 and 6 (fig. 10) showed a difference in behavior similar to that of the specimens in experiment No. 17. From February 4 until just before death on the 18th, specimen No. 5 was open an average of 10.4 hours per day. On the 19th this oyster was gaping wide and motionless, having died after 15 days in the solution. Specimen No. 6, from the 4th until the 16th, averaged 22.54 hours per day open, and was dead on the 17th, 13 days after the liquor was introduced. These specimens showed no indication of shell growth and threw out no waste matter to indicate that feeding was going on.

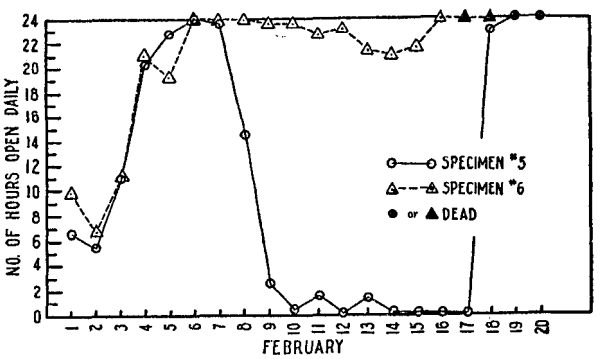

FIGURE 10.—Graph showing the records of two specimens in experiment No. 16 (5 parts per 1,000). The oyster which remained closed most lived longer than the other specimen. Compare with Figure 9 showing the record of controls

Experiments Nos. 18 and 19 (February 1 to March 3, 1930).—Records were started on February 1 at 1.45 p. m., the specimens in sea water alone. The pH of the sea water was always about 7.8. The temperature of the water from the 1st to the 3d, inclusive, varied from 4° to 8° C., after which the water was heated and fluctuated from 12° to 18° C., being usually from 14° to 17° C. The stock liquor consisted of 1 part pure liquor to 4 parts sea water.

Experiment No. 19 (2.4 parts per thousand).—Water entered the mixing chamber at the rate of 139 cubic centimeters per minute and liquor (1:4) at the rate of 1.67 cubic centimeters per minute. The pH of the solution in the experimental chamber varied from 6.6 to 7.1.

The results with specimens Nos. 1 and 2 (fig. 11) are closely parallel. Specimen No. 1 was open, from February 4 to 27, when it started gaping wide and making a

movement only every few hours, an average of 14.25 hours per day, while for the same period specimen No. 2 averaged 16.14 hours per day open. At the end of the experiment both specimens were gaping wide open and No. 2 had ceased to move. Specimen No. 2 had died after 27 days of treatment. Within one or two days more, at most, judging from the infrequent shell movements, specimen No. 1 would have died. For the purpose of record the latter is considered to have died in 29 days.

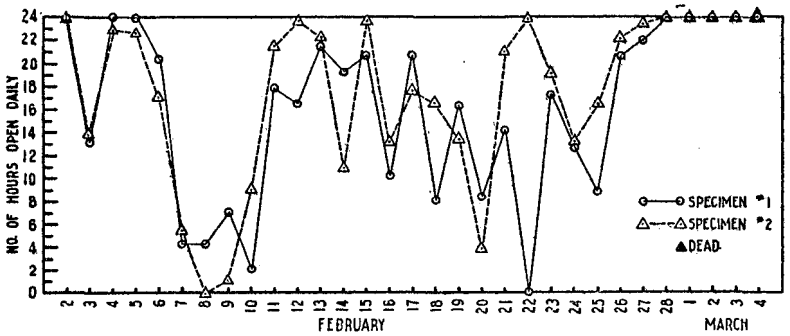

FIGURE 11.—Graph showing the records, in hours open daily, of the two specimens employed in experiment No. 19 (2.4 parts per 1,000). Compare with the controls (fig. 12)

At the same time the control specimens Nos. 3 and 4 (fig. 12) averaged 20.61 and 17.85 hours per day open. Between the 23d and 28th these specimens remained closed a large part of the time. It was suspected that a leak between one of the experimental chambers and the control chamber had occurred. The level of water in the control chamber was raised a little to prevent flow of contaminated water into it, and the specimens again behaved normally. After the experiment had been completed such a small leak was found to have occurred. A small quantity of the liquor

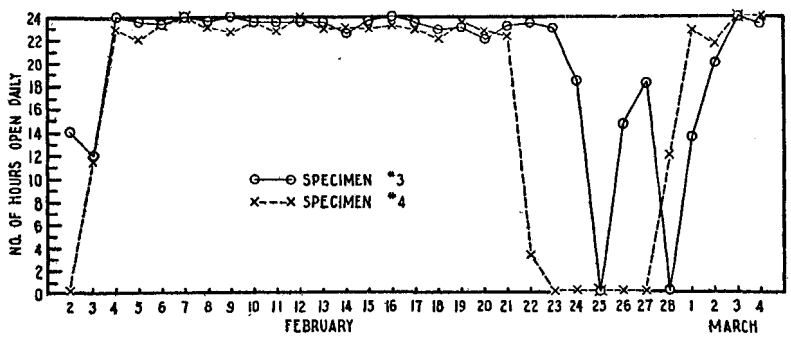

FIGURE 12.—Graph presenting the records of control specimens for experiments Nos. 18 and 19. The low records toward the end were due to a leak from one of the aquaria containing liquor solution. Compare with Figures 11 and 13, experiments Nos. 18 and 19

solution had probably seeped through and produced the striking change in behavior. That this was due to some unusual condition in the control chamber alone is shown by the fact that the records of the experimental oysters show no unusual variations during the same period.

Experiment No. 18 (3.2 parts per thousand).—The rate of flow of water was 123 cubic centimeters per minute and that of liquor solution (1:4) 2 cubic centimeters per minute, producing a final concentration of 3.2 parts per thousand pure liquor to

sea water. The pH of the solution varied from 6.5 to 7.0. Liquor was started at 3.50 p. m. February 3, and continued until the end of the experiment.

The records (fig. 13) show the time of death of the two specimens, as well as the number of hours per day that they remained open. Specimen No. 5, during the period from February 4 until just before it started gaping wide on the 14th, was open an average of 16.62 hours per day and was dead on the 16th, 12 days after the liquor was started. On the other hand, specimen No. 6 averaged 13.32 hours per day open from the 4th until the 24th, after which death occurred following 21 days of treatment.

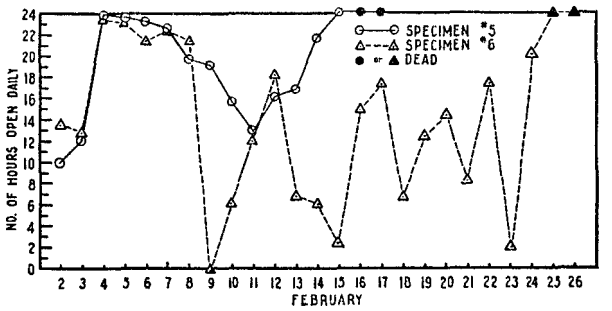

FIGURE 13.—Graph showing the number of hours per day which the specimens in experiment No. 18 (3.2 parts per 1,000) were open. Compare with the controls (fig. 12)

Experiments Nos. 12 and 13 (January 3 to January 10, 1930).—This is an incomplete series due to the fact that the water system was frozen on January 10 and experiments had to be stopped. However, it shows some effect of the liquor in the short time that the test was continued. The water was heated and the temperature varied between 14° and 18° C. The stock liquor solution consisted of 1 part pure liquor to 19 parts sea water.

Experiment No. 13 (3.8 parts per thousand).—The rate of flow of water was 61 cubic centimeters per minute and that of liquor solution 4.66 cubic centimenters per minute, producing a concentration of about 3.8 parts pure liquor per thousand parts water. The pH varied between 6.6 and 7.0.

From January 3 until the 7th at 4.30 p. m., when liquor was started, the specimens were in pure, running water, and both control and experimental oysters (fig. 14) were open a large part of the time. After the liquor was started both experimental specimens showed an immediate reaction. During the three days from the 8th to the 10th, specimen No. 1 averaged 11.46 hours per day open, while specimen No. 2 averaged 18.96 hours per day open. At the same time the control specimens Nos. 3 and 4 were open, respectively, 23.73 and 23.5 hours per day. This is too short a treatment for these averages to be of much significance, but the appearance of the records showing the immediate effect of the liquor on specimens Nos. 1 and 2 is important.

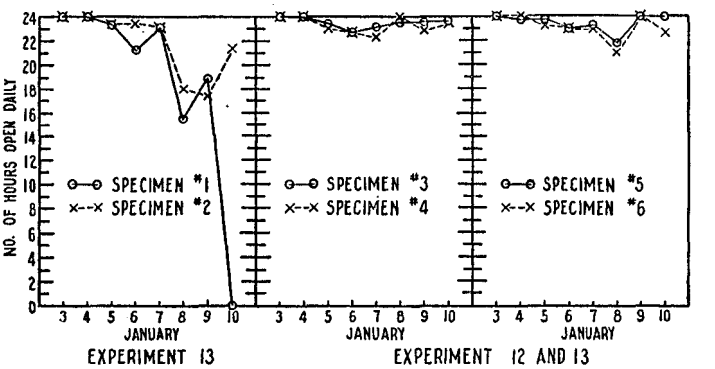

FIGURE 14.—Graphs showing the reactions of the specimens in experiments Nos. 12 (1.8 parts per 1,000) and 13 (3.8 parts per 1,000) and controls

Experiment No. 12 (1.8 parts per thousand).—Water flowed at 116 cubic centimeters per minute and liquor solution (1:19) at 4.25 cubic centimeters per minute, producing the above-stated concentration in parts of pure liquor per thousand. The pH was usually between 6.8 and 7.1. This concentration is lower than that

used in experiment No. 13 above and the sudden drop in the curves of hours per day open (fig. 14) is not so marked. This is not considered to be conclusive in any respect, and is presented to show that the reaction to relatively low concentrations is not immediate.

Experiments Nos. 20 and 21 (February 26 to March 30, 1930).—During these experiments the temperature of the water was maintained, with the exception of a few days, at between 14° and 19° C. The stock liquor solution consisted of 1 part pure liquor to 9 parts sea water. Records were started on February 26 with all specimens in pure running water of pH 7.7 to 7.9.

Experiment No. 21 (1.3 parts per thousand).—The rate of flow of water entering the mixing chamber was 90 cubic centimeters per minute and that of liquor solution (1: 9) 1.2 cubic centimeters per minute, resulting in a final concentration of 1.33 parts per thousand. The pH varied between 6.6 and 7.1.

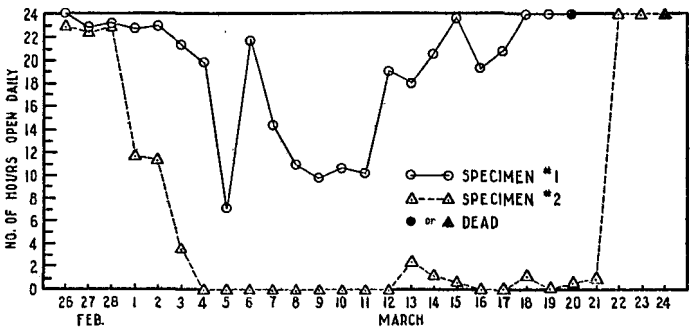

FIGURE 15.—Graph showing records of specimens in experiment No. 21 (1.3 parts per 1,000). The marked individual difference in reaction is clear. Compare Figure 16, showing records of the control oysters

The graph (fig. 15) presents the activity of these specimens in hours per day that they remained open. Before liquor was started the specimens were open about 23 hours per day. Specimen No. 1 did not show an immediate marked change in activity, but the effect appeared after a few days. From March 1 to March 17 this oyster was open an average of 17.15 hours per day. On the following two days it was gaping wide and showing very little movement, ending with death by the 20th,

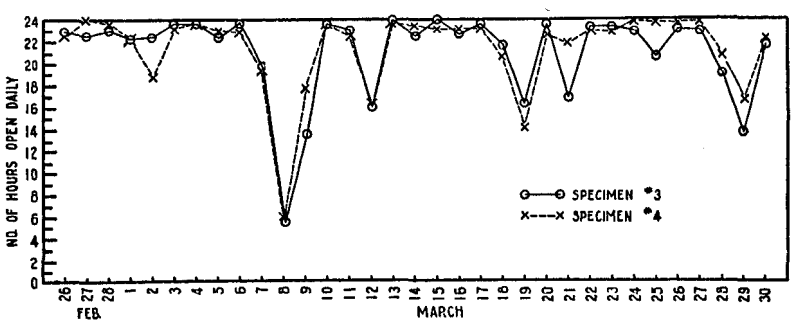

FIGURE 16.—Graphs showing records of control specimens for experiments Nos. 20 and 21. The marked drops in the records were due to sudden temperature changes caused by stopping of heater. Compare with Figures 15 and 17, giving records of experimental oysters

after 19 days of treatment. Specimen No. 2, on the other hand, was not dead until 23 days of treatment, but, from March 1 to 21 averaged only 1.66 hours per day open.

While both of the experimental specimens died, the control specimens (fig. 16) during the entire period of treatment averaged respectively 20.91 and 21.1 hours per day open. At the end of the experiment it was noted that both of these specimens showed new shell growth and that large masses of fecal matter, discarded silt, etc., were left. In the experimental specimens there was no growth and very little refuse matter was thrown out.

Experiment No. 20 (2.0 parts per thousand).—The rate of flow of water was 124 cubic centimeters per minute and that of liquor solution (1 : 9) 2.5 cubic centimeters per minute. The pH of the solution in the experimental chamber was from 6.8 to 7.2. Liquor was started dripping into the mixing chamber on February 28 at 12.30 p. m. On the preceding two days both specimens were open most of the time, but after the liquor was started both remained closed more and more of the time until finally they gaped open and steadily ceased activity. (Fig. 17.) Specimen No. 5 averaged, from March 1 to 15, when it started gaping, 12.93 hours per day open and was finally dead on the 25th, following 24 days of treatment with the solution. Specimen No. 6 also died after 24 days of treatment and during the period from March 1 to 20 remained open an average of only 9.9 hours per day. Compare the records of the two control specimens. (Fig. 16.)

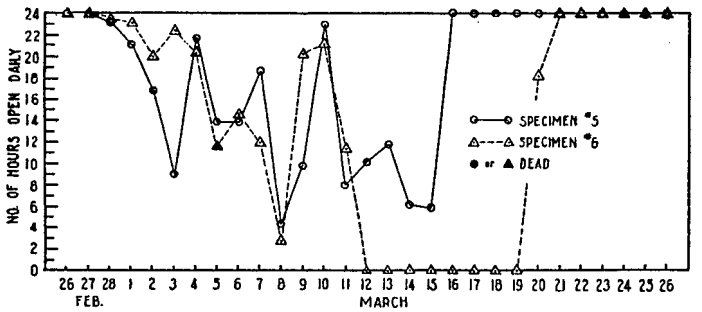

FIGURE 17.—Graph showing the records of the two specimens in experiment No. 20 (2 parts per 1,000). Compare records of controls (fig. 16)

Experiments Nos. 22 and 23 (March 12 to April 11, 1930).—During this series the temperature of the water was 14° to 17° C., except for certain short intervals when the heater was out of order, until April 1 when it was no longer heated and the temperature varied from 8° to 12° C. The pH of the water was 7.7 to 7.9. The stock liquor solution consisted of 1 part pure liquor to 9 parts sea water.

Experiment No. 23 (0.67 parts per thousand.)—Water entered the mixing chamber at the rate of 136 cubic centimeters per minute, and liquor solution (1 : 9) at the rate of 0.9 cubic centimeters per minute. The pH of the solution in the experimental tank varied from 7.1 to 7.5.

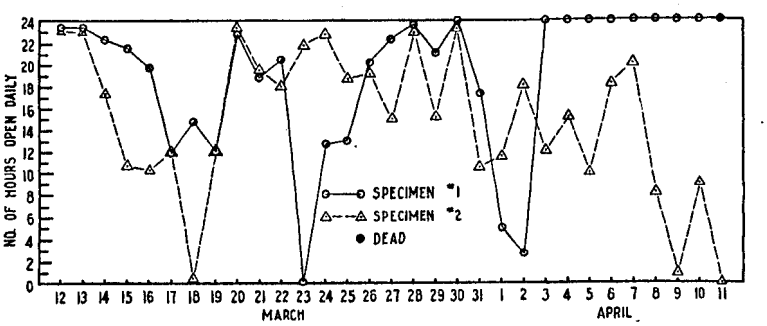

FIGURE 18.—Graph of records of specimens in experiment No. 23 (0.67 part per 1,000). There is considerable fluctuation in the curves, and only one of the specimens died before the test was discontinued. Compare with the records of the control oysters

Both specimens Nos. 1 and 2 (fig. 18) were open about 23 hours per day before liquor was started dripping into the mixing chamber on March 13 at 10.30 a. m. Then both oysters showed marked disturbances. From March 14 until April 2 specimen No. 1 averaged 16.21 hours per day open. From April 3 to 10 the specimen was gaping wide and making infrequent movements, at the end of which time death occurred after 28 days of treatment. Specimen No. 2 did not die during the period of the experiment, but averaged only 14.49 hours per day open from March 14 to April 11, during the 29 days of treatment.

The control specimens behaved in a strikingly different manner. (Fig. 19.) From March 14 until the experiment was stopped (29 days) specimen No. 3 was

open an average of 22.9 hours per day and specimen No. 4 remained open 21.78 hours per day on the average. At the end of the experiment these oysters showed new shell growth and had thrown out considerable quantities of débris, neither of which was true of the experimental specimens save that they left very small amounts of débris.

Experiments No. 22 (1.0 part per thousand).—The rate of water flow was 120 cubic centimeters per minute and that of liquor solution (1 : 9) was 1.2 cubic centimeters per minute. The pH of the solution in the experiment chamber was 6.8 to 7.3.

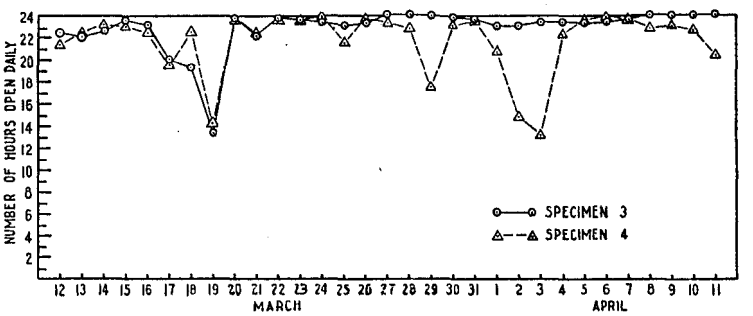

FIGURE 19.—Graph presenting the records of the control specimens of experiments Nos. 22 and 23. Compare with Figures 18 and 20

The results obtained from these two specimens (fig. 20) are very different from one another. Specimen No. 5 remained open an average of 23.66 hours per day from March 14 until the 24th, when it showed marked signs of gaping and cessation of activity. The last movement occurred on the 28th, 15 days after treatment had started. Specimen No. 6, however, remained closed most of the time, averaging only 6.74 hours per day open from March 14 to April 11, when the series ended. This behavior is definitely abnormal, as will be seen by comparing this record with those of the controls. (Fig. 19.)

Experiments Nos. 8 and 9 (December 13, 1929 to January 1, 1930).—Stock liquor for this series consisted of 1 part pure liquor to 9 parts sea water. During only part of the time was the water heated. Because of an apparent change of behavior due to temperature variation a graph (fig. 24) is presented giving average daily temperatures of the water, calculated from hourly thermograph readings. This will be referred to below. The pH of the water was 7.8. One kymograph sheet of the series was unfortunately destroyed.

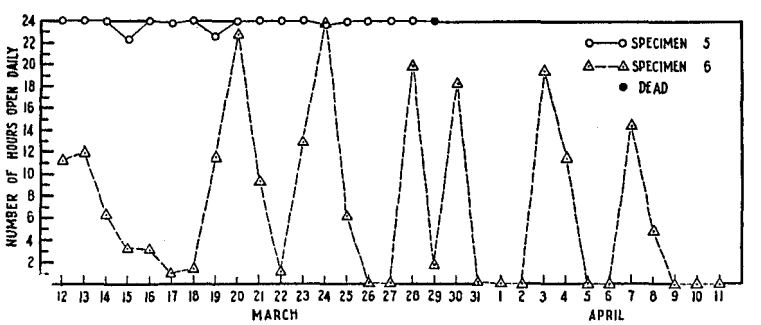

FIGURE 20.—Showing a graph of the records of the two specimens in experiment No. 22 (1 part per 1,000). One specimen remained open most of the time and died, while the other one was still living when the experiment was discontinued. Compare with the controls (fig. 19)

Experiment No. 9 (0.7 part per thousand).—This solution was made by water flowing into the mixing chamber at the rate of 3.48 cubic centimeters per minute, and liquor solution (1 : 9) at 2.5 cubic centimeters per minute. Both specimens reacted normally in pure water before liquor was started on December 14 at 9 p. m. Soon thereafter both began to stay open less hours per day. The graph (fig. 21) shows the behavior up until the experiment was ended. Specimen No. 1 averaged during the

period of treatment 15.82 hours per day open, and specimen No. 2 averaged only 8.11 hours per day open. At the same time, however, the controls (fig. 22) did not remain open as long as expected. While specimen No. 3 averaged 17.42, specimen No. 4 averaged 18.22 hours per day open, during the period in which the experimental specimens were in liquor solution. Both controls, however, gave a higher average than either of the experimental oysters. The reason for the confusion appears in Figure 24, in which daily temperatures are presented. The curves of all specimens in both experiments show almost perfect parallel to the temperature fluctuations. They were open more at high than at low temperatures. The effect of temperature on shell movements is discussed more fully in another publication. (Hopkins, 1931.) This explains the low records of the controls, but it does not explain the distinctly lower records made by the experimental oysters, which show that the presence of the liquor caused an additional effect. All specimens were living when the test was ended.

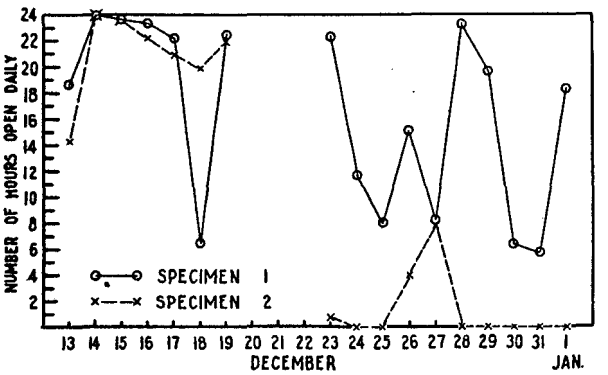

FIGURE 21.—Graph showing records of the two specimens in experiment No. 9 (0.7 part per 1,000). Kymograph sheet covering records for three days were lost. Compare with controls (fig. 22) and with the record of daily temperature, the fluctuations of which caused much of the variability in the behavior of the specimens

Experiment No. 8 (0.44 parts per thousand).—In this case water flowed at the rate of 368 cubic centimeters per minute and liquor (1:9) at 1.6 cubic centimeters per minute. The two specimens (fig. 23) gave inconclusive results during the short experimental period. Specimen No. 5 averaged 19.21 and specimen 6, 13.0 hours per day open. These figures are not different enough from the controls to be significant. For such a low concentration a longer period of treatment appears to be necessary.

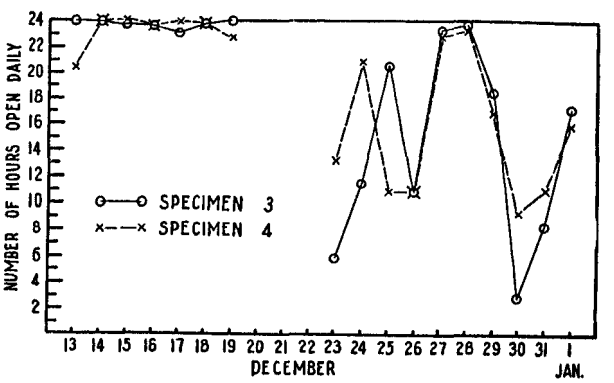

FIGURE 22.—Graph giving records of the control oysters in experiments Nos. 8 and 9 (figs. 21 and 23). The variability in height of records is parallel to fluctuations in water temperature (fig. 24)

Experiments Nos. 2 and 3 (October 20 to November 22, 1929).—These tests were carried on in unheated water of pH, seldom varying from 7.8. The temperature of the water was between 12° and 13° C. at the beginning but by the time the experiments were stopped had fallen to around 5° C. The stock liquor solution was made up of 1 part pure liquor to 19 parts sea water.

Experiment No. 2 (0.52 parts per thousand).—Water entered the mixing chamber at the rate of 84 cubic centimeters per minute and liquor (1:19) at 0.88 cubic centimeters per minute. The pH of the mixture was 7.2 to 7.6 in the experiment chamber.

Effect of the liquor is clearly shown in the graphs (fig. 25) of these two specimens as compared with those of the two controls (fig. 26). After the liquor was introduced

on October 23 and until the end of the experiment, specimen No. 1 averaged 6.76 while specimen No. 2 averaged 7.06 hours per day open. For a comparable period of time (October 22 to November 23) control No. 3 averaged 19.86, while No. 4 averaged 16.44 hours per day open. Even the controls show a more or less progressive lowering of the curves. This was due, as in the case of experiments Nos. 8 and 9, to temperature fluctuation. During the tests the average daily water temperature fell gradually from about 13° C. at the beginning to about 5° C. at the end of the series. This, however, does not interfere with the well warranted conclusion that the presence of the liquor was the cause of the experimental oysters remaining open less, for the controls were open more than twice as much.

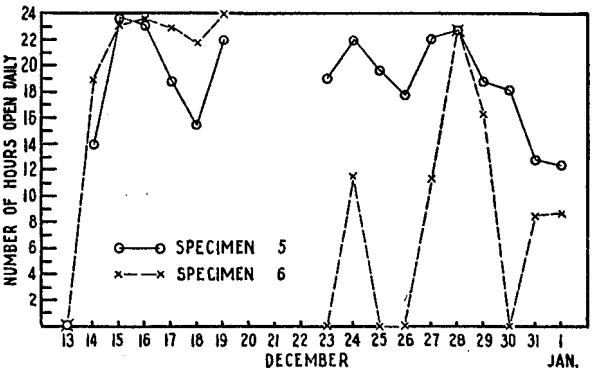

FIGURE 23.—Graph showing records of the two specimens in experiment No. 8 (0.44 part per 1,000). Compare with the controls (fig. 22) and the temperature record (fig. 24)

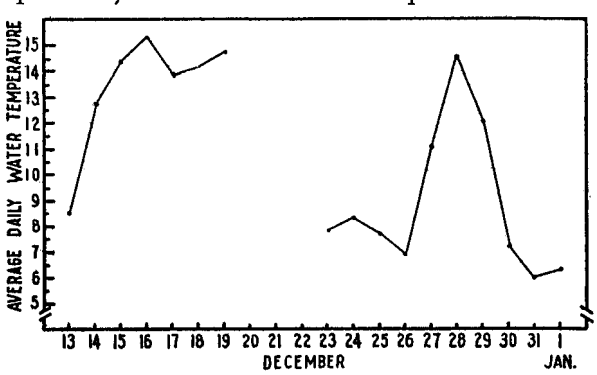

FIGURE 24.—Graph showing average daily temperature of the water in experiments Nos. 8 and 9. At times the water was heated, while during other periods it was cold. These temperature changes are accompanied by changes in the behavior of the oysters (figs. 21 to 23)

Experiment No. 3 (0.83 parts per thousand).—The water rate was 300 cubic centimeters per minute and that of liquor (1:19) was 5.13 cubic centimeters per minute. The pH of the solution varied between 7.0 and 7.5. The results compare favorably with those obtained in experiment No. 2 and show a definite reduction in number of hours per day open. (Fig. 27.) During the experimental period specimen No. 5 averaged 8.51 and specimen 6, 9.49 hours per day open, as compared with controls Nos. 3 and 4, which remained open 16.44 and 19.86 hours per day, respectively. Comparison of the record of specimen No. 5 with the records of other

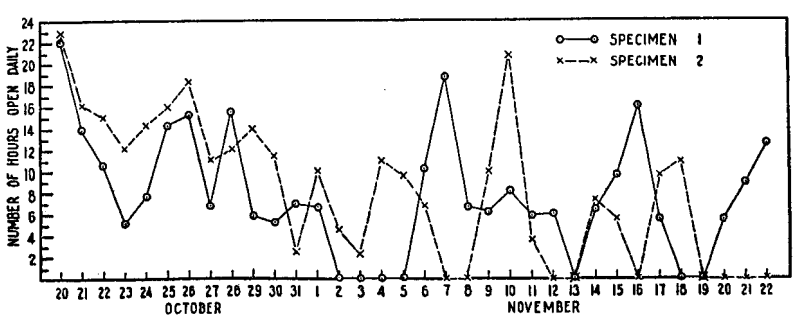

FIGURE 25.—Records of the oysters used in experiment No. 2 (0.5 part per 1,000). As compared with the controls (fig. 26), these specimens remained closed a large proportion of the time

oysters which died under treatment (figs. 8, 10, 11, 13, 15, 17, 18) suggests that this specimen, after having remained closed for a long time, was opening slowly to die.

In order to show the results obtained with all six specimens used in experiments Nos. 2 and 3, Table 1 is appended. The difference between the length of time that

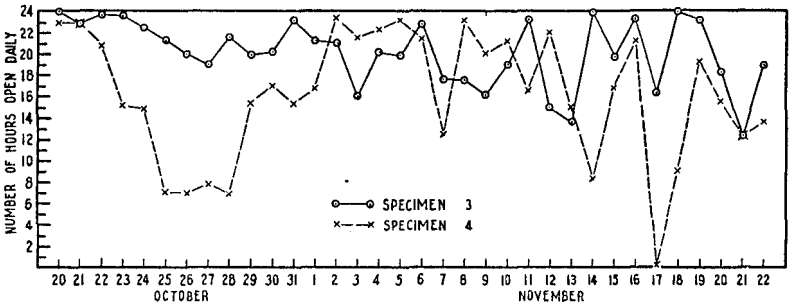

FIGURE 26.—Records of the controls in experiments Nos. 2 and 3 (figs. 25 and 27). The records are not as high as expected, though much higher than those of the experimental specimens. This was due, apparently, to the relative gradual fall in temperature from about 13° C. at the beginning to about 5° C. at the end of the series

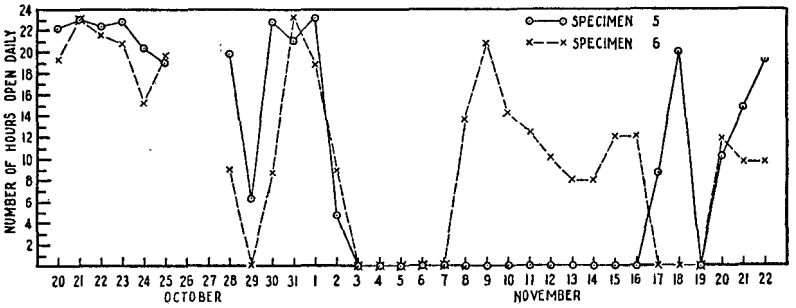

FIGURE 27.—Records of specimens in experiment No. 3 (0.83 part per 1,000). Compare with the controls (fig. 26)

the oysters in the three groups remain open is striking, expecially when the tendency for the treated specimens to remain closed continuously for several days is noted.

TABLE 1.—*Experiments 2 and 3 and controls. Length of time specimens remained open as influenced by the presence of sulphite liquor. Liquor was started on October 23*

Date	Number of hours open					
	Experiment No. 2, 0.5 part liquor per thousand		Controls, sea water		Experiment No. 3, 0.83 part liquor per thousand	
	Specimen No. 1	Specimen No. 2	Specimen No. 3	Specimen No. 4	Specimen No. 5	Specimen No. 6
Oct. 20	21.9	23.0	24.0	23.0	22.3	19.4
21	13.8	16.2	22.9	22.9	23.1	23.2
22	10.5	15.0	23.7	20.8	22.4	21.8
23	5.2	12.1	23.6	15.2	22.9	20.8
24	7.7	14.1	22.5	15.1	20.4	13.3
25	14.1	15.9	21.5	7.0	19.0	19.8
26	15.2	18.3	20.0	7.0	----------	----------
27	6.7	11.0	19.1	7.9	----------	----------
28	15.4	12.1	21.6	7.0	19.8	8.9
29	5.7	13.1	20.0	15.4	6.3	0
30	5.2	11.4	20.3	16.9	22.7	8.7
31	7.0	2.4	23.4	13.5	21.3	23.2
Nov. 1	6.6	10.0	21.4	16.9	23.1	18.8
2	0	4.5	21.0	23.4	4.6	8.9
3	0	2.4	16.1	21.7	0	0
4	0	10.9	20.4	22.3	0	0
5	0	9.8	19.9	23.2	0	0
6	10.2	6.8	22.8	21.4	0	0
7	18.6	0	17.7	12.4	0	0

TABLE 1.—*Experiments 2 and 3 and controls. Length of time specimens remained open as influenced by the presence of sulphite liquor. Liquor was started on October 23*—Continued

Date	Number of hours open					
	Experiment No. 2, 0.5 part liquor per thousand		Controls, sea water		Eperiment No. 3, 0.83 part liquor per thousand	
	Specimen No 1.	Specimen No. 2	Specimen No. 3	Specimen No. 4	Specimen No. 5	Specimen No. 6
Nov. 8	6.5	0	17.6	23.3	0	13.5
9	6.2	10.2	16.2	19.9	0	20.8
10	8.1	20.5	19.1	21.2	0	14.3
11	5.8	3.6	23.3	16.4	0	12.4
12	5.9	0	15.0	22.1	0	9.9
13	0	0	13.7	14.9	0	8.0
14	6.4	7.3	24.0	8.3	0	7.9
15	9.6	5.6	19.8	16.7	0	12.1
16	16.0	0	23.4	21.3	0	12.1
17	5.5	9.9	16.5	0	8.7	0
18	0	10.7	24.0	9.0	19.8	0
19	0	0	23.3	19.3	0	0
20	5.3	0	18.3	15.4	10.3	11.9
21	8.9	0	12.3	12.3	14.7	9.8
22	12.5	0	19.0	13.5	19.4	9.7
Total, 34 days	260.5	276.6	687.4	546.6	[1] 300.8	[1] 330.2
Average for 31 days of liquor treatment	6.76	7.06	19.86	16.44	8.51	9.49

[1] Total for 32 days only.

EXPERIMENTS WITH NEUTRALIZED LIQUOR

After sulphite liquor has been in sea water for some time the mixture ceases to give an acid reaction, due partially to neutralization by substances in the sea water and partially to loss of sulphur dioxide. It is necessary to be sure that, in the laboratory experiments, the effect of the liquor was not due to acid content.

A series of experiments was arranged to consist of 2 control oysters; 2 in a solution of 10 parts per thousand, and 2 in a solution of 5 parts per thousand of neutralized liquor in sea water. To the digester liquor was added concentrated NaOH solution until the pH of sea water was not changed by addition of the liquor. The liquor was then allowed to set for from 12 to 24 hours to permit the precipitate, probably chiefly calcium sulphate, to settle. Then it was decanted and filtered through several layers of cheesecloth, which removed most, but not all, of the precipitate.

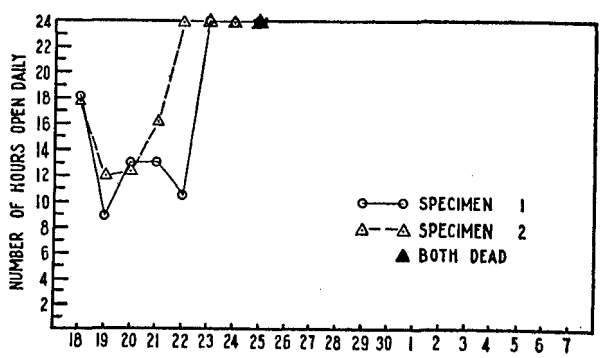

FIGURE 28.—Records of oysters in experiment No. 25 (10 parts per 1,000) in which neutralized sulphite liquor was used. Compare with the controls (fig. 29) which remained open and normally active long after these were dead

Experiments Nos. 24 and 25 (April 18 to May 8, 1930).—Throughout the series, for either concentration employed, the pH of the solution in the experimental tanks did not vary more than 0.2 points from the pH of the control water (7.8). The temperature of the water varied from 10° to 15° C.

Experiment No. 25 (10 parts per thousand).—The rate of flow of water was 103 cubic centimeters per minute and that of liquor solution (1:4) was 5.1 cubic centimeters per minute. Both oysters (fig. 28) were normally open and active when liquor

was started on April 18, at 2.20 p. m., and thereafter both specimens showed marked reactions.

Specimen No. 1, from April 19 to 22, after which it began to gape wide and movements became less frequent, was open an average of only 5.55 hours per day. The last movement occurred on the 24th, after 6 days of treatment. From the 19th until specimen No. 2 began to gape at the end of the 21st, this oyster was open an average of 13.57 hours per day.

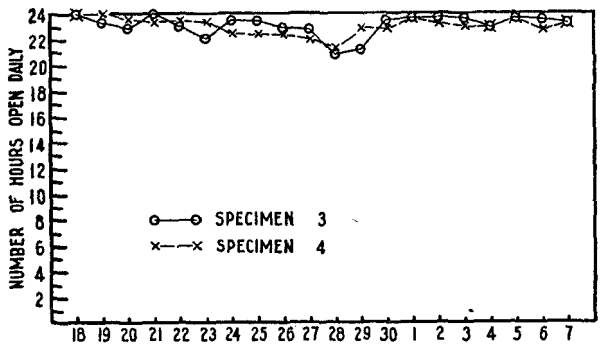

FIGURE 29.—Records of the control specimens in experiments Nos. 24 and 25. Compare with the experimental oysters (figs. 28 and 30)

During the same period of time both controls were open and normally active about 23 hours per day. (Fig.29.)

Experiment No. 24 (5 parts per thousand).—The rate of flow of water was 111 cubic centimeters per minute and that of liquor solution (1:4) was 2.8 cubic centimeters per minute. Both specimens tended to remain closed after the liquor was started. During the time of treatment, from April 19 until it started gaping after May 7, specimen No. 5 (fig. 30) averaged only 4.41 hours per day open. Similarly, specimen No. 6 remained open an average of 5.86 hours per day up until gaping started on May 6. Specimen No. 5 was considered dead after 20 days of treatment and specimen No. 6 after 18 days. Compare these results with the records of the controls. (Fig. 29.)

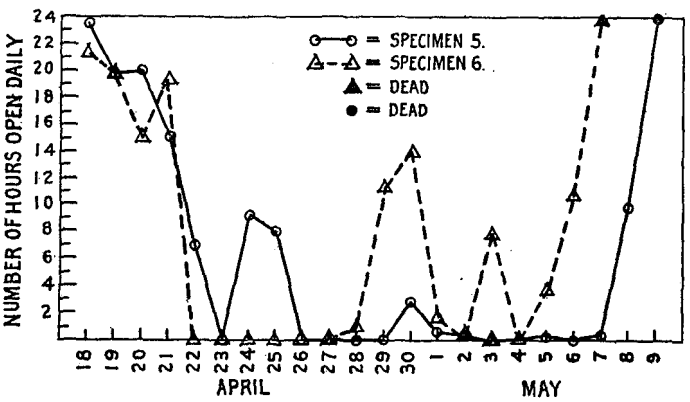

FIGURE 30.—Records of specimens in experiment No. 24 (5 parts per 1,000, neutralized sulphite liquor). Compare with the controls (fig. 29)

From these results, obtained with neutralized liquor, it is clear that it is not the acid content of the liquor which exerts the major unfavorable influence upon oysters. The time required for the specimens to die is easily within that which would be expected from a comparison of the death periods of specimens treated with acid liquor. In Figure 33 and Table 3 it is shown that when the length of time that specimens remain open is taken into consideration the results of these specimens agree substantially with those of other experimental oysters.

In the tests with unneutralized liquor it was noted that the pH of the solutions in the experimental chambers was not highly acid, as compared to the freshly mixed solutions used in the sensory stimulation experiments. This is due partially to the fact that stock liquor was mixed with sea water and during the few days that a bottle of this lasted was slowly partially neutralized. But it is chiefly due to the exposure of the liquor to air in the dripping apparatus. This allowed most of the sulphur dioxide gas to escape, so that the relatively slight acidity of the final solution could hardly be expected to produce any unfavorable results.

SENSORY REACTION TO LIQUOR

Along the edges of the mantle of the oyster are two rows of delicate tentacles which are sensory in function, and presumably serve to test the character of the entering water. When certain chemical solutions are brought into contact with these tentacles they retract sharply; and, if the chemical be sufficiently irritating, the retraction spreads to adjacent portions of the mantle and finally to the entire organ. Following such stimulation the adductor muscle contracts, protecting the oyster by closing the valves.

A method of measuring the sensitivity of the tentacles was devised. It need not be described fully here, but it consisted in measuring the length of time, or latent period, after the solution touches the tentacles, that they retract. These measurements were made over a relatively small temperature range, from 16° to 18° C.

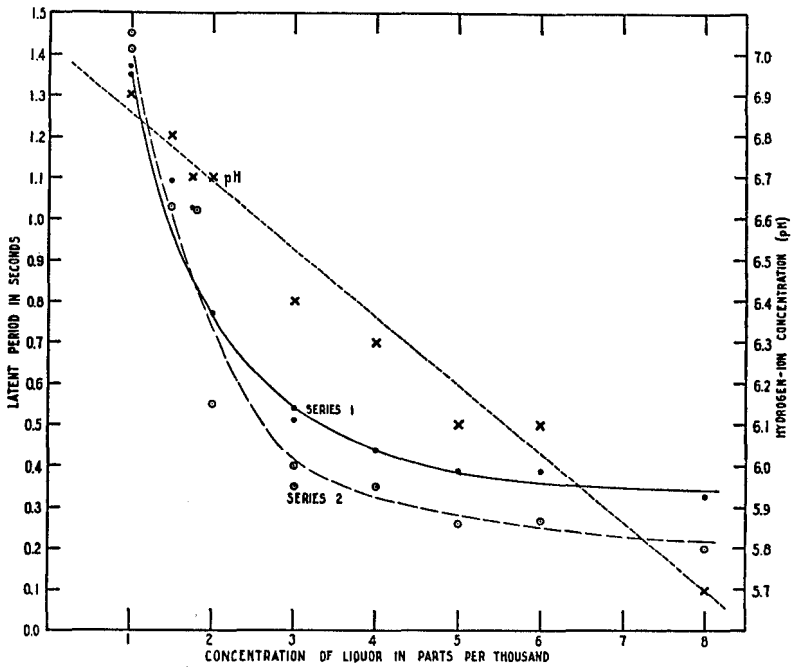

FIGURE 31.—The latent period of the reaction of the tentacles of the oyster to sulphite liquor. Two series are shown, as well as the pH of the solutions. See description in text

Two series of tests with different specimens are presented in Figure 31. Each point represents an average of at least 10 readings. On the same graph the pH values of the liquor solutions are plotted. The sea water in which the specimens were immersed was of pH 7.8.

The resulting latent period curves are typically logarithmic, but need no analysis here. The latent period is extremely short for a concentration of 8 parts per thousand, where the curves appear to be almost horizontal. Between this concentration and the minimum which produced a very sharp reaction (1 part per thousand), the curve increases the angle it makes with the horizontal until it becomes almost vertical. The measurable latent period of the tentacular reaction is limited to concentrations of 1 part per thousand or higher. Control tests with liquor neutralized with NaOH gave the same latent period values, though the reaction was less clearly defined and the error therefore greater.

It is interesting to compare these curves with those presented in the accompanying report by Galtsoff on the influence of liquor on the rate at which the oyster takes

in water. He found that the effect disappears at about 1 part per thousand, and that no water is pumped at about 8 parts per thousand. The latent period curves become approximately straight at these two limits, suggesting that the effect of the liquor noted by Galtsoff is produced directly through sensory or nervous channels.

DISCUSSION OF RESULTS

It is unfortunately impossible to present in a single graph all of the results which have been described in the foregoing account. In the first place the results fall into two groups, depending upon whether the specimens died during the period for which the tests were continued, or whether at this time they were still living but reacting in some other manner to the liquor. Large individual variations in susceptibility make it difficult to express the results mathematically.

In Table 2 the essential points of the results are given to show the difference between the activity of the experimental oysters and that of the controls. The records of the two control specimens of a series are averaged to serve as a basis for comparison with the treated oysters. The percentage of reduction, from the normal or control records, in the number of hours per day that treated specimens remain open is given for each oyster. In these values there is a great variation. This is due, in the first place, to the fact that all experiments were not continued equally long, and also to the marked individual difference in behavior of two specimens in the same solution. In the descriptions of the individual experiments it was pointed out that, when treated with a certain concentration of liquor, one specimen might remain open as much, or almost so, as the controls, and die within a few days, while the other specimen might protect itself by remaining closed a large proportion of the time, with the result that it would live longer. Why this variation should be so great is not clear.

TABLE 2.—*Summary of data on the effect of sulphite liquor on the Olympia oyster, showing death time and percentage of difference in time open between control and experimental specimens*

[In certain cases, designated by (—), the controls were open slightly less than the experimental oysters (see text description)]

Experiment number	Concentration parts per thousand	Specimen number	Death time in days	Hours per day open	Controls average hours per day open, 2 specimens	Difference (in hours per day open) between experimental and control specimens	Per cent difference
1	6.0	3	2	14.3	21.03	6.73	32.0
17	10.0	1	14	7.7	22.85	15.15	66.3
		2	19	2.4		20.45	89.4
16	5.0	5	15	10.4	22.85	12.45	54.5
		6	13	22.5		.35	1.5
24	5.0	5	20	4.41	23.08	18.67	80.4
		6	18	5.86		17.22	74.6
25	10.0	1	6	5.55	23.08	17.53	75.9
		2	6	13.6		9.48	41.1
19	2.4	1	29	14.25	19.28	5.03	26.1
		2	27	16.14		3.14	16.3
18	3.2	5	12	19.6	19.28	—.32	—1.5
		6	21	13.3		5.98	31.0
21	1.3	1	19	17.2	21.0	3.8	18.1
		2	23	1.7		19.3	91.9
20	2.0	5	24	12.93	21.0	9.07	43.2
		6	24	9.9		11.1	52.9
23	.67	1	28	16.21	22.34	6.13	27.4
		2		14.49		7.85	35.1
22	1.0	5	15	23.66	22.34	—1.32	—5.9
		6		6.74		15.60	69.8
13	3.8	1		11.46	23.61	12.15	51.4
		2		18.96		4.65	19.7
12	1.8	5		23.3	23.61	.31	1.3
		6		22.53		1.08	4.6
9	.7	1		15.82	17.82	2.00	11.2
		2		8.11		—9.71	54.4
8	.44	5		19.21	17.82	—1.39	—7.8
		6		13.0		4.82	27.1
2	.5	1		7.06	18.15	11.09	61.1
		2		6.76		11.39	62.8
2	.83	5		8.51	18.15	9.64	53.1
		6		9.49		8.66	47.7

However, in spite of this, certain results stand out sharply. In the first place, many treated specimens actually died in the liquor solutions. Secondly, those specimens which were treated for any considerable length of time with liquor in any concentration employed, from 0.5 to 10.0 parts per thousand, and did not die, were caused to reduce their normal number of hours per day of feeding, as judged on the basis of the activity of the control oysters.

In all, 19 specimens died as a result of the sulphite liquor solutions to which they were subjected. Oysters died after different periods of time in solutions of liquor ranging from 0.67 to 10.0 parts per thousand; that is, 1 part liquor to 1,500 to 1 part to 100.

In Figure 32 a graph is given to show the number of days of treatment after which specimens died in the series of concentrations employed. Although the points on the graph are widely distributed, they appear to fall into a sort of curve of the general form of those presented in Figure 31 for the sensory reaction. There are too few points to allow such an analysis as might permit the prediction of the length of time required for death to occur in even lower concentrations of liquor than those employed. There is a striking difference between the death time of oysters in 10 parts per thousand and that of specimens in about 1 part per thousand. Naturally, as the concentration is lowered the death time increases toward infinity. It is impossible to predict certainly from this whether 0.1 part per thousand, for example, would kill oysters, but it is obvious that, should it do so, it would on the average require considerably over 30 days.

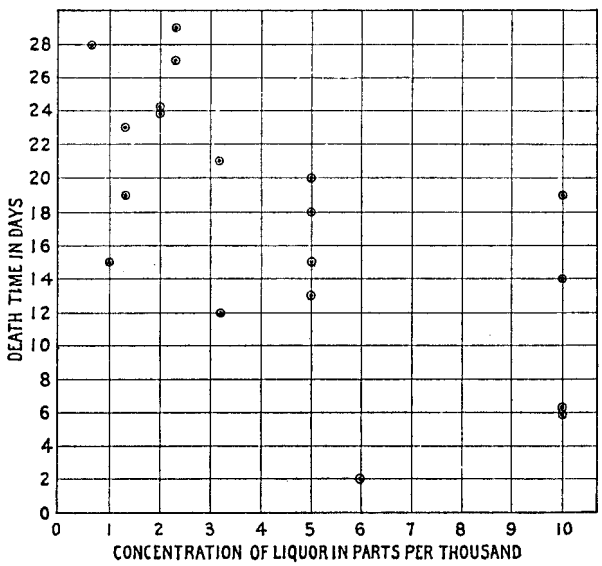

FIGURE 32.—Time required for death in solutions of sulphite liquor in the series of concentrations. The points in general show that the death time increases with diminishing concentration. See Figure 33 and Table 3

Only one specimen died much more quickly than the others, in respect to the concentration used. This was the specimen treated with a solution of 6 parts per thousand which died at the end of two days. The graph (fig. 32) clearly shows, also, that death of all these specimens was due to a particular variable factor in the water. If due to any variable other than the sulphite liquor the points in the graph would be scattered in all directions, regardless of the concentration of liquor used. Further, that the control specimens lived, and grew, and fed well under laboratory conditions demonstrated that these conditions were certainly not unfavorable.

If the death periods of oysters treated with different concentrations of sulphite liquor be plotted in such a manner as to take into consideration the differences in activity of the specimens, a somewhat more complete picture is obtained. In order to do this (fig. 33) the record of each specimen which died was analyzed and the number of hours per day that it remained open computed as percentage of the 24-hour period. The product of this value (average percentage of the time open) multiplied by the number of days required for death, is then considered to represent total effect

of the liquor. (Table 3.) As has been pointed out, the oyster which stays open least during treatment lives longest. Such a composite figure, as stated above, assumes that the fact that an oyster remains closed results in protection. That the assumption is justified will appear on examination of the records of individual oysters. The graph (fig. 33) shows more clearly the decrease in effectiveness of the liquor as the concentration is lowered, and gives a picture of the results not otherwise obtainable. There is, certainly, a fairly wide distribution of the points, but most of them fall into a definite curve.

TABLE 3.—*Data concerning the specimens which died as a result of treatment with sulphite liquor solutions. These data are shown graphically in Figures 32 and 33*

Experiment number	Specimen number	Death period in days	Average hours per day open	Per cent hours per day open	Mortality factor [1]	Concentration liquor parts per thousand
1	3	2	14.3	60.0	120.0	6.0
17	1	14	7.7	32.1	449.4	10.0
	2	9	24.0	100.0	900.0	10.0
16	5	15	10.4	43.3	649.5	5.0
	6	13	22.5	93.7	1218.1	5.0
19	1	29	14.25	59.4	1722.6	2.4
	2	27	16.14	67.2	1814.4	2.4
18	5	12	19.6	81.6	979.2	3.2
	6	21	13.3	55.4	1163.4	3.2
21	1	19	17.2	71.6	1360.4	1.3
	2	23	1.7	7.1	163.3	1.3
20	5	24	13.0	54.1	1298.4	2.0
23	6	24	10.0	41.6	998.4	2.0
22	1	28	16.2	67.5	1890.0	.67
	5	15	23.6	98.3	1474.5	1.0
25	1	6	5.55	23.1	138.6	10.0
	2	6	13.6	56.6	339.6	10.0
24	5	20	4.41	18.3	366.0	5.0
	6	18	5.86	24.4	439.2	5.0

[1] Mortality factor=death time (days) × per cent of hours per day open.

As stated above, death occurred in some of the specimens in concentrations as low as 0.67 parts per thousand. To this concentration two specimens were subjected (experiment No. 23, fig. 18), but only one died during the time the experiment was continued. The other oyster was still living at the end of the test, but the activity of this specimen could certainly not be called normal. During the entire experimental period this oyster averaged only 14.5 hours per day open, while the two control specimens averaged 22.9 and 21.8 hours per day open, respectively. This experimental oyster was open 35 per cent less time than the controls. Then this oyster was under a disadvantage in that it could take in no more than 65 per cent as much food as normal oysters. This would be the case even if the liquor exerts no direct lethal, or toxic, effect upon the oyster in other respects. In experiment No. 22 one specimen died while one continued to live during 29 days of treatment with 1 part per thousand. However, the latter, during that period, remained open only 6.74 hours per day on the average. It was able to feed a maximum of only 30 per cent as much as the control oysters.

In experiment No. 2 (0.5 part per thousand) neither specimen died during the 31 days that the test was continued, but both together averaged only 6.9 hours per day open, as contrasted with the controls, which remained open an average of 18.15 hours per day. Feeding time of the experimental oysters was thereby reduced by about 62 per cent.

Experiment No. 3 (0.83 part per thousand) is similar in that the two specimens averaged only 9 hours per day open, a reduction from the records of the control oysters of about 50 per cent.

Table 1 summarizes such data. All specimens which were given a fair test in any concentration, from 0.5 to 10 parts per thousand reacted very unfavorably either by dying or by remaining closed a large part of the time. Lower concentrations were not studied. Sulphite liquor, then, in these concentrations, appears to be definitely harmful to oysters.

These effects are clearly not due to the acid content of the liquor, for the four specimens treated with neutralized liquor died after periods of time entirely comparable to those of specimens treated with acid liquor.

At the beginning of this report attention was called to the fact that in Oakland Bay the oysters showed three peculiarities to an abnormal degree, namely, high mortality rate, poor meats, and lack of growth. Either directly or indirectly these conditions have been reproduced in the laboratory by subjecting normal oysters to various concentrations of sulphite liquor. The death of oysters as a result of the presence of the liquor in concentrations above 0.67 part per thousand has just been described.

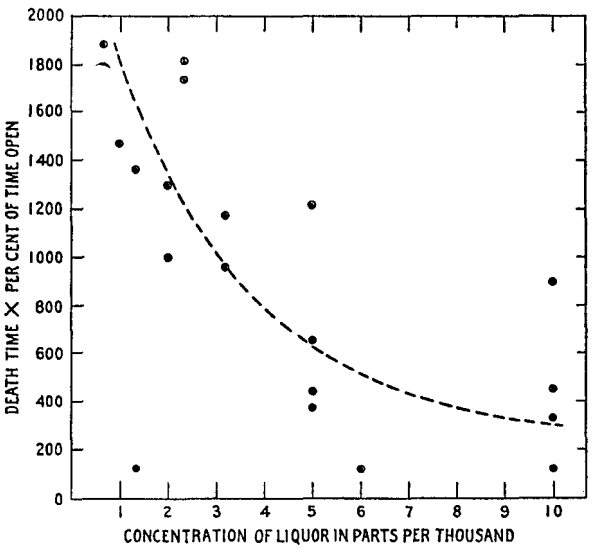

FIGURE 33.—Showing the data given in Figure 32 calculated to include the influence of the proportion of time the shells were open. The points represent death time in days × the percentage of time the valves were open. Since death occurs more quickly when the specimens remain open than when they stay closed, the values calculated as above show more clearly than Figure 32 the inverse relationship between concentration and death time, although individual variations are large

It was also stated that concentrations as low as 0.5 part per thousand caused specimens to remain closed more than the presumably normal control oysters. Any factor which causes oysters to remain closed abnormally at the same time deprives them of their due amount of food, for food-bearing water can not enter with the valves closed. The result would be that specimens subjected to liquor in such concentrations as above stated would be unable to take in as much food as normal oysters, and would consequently be expected to be relatively thin and watery. The results of Galtsoff, in his accompanying report, should be consulted in this connection, for he showed that an open oyster, in certain concentrations of liquor, does not take in water, and food with it, as rapidly as normal specimens.

In the experiments in which water of a temperature around 15° C. was used, the control specimens within two to four weeks developed 1 to 3 millimeters of new, delicate shell growth. Oysters were brought in winter to the laboratory from the cold water in the dikes, where growth could not go on because of the low temperature, so there was no possibility of mistaking the new shell for some already existing. The control specimens, however, were the only ones which grew new shell. In no case was new shell growth observed on an experimental oyster. One reason for this is that when the oysters are in liquor solution, the very sensitive edge of the mantle, which secretes

the new shell, does not extend outward as in the normal oyster, but withdraws several millimeters back into the shell. In addition, there may be more fundamental physiological reasons why treated specimens do not grow, but the above observation has been repeatedly made during these tests.

In an early part of this report it was pointed out that most of the works dealing with sulphite pollution have been concerned with the effect of diminished oxygen supply on fishes. It is certain that sulphite liquor is a strong reducing agent. Some fishes are very sensitive to reduced oxygen content of the water, and it may well be that this constitutes the major harmful effect on them of the liquor. In the case of oysters, however, dissolved oxygen is of secondary importance in this matter, for it is well known that the oxygen requirement of oysters is low. Because of this fact, oysters can be kept out of water for long periods of time with little harmful effect.

Verrill (1885) observed that oysters which were out of water lived for about eight weeks, during which time they necessarily remained closed, or the inclosed water would have been lost. Mitchell (1912) found that medium-sized oysters at between 19° and 28° C. used from 7 to 35 decimilligrams of oxygen per 100 grams of total weight, the amount used varying with temperature. He determined that completely closed oysters take in no more oxygen from the medium than do the shells alone. In one case an oyster lived in an almost oxygen free medium for 7 days without apparent ill effect, although it had absorbed only 1.2 milligrams of oxygen during the period.

Nozawa (1929) showed that the oxygen consumption of the oyster is independent of the oxygen tension until reduced to 0.1 per cent or lower. Even after oxygen consumption is reduced to none, carbon dioxide is still produced, and he agreed with Barkeley (1923) that the crystalline style plays a rôle in this anaerobic respiration. A more complete review of the physiology of respiration in the oyster may be found in the recent publication of Galtsoff and Whipple (1930), who found that oxygen consumption depends upon oxygen tension only when the amount of oxygen in the medium is below 2.5 cubic centimeters per liter.

TABLE 4.—*Showing lengths of time specimens remained closed continuously*

[Only periods of four days or more are included]

Experiment number	Specimen number	Number days closed consecutively	Condition following period of closure
2	1	4	Still living 18 days later.
2	1	6	Still living.
3	5	14	Still living 6 days later.
3	6	6	Still living 15 days later.
16	5	4	Died 1 day later.
17	1	8	Died 6 days later.
18 and 19	4	5	Still living 4 days later.
20	6	8	Died 5 days later.
21	2	−9	On the 1st, 2d, and 7th days the specimen was open 0.1 hour each. Died 11 days later.
24	5	4	Died 8 days later.
24	6	6	Died 10 days later.

When the valves of the oyster are closed, the normal flow of water is stopped, and very little, if any, oxygen is able to enter. In the experiments just described, it frequently occurred that specimens would remain closed for many consecutive days without once opening the valves to take in new water. Table 4 summarizes the most important examples of this reaction. Only periods of closure of more than

three days are recorded here, for the purpose of the table is to demonstrate that oysters can remain closed for many days and then still live for a considerable time. These oysters were in solutions of sulphite liquor in sea water. The control specimens in pure sea water remained open an average of more than 20 hours per day, and in no case did one of them die as a result of the tests.

One oyster (experiment No. 3, specimen No. 5) remained constantly closed for 14 days, after which it opened and lived for at least 6 more days. Others stayed closed for shorter periods of time without damage as due apparently to this cause. It has already been pointed out that specimens in liquor solutions live longer the greater portion of the time they remain closed. If death were due to lack of oxygen, this would not be expected. When such a specimen closes after having been in the liquor solution for some time, the fluid filling the mantle and gill chambers is the same as that in the experimental tank. The liquor would slowly use up all of the available oxygen in this inclosed water, and it would be expected, therefore, that the oysters which remain closed most would die most quickly, if death is due to lack of oxygen. Such, however, is not the case. In spite of the fact that newly pumped sea water was constantly flowing into the experimental chamber, carrying with it dissolved oxygen, the oysters which remained open and in contact with the new water died more quickly than those which closed. (See fig. 33.)

Oxygen determinations were not made, but instead of this the experiments were so arranged that a large supply of fresh sea water was constantly entering the experimental tanks. The lowest rate of flow of water was 61 cubic centimeters per minute (experiment No. 13) and the highest, 368 cubic centimeters per minute (experiment No. 8). In nearly all cases water entered the experimental tank at well over 100 cubic centimeters per minute. The capacity of each tank was about 3,000 cubic centimeters, so there was ample exchange. When solutions concentrated enough to show a marked color were used, it was observed that within less than one hour after stopping inflow of liquor into the mixing chamber, the color disappeared completely from the solution in the experimental tank.

As explained above, if the harmful effect of the liquor were due to lack of dissolved oxygen, it would not be expected that there would be such a difference in the duration of life of specimens which remained open most of the time and those which stayed closed more. Figure 33 indicates the correlation between time required to kill and concentration of liquor when this factor of shell behavior is taken into consideration. The effect of the liquor appears to be the consequence of mass action. It is progressive and steady when the oysters are open, and the liquor is constantly in contact with the tissues; but that portion of the toxic agent which is inclosed when the oyster closes may soon become exhausted and the tissue is then immersed in a relatively nontoxic medium.

The nature of the toxicity is not known and would be difficult to establish. The work of Galtsoff (accompanying report) demonstrates that the liquor has an immediate harmful effect on the activity of the gill mechanism. This effect, however, appears to be marked only in the relatively high concentrations. As contrasted to the immediate reaction of the ciliary mechanism, the oyster as a whole slowly succumbs as if by progressive poisoning.

The normal oyster under favorable conditions remains open most of the time, the shells closing and opening again periodically. The frequency of these closures is highly variable and the periodicity complicated. While it is difficult to express this activity mathematically, a simple comparison of the normal and experimental speci-

mens in this respect will suffice to show the progressive departure of the sulphite-treated oysters from the normal. Nozawa (1929) said that while the periodic closures of the shells occur in the normal Japanese oyster at about six per hour, after reduction of dissolved oxygen to 2 to 3 cubic centimeters per liter the frequency of shell movements is much reduced, as the time required for opening and closing becomes longer.

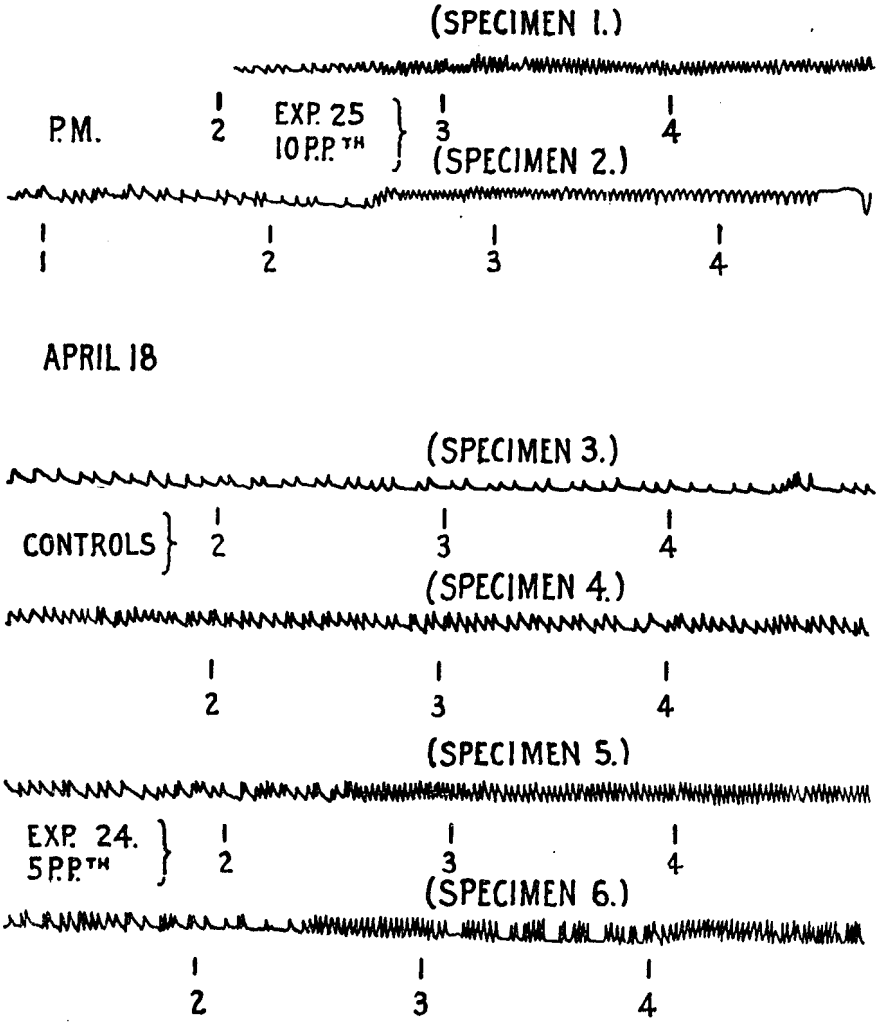

FIGURE 34.—Reproduction of portion of kymograph sheet showing records of shell movements of oysters used in experiments Nos. 24 (5 parts per 1,000; specimens Nos. 5 and 6 at bottom) and 25 (10 parts per 1,000; specimens Nos. 1 and 2 at top). The middle two records were made by the controls (specimens Nos. 3 and 4). Intervals of one hour are indicated. At 2.20 p. m. the liquor solution was started running into the experimental chambers. Note the more frequent shell movements of specimens Nos. 1, 2, 5, and 6 immediately thereafter. See Figures 35 to 38

The primary effect of sulphite liquor solution on the oyster is just the reverse. It stimulates the oyster to close and open more frequently, except shortly before the specimen finally dies. In order to show the typical changes in shell activity from beginning of an experiment until death of the oysters, several reproductions are given of portions of the kymograph records obtained in experiments Nos. 24 and 25. (Figs. 34 to 38.) Figure 34 shows the reactions during the time when liquor was started. There is a distinct change of activity which consists in increased frequency of closures.

The difference between the control and the experimental oysters is very clear, although all specimens are quite active, as is typical of oysters which are immersed after being in air for some time.

Soon another type of shell reaction begins, namely, the tendency to close and remain closed for increasingly long periods. In Figure 35, a few hours farther advanced than Figure 34, this tendency is shown. However, the initial effect, very

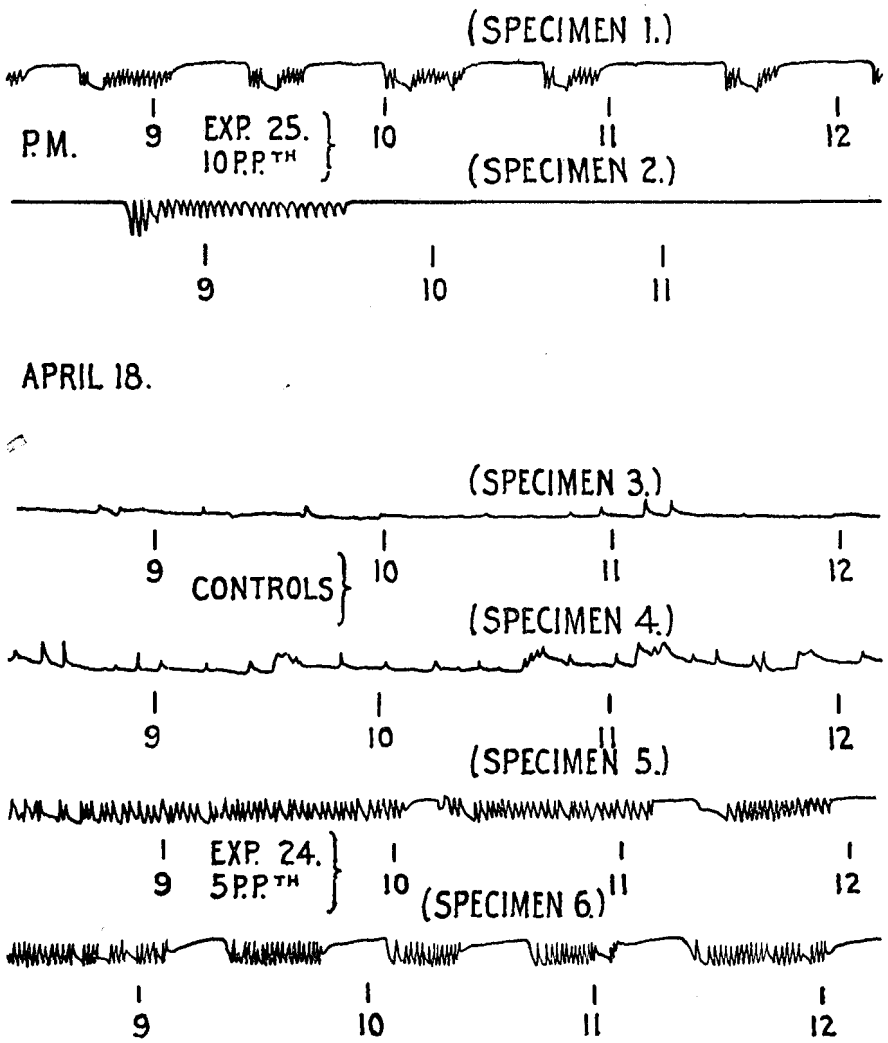

FIGURE 35.—Reproduction of portion of kymograph sheet showing records of specimens used in experiments Nos. 24 and 25, a few hours later than that shown in Figure 34. The tendency to remain closed (high straight lines) and the rapid movements when open are well shown

frequent closures, is still to be observed during the periods of activity or "openness." This continues for some time, depending upon the concentration of liquor and the consequent rapidity with which the toxic action occurs. Then (fig. 36) the periods of remaining closed become shorter and the reactions while open less frequent. At the same time the adductor muscle slowly loses its power to close the shells completely. In the figure this is more obvious in the second record than in the first. That the closure is incomplete after the short opening period is shown by the lower

level of the record as compared with that before opening. Such a specimen is beginning to gape, the adductor muscle losing its tonus, so that the shells open wider than normally. The muscle also loses ability to make a complete contraction. There follows then a period during which gaping becomes more and more pronounced,

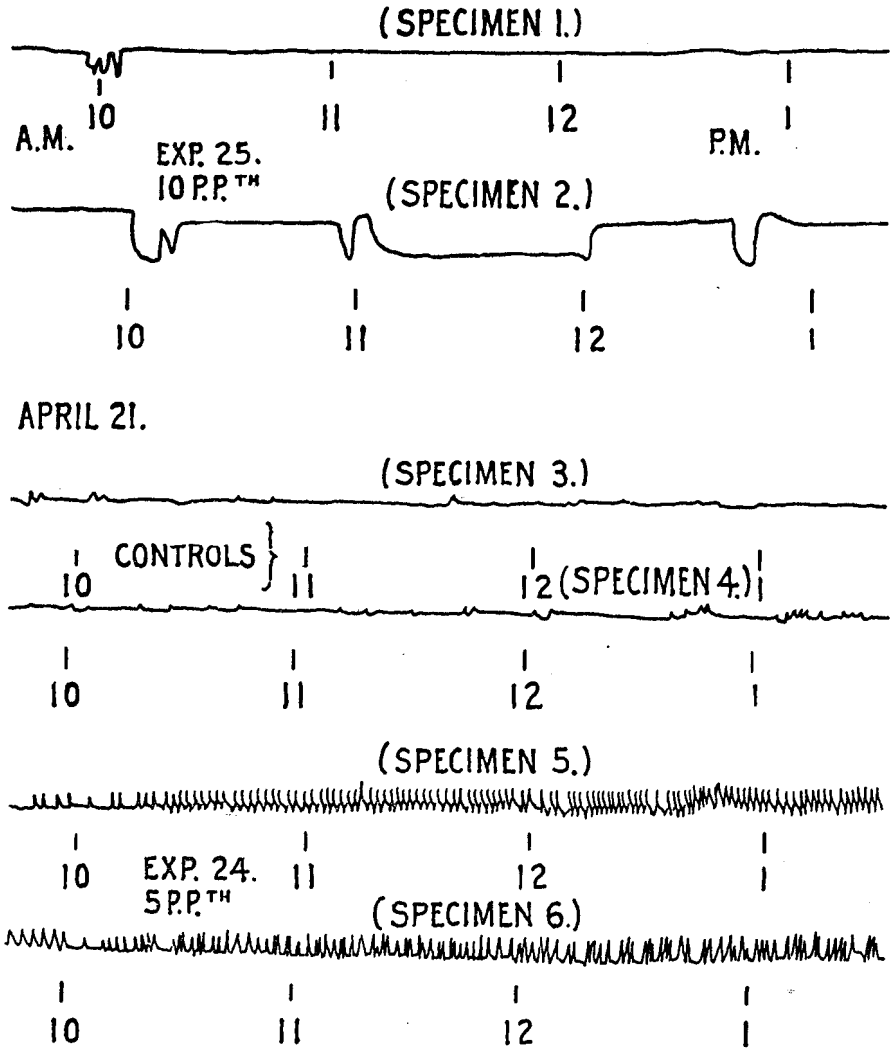

FIGURE 36.—Reproduction of portion of kymograph record of experiments Nos. 24 and 25 about two and one-half days later than that shown in Figure 35. The inability of specimens Nos. 1 and 2 to close completely is shown. The record of specimen No. 1 is wavering, showing that closure was incomplete. The record of specimen No. 2 shows the differences in height of the lines, or differences in degree of closure. Specimens Nos. 5 and 6 in a weaker concentration of liquor are still more active than the controls

complete closure never occurring, and even the partial closures become less frequent. (Fig. 37.)

As the oyster gapes, the constantly less frequent shell movements are also of less amplitude. It will be observed in Figure 37 that the contractions are very weak, but that the muscle acts as if attempting to hold the shells together, instead of relaxing immediately after a contraction, as is typical of the control records. When the oysters are in this condition they are almost at the death point. The last figure of

the series (fig. 38) shows the two records of the experimental oysters as straight lines. All movement has ceased and they are gaping wide open, motionless.

In the experiment just described, the different phases of the oyster's death process followed upon one another within a few days. In tests in which lower concentrations of sulphite liquor were employed, much longer periods of exposure were required before the specimens died. (Bottom two records, figs. 34 to 38.) However, the same

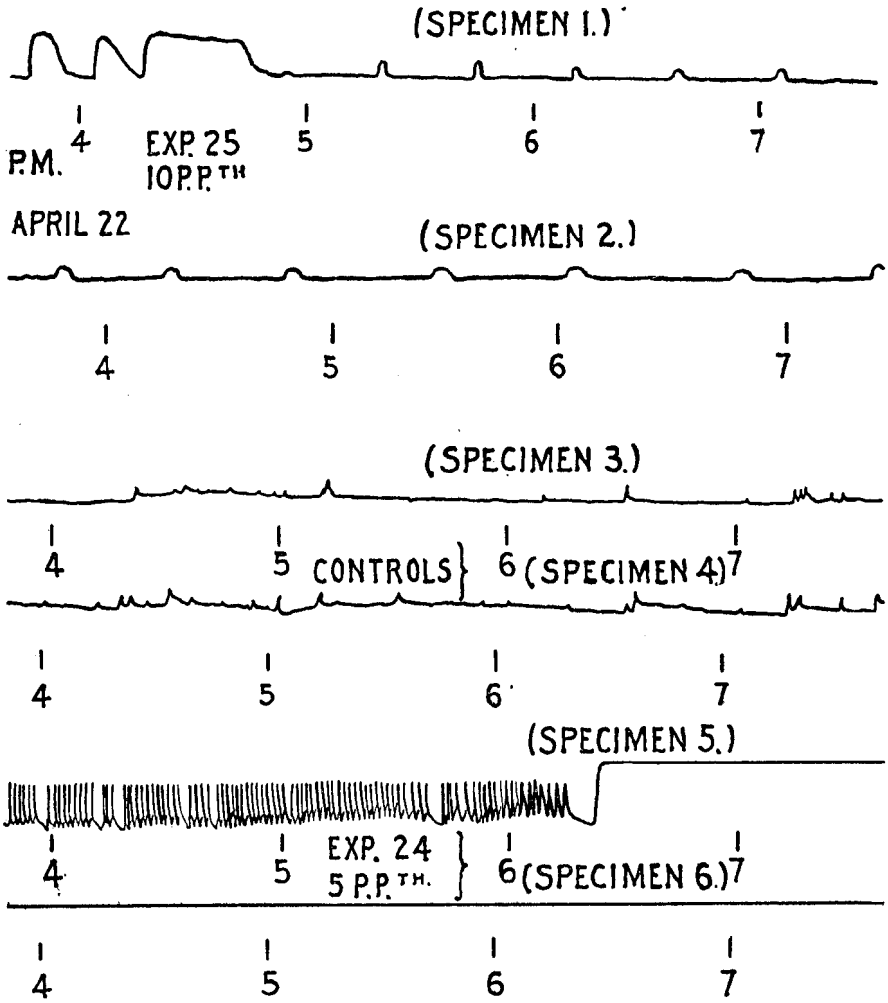

FIGURE 37.—Reproduction of portion of kymograph record of experiments Nos. 24 and 25, one day later than that shown in Figure 36. Specimens Nos. 1 and 2 have lost their ability to close completely and movements are relatively slight and infrequent. Specimens Nos. 5 and 6, in weaker concentration of liquor remain closed a large part of the time, and when open are very active. The behavior of these is similar to that of specimens Nos. 1 and 2 about three days previously, as shown in Figure 35

sequence of the different phases of the activity of the oysters during treatment was observed to hold, although each phase was very much prolonged.

The most striking characteristics of the reactions before the approach of gaping were the tendency to remain closed for long periods and, while open, to make abnormally frequent temporary closures. If there had been a marked deficiency of oxygen, the specimens would have become less active from the beginning, according to the

observations of Nozawa (1929). The reaction which he described was observed to occur, as described above, as one of the late stages just preceding gaping. This diminished activity is probably typical of an oyster dying from any cause.

It would be of considerable importance to determine what components of the sulphite liquor are the actual toxic agents. It may well be that some substance is contained in the liquor in such small quantities as to pass unnoticed in ordinary chemical analyses, but which exerts a toxic effect upon the oyster. Such a substance

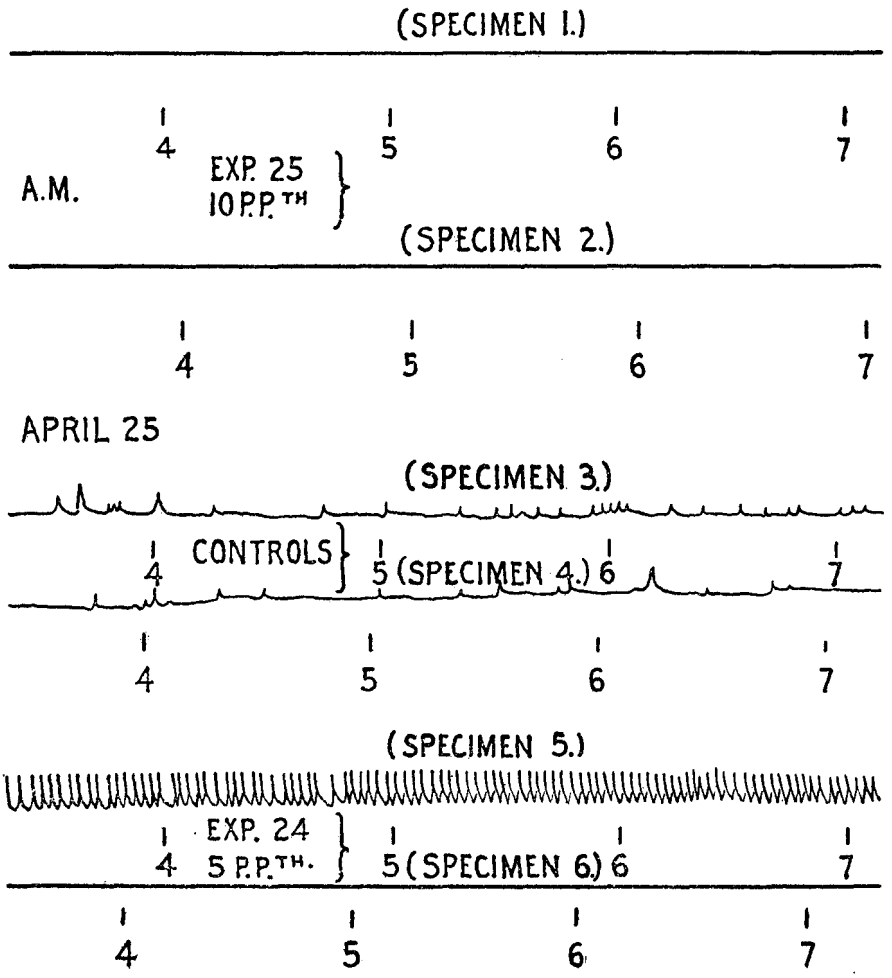

FIGURE 38.—Reproduction of portion of kymograph record of experiments Nos. 24 and 25, about two and one-half days later than that shown in Figure 37. Specimens Nos. 1 and 2 are dead and the shells are gaping open without movement. Specimen No. 5 is open and highly active while specimen No. 6 is closed. The controls (3 and 4) show normal activity

might be extremely dilute and yet toxic. To some substances aquatic animals may be highly sensitive. Marsh (1908) found, for example, that silver nitrate killed salmon fingerlings in 24 hours at a dilution of 1 to 22½ million. The toxicity of this substance as compared with that of sulphite liquor is tremendous.

That the acid content of the liquor was not responsible for the harmful effect was shown by the fact that oysters died in neutralized solutions, as described above, as well as by the observations of Galtsoff, as reported in an accompanying paper, that

neutralized liquor produced a diminished feeding rate. Further, the oxygen requirement of the liquor, while it might be of significance if experiments were carried on in stagnant water, is not the important factor. Both in the above described experiments, in which running, newly pumped water was continuously provided, and in the experiments of Galtsoff, in which the solution was constantly stirred and aerated, the possibility of insufficient dissolved oxygen was eliminated.

Acidity and oxygen demand have generally been considered to be the cause of the harmful effect of sulphite pollution. While this may be the case to a great extent in fresh-water streams, it is of little significance from the point of view of pollution of waters near oyster grounds. The customary recommendations that liquor be neutralized and exposed to air in ponds before allowing it to enter bodies of water may not be made with any confidence where the body of water concerned contains oyster beds. Until all toxicity is removed from such wastes, if such is possible, they should be completely excluded from waters where oysters are grown.

In order to work out all of the problems connected with removal of toxicity from the liquor, a great amount of research would be required. It would be of importance to determine whether the toxicity is slowly destroyed in sea water. It might possibly be expected that oxidation would slowly eliminate such substances. However, in this case there is no reason for assuming that this would occur, since the actual toxic agents are unknown. The results of the present work as correlated with those of McMillin and Galtsoff, in accompanying reports, rather indicate that destruction of the toxicity of the liquor in Oakland Bay has not progressed very rapidly.

McMillin was able to calculate, with reasonable accuracy, the maximum concentration of liquor which would develop in Oakland Bay following regular dumping of known quantities by the mill. He found that when an average of about 70,000 gallons of liquor is dumped daily, the equilibrium developed in the bay would be stronger than 1 part per thousand, and that in the neighborhood of a year would be required to reach this equilibrium. These calculations assume that no destruction of the toxic agents takes place. In the laboratory experiments described above, it was shown that liquor in concentrations of 1 part per thousand or above kill oysters within less than a month. Lower concentrations produce death after a longer period of time. There appears to be a clear relationship between the time required to kill and the concentration of liquor. Oysters died in concentrations as low as 0.67 part per thousand.

While the oysters in the laboratory were constantly subjected to the same concentration, this would be true of the oysters in the bay only if complete mixing took place. However, complete mixing and distribution of the liquor throughout the bay would be a slow process. Further, at low tide the oysters are subjected to the relatively pure seepage water, which would allow for some recovery. In Oakland Bay oysters were first observed to be dying about a year after the mill started operations, which is about the same time as McMillin calculated would be necessary for development of equilibrium if complete mixing and distribution should take place at a rapid rate.

The close correlation, therefore, between the time of theoretical building up of the equilibrium concentration, the beginning of mortality of oysters in the bay, and the results of laboratory experiments on the effect of different concentrations, appears to indicate that toxic substances in the liquor were at least partly responsible for the abnormal mortality of oysters in Oakland Bay. This agreement between the results of the several phases of these investigations is of the utmost importance, for on the

basis of these principles it would be theoretically possible to predict the effect of sulphite liquor on oysters in any other body of water on which a similar mill might locate.

Since sulphite liquor is poisonous to oysters, either killing them or causing them to take in less food, it should be totally excluded from tidal areas in which oysters are cultured. There is a fundamental difference between the results of dumping wastes into a flowing stream on the one hand, and into a tide-controlled bay or estuary, on the other. In the former case, the waste matter is diluted and washed away by constantly flowing, unpolluted water. In the latter case the liquor becomes mixed with water the movement of which, for the most part, consists in back and forth fluctuations. The same water, with relatively minor variations, remains day after day. To what extent this would be true depends upon the degree to which the body of water in question is inclosed and its total volume, the amount of fresh water entering, the difference between the tides, and the consequent actual loss of water. Other factors, such as direction and rate of currents with respect to location of oyster grounds and source of pollution, would have to be given consideration.

The extent of the damage to oysters in any such location might be predicted if the equilibrium concentration calculated should be as high as the concentrations studied in the laboratory. For lower theoretical equilibrium concentrations, however, low they might be, it would never be safe to say that no damage would be done, for all of the effects observed in the laboratory occurred within the relatively short time of one month. The dilution at which toxicity ceases, when long periods of exposure are considered, can not be stated. Only complete exclusion of liquor from oyster-producing waters can be considered as safe.

SUMMARY AND CONCLUSIONS

(1) Sulphite liquor, when added to sea water in concentrations from 0.5 to 10.0 parts per thousand, is decidedly unfavorable to oysters (*Ostrea lurida*).

(2) In concentrations from 0.67 to 10.0 parts per thousand most of the specimens died after being treated for from 2 to 29 days, depending upon the concentration of liquor.

(3) In all concentrations tested (0.5 to 10.0 parts per thousand) for a reasonably long period of time the specimens either died, or they remained closed much longer daily on the average than did control specimens in presumably uncontaminated water.

(4) When the temperature of the water in the laboratory was sufficiently high, in the vicinity of 15° C., the control specimens showed 1 to 3 millimeters of new shell growth within 2 to 4 weeks. Specimens in water containing liquor did not show any perceptible growth. The mantles of treated oysters remained withdrawn into the shell instead of protruding slightly at the edge of the shell where new shell is secreted.

(5) The effect of sulphite liquor on oysters is not due to acidity, for its potency is not disturbed by neutralization with NaOH.

(6) The chief characteristics of the abnormal oysters in Oakland Bay (namely, high mortality rate, poor meats, and lack of shell growth) have been produced either directly or indirectly in the laboratory by adding various amounts of sulphite liquor to the water.

(7) Concentrations of from 0.5 to 10.0 parts per thousand only were adequately tested in the laboratory, but it is not to be assumed, therefore, that in less concentrated solutions the liquor is harmless to oysters. The solutions tested required only about 35 days, at most, to produce the effects described in this report, and higher

dilutions might well be expected to produce an unfavorable effect after a longer period of time.

(8) From the calculations of McMillin in an accompanying report it is seen that the concentration of liquor which would be expected in Oakland Bay is within the limits of the concentrations tested in the laboratory. At such concentrations the liquor is definitely harmful.

(9) It is recommended that pulp mills using the sulphite process totally exclude waste liquor from waters in which oysters are grown. It is not impossible to dispose of waste liquor by means of by-products plants or by evaporating and burning, and such measures should be employed in the interest of such natural resources as oysters.

BIBLIOGRAPHY

BERKELEY, C.
1923. On the crystalline style as a possible factor in the anaerobic respiration of certain marine mollusks. Journal of Experimental Zoology, vol. 37, 1923, pp. 477–488. Philadelphia.

CARPENTER, KATHLEEN E.
1930. A biological survey of the Champlain watershed. Section IX. Fish life in relation to the polluting influences in the Lake Champlain watershed. Supplement to the Nineteenth Annual Report, State of New York Conservation Department, 1929 (1930), pp. 186–209. Albany.

FAIGENBAUM, HAROLD M.
1930. A biological survey of the Champlain watershed. Section XI. Chemical investigation of the Champlain watershed. Supplement to the Nineteenth Annual Report, State of New York Conservation Department, 1929 (1930), pp. 220–240, 5 figs. Albany.

GALTSOFF, PAUL S.
1926. New methods to measure the rate of flow produced by the gills of the oyster and other Mollusca. Science, new series, Vol. LXIII, No. 1626, February 26, 1926, pp. 233–234, 1 fig. New York.
1928. Experimental study of the function of the oyster gills and its bearing on the problems of oyster culture and sanitary control of the oyster industry. Bulletin, United States Bureau of Fisheries, Vol. XLIV, 1928 (1929), pp. 1–39, 13 figs. Washington.

GALTSOFF, PAUL S., and DOROTHY V. WHIPPLE.
1931. Oxygen consumption of normal and green oysters. Bulletin, United States Bureau of Fisheries, Vol. XVLI, 1930 (1931), pp. 489–508, 4 figs. Washington.

HOPKINS, A. E.
1931. Temperature and the shell movements of oysters. Bulletin, United States Bureau of Fisheries, Vol. XLVII, No. 1, 1931, pp. 1–4, 10 figs. Washington.

KNIGHT, A. P.
1901. The effects of polluted waters on fish life. Contributions to Canadian Biology, Supplement to the 32nd Annual Report of the Department of Marine and Fisheries. Sessional Paper No. 22a, 1901, pp. 9–18.

MARSH, M. C.
1907. The effect of some industrial wastes on fishes. Water Supply and Irrigation Paper No. 192: The Potomac River Basin, United States Geological Survey, 1907, pp. 337–348. Washington.
1908. Notes on the dissolved content of water in its effect upon fishes. Bulletin, United States Bureau of Fisheries, Vol. XXVIII, part 2, 1908 (1910), pp. 891–906. Washington.

MITCHELL, PHILIP H.
1912. The oxygen requirements of shellfish. Bulletin, United States Bureau of Fisheries, Vol. XXXII, 1912 (1914), pp. 207–222. Washington.

NIGHTINGALE, H. W., and V. LOOSANOFF.
1928. The effects of waste sulphite liquor on early stages of the chinook salmon, and means of prevention by disposal methods. Transactions, American Fisheries Society, vol. 58, 1928, pp. 232–244. Hartford, Conn.

NOZAWA, A.
1929. The normal and abnormal respiration in the oyster, *Ostrea circumpicta* Pils. Science Reports, Tohoku Imperial University, Fourth Series (Biology), Vol. IV, No. 1, fasc. 2, pp. 315–325. Tokyo and Sendai, Japan.

OKU, K.
1904. The action of calcium sulphate on living oysters. Journal of the Fisheries Bureau, Vol. XIII, No. 1, pp. 20–35. Tokyo.
OKU, K., K. ITO, and M. FUJITA.
1901. The action of calcium sulphate on living oysters. Journal of Fisheries Bureau, Vol. X, No. 2, 1901, pp. 13–29. Tokyo.
PHELPS, EARLE BERNARD.
1909. The pollution of streams by sulphite pulp waste. A study of possible remedies. Water Supply Paper No. 226, United States Geological Survey, 1929, 36 pp., 1 plate. Washington.
SUTER, RUSSELL, and EMMELINE MOORE.
1922. Stream pollution studies. State of New York, Conservation Commission, 1922, pp. 1–27. Albany.
SUTERMEISTER, EDWIN.
1929. Chemistry of pulp and paper making. 2nd edition, rewritten, 1929, X+565 pp. John Wiley and Sons, Inc. New York.
VERRILL, A. E.
1885. How long will oysters live out of water. Bulletin, United States Fish Commission, vol. 5, No. 11, April 25, 1885, pp. 161–162. Washington.
WHIPPLE, GEORGE C.
1922. Pollution of streams by pulp mill wastes. Proceedings, American Society of Civil Engineers, vol. 48, August 1922, pp. 1385–1392.
WISCONSIN STATE BOARD OF HEALTH.
1927. Stream pollution in Wisconsin. Special report. A joint report of the Conservation Commission and State Board of Health of Wisconsin concerning activities in the control of stream pollution, from July 1, 1925, to December 31, 1926 (1927) 328 pp., 80 figs. Madison, Wis.

II. THE EFFECT OF SULPHITE WASTE LIQUOR ON THE RATE OF FEEDING OF *OSTREA LURIDA* AND *OSTREA GIGAS*

BY PAUL S. GALTSOFF

The mode of feeding of oysters and other lamellibranch mollusks consists in producing a strong current of water which passes through the gills and in catching, conveying toward the mouth, and ingesting the suspended particles which were brought in with the stream of water. The maintenance of a steady current is dependent on the ciliary motion of the lateral cilia which, by beating inward, that is, at a right angle to the surface of the gill, produce the necessary pressure inside the gill cavity. It has been shown in previous investigations of the author that two factors, the rhythm of the ciliary beats and the coordination of the ciliary activity throughout the whole layer of ciliated epithelium, control the rate of flow of water through the gills. Both of these factors can be affected by physical and chemical changes in the surrounding medium. Some of them (as for instance, mechanical stimulation) may have no direct influence on the rate of beating of the cilia, yet they may produce a pronounced effect on the coordination of ciliary motion and result in a loss of head pressure in the gills and a subsequent sharp decrease in the rate of flow. Changes in chemical composition of the sea water may affect both factors simultaneously and cause disturbances in the operation of the ciliated mech anism. One would expect, therefore, that the discharge into the sea of large quantities of any waste product, even nontoxic, would upset the chemical equilibrium in the solution of salts in the sea water and would interfere with the normal activity of the organisms growing in it. A study of the effect of pulp mill wastes on the activity of the ciliated epithelium of the oyster presented, therefore, a problem which was both interesting from a scientific point of view and important because of its practical application.

This study was carried out in October, 1929, at the Jaques Loeb Laboratory at the Hopkins Marine Station, Pacific Grove, Calif. Oysters for experimental work were received from Olympia (*Ostrea lurida*) and from Samish Bay (*O. gigas*, the Japanese oyster). Olympia oysters, shipped by boat from Olympia to San Francisco and by train from San Francisco to Pacific Grove, arrived on the fifth day; Japanese oysters were expressed and were en route four days. In both cases the oysters arrived in good condition and apparently did not suffer from transportation. They were placed in large tanks with running sea water where they were kept for four weeks. There was no indication of unusual mortality among the oysters. The temperature of the water in the tanks (recorded three times a day) fluctuated from 15° to 17.5° C.; its salinity (daily observations) varied from 33.40 to 33.84 parts per thousand.

The method employed in the present investigation consisted in measuring the velocity of the current of water in the circular glass tubing introduced into the gill cavity. Since this method was fully discussed in previous papers by the author (Science, 1926, Vol. LXIII, pp. 233–234; Journal of General Physiology, 1928, Vol. XI, pp. 415–431; Bulletin of the Bureau of Fisheries, 1928, Vol. XLIV, pp. 1–39) a description of it here is omitted. Because of the small size of the Olympia oyster, the glass tubing used in the experiments with this species was of smaller diameter than the tubing used in the experiments with Japanese and Eastern oysters. During the experiments, oysters were kept in enamel trays containing 5 liters (Olympia oyster) and 10 liters (Japanese oyster) of water. The water was continuously stirred and aerated. No attempts were made to keep the temperature constant; it fluctuated within 2° C. "Red liquor" was received from the Shelton pulp mill; its specific gravity at 15.6° C. was 1.046. Various amounts of red liquor were added to the water in which the oysters were kept, and the rate of flow of water was measured after the oysters were allowed to remain for at least 15 minutes in a given concentration. Each experiment lasted several hours, depending on the number of observations. The values of the rate of flow given in figures are the means of 10 or 20 measurements. The limits of fluctuation are shown in the tables. Numerous controls show that under the conditions of the experiments, the rate of flow through the gills of an oyster, which was kept in pure sea water for several hours remained constant. Thus the decrease in the rate of flow of water observed during the present investigation can be attributed to the effect of the red liquor.

The results of the experiments are shown in Tables 5–7 and in Figures 39–41.

For computing the rate of flow in cubic centimeters per hour, the following formula was used:

$$V = 450\pi D^2 S$$

where D is the diameter of the glass tubing in centimeters and S is the velocity of current at the axis in cms/sec. In the case of *Ostrea lurida*, the diameter of the glass tubing D was 0.33 centimeter; in the case of the Japanese oyster, D equaled 0.68 centimeter.

An examination of the results of the experiments (Tables 5 and 6, figs. 39 and 40) shows that the addition of 2 parts per thousand of red liquor causes a decrease in the rate of flow of water through the gills. At the concentration of 6 parts per thousand, the flow of water in *Ostrea lurida* is about one-fifth of its normal rate; at 9 parts per thousand, it constitutes only 7.3 per cent of the normal rate of a given specimen. It is interesting to note that, beginning with the concentration of 4 parts per thousand, the current becomes less regular, the irregularity increasing with the increase in concentration.

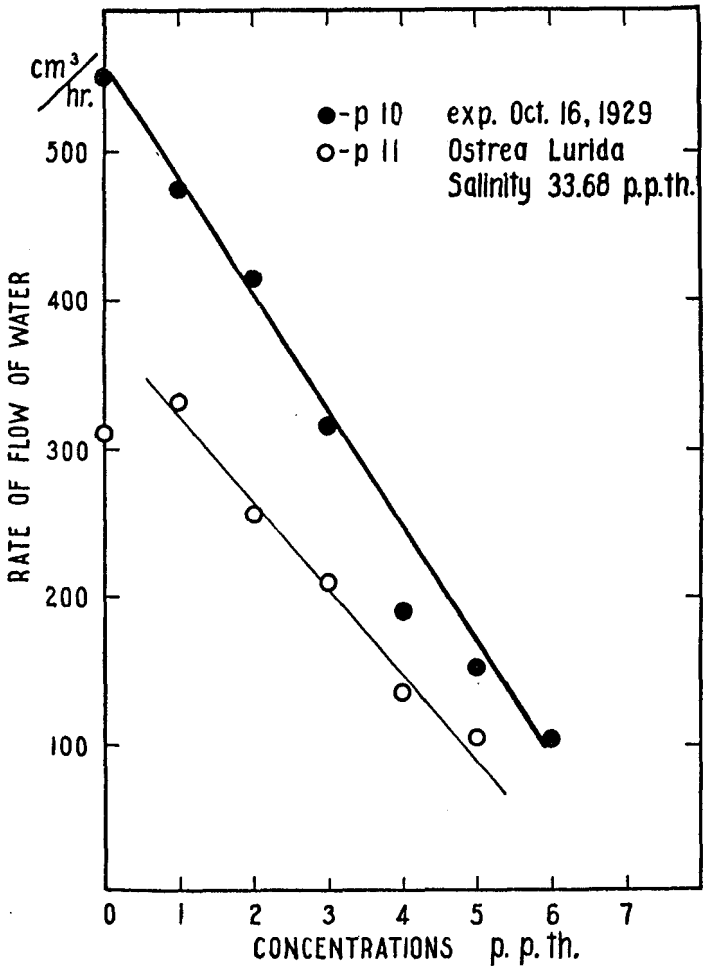

FIGURE 39.—Effect of sulphite liquor on the rate of feeding of *Ostrea lurida*. Temperature, 16.8–17.5° C.; pH, 7.9–6.2

TABLE 5.—*Effect of red liquor (specific gravity 1.046) on the rate of feeding of Ostrea lurida (oyster P–10 is 4.8×3.3 centimeters; P–11 is 3.7×3.4 centimeters). Salinity—33.68 parts per thousand*

[October 16, 1929, Pacific Grove, Calif.]

Time	Temperature	Concentration parts per thousand	pH	Rate of flow, cubic centimeters per hour			Remarks
				Average	Maximum	Minimum	
P-10	°C.						
2.20 p. m.	16.8	0	7.9	550	573	459	
2.37 p. m.	16.8	1	7.4	474	521	459	
3.10 p. m.	17.0	2	7.0	413	418	370	
3.40 p. m.	17.6	3	6.7	312	336	286	
4.03 p. m.	17.5	4	6.6	199	261	99	Irregular current.
4.27 p. m.	17.8	5	6.3	153	230	115	Do.
5.15 p. m.	17.5	6	6.2	107	143	84	Do.
P-11							
2.25 p. m.	16.8	0	7.9	306	327	286	
2.40 p. m.	16.8	1	7.4	310	318	277	
3.15 p. m.	17.0	2	7.0	230	243	208	
3.45 p. m.	17.6	3	6.7	214	220	144	
4.08 p. m.	17.5	4	6.6	138	167	110	Irregular current.
4.31 p. m.	17.8	5	6.3	107	153	90	Do.
5.20 p. m.	17.5	6	6.2	118	121	115	Do.

TABLE 6.—*Effect of neutral red liquor (specific gravity 1.046) on the rate of feeding of Ostrea lurida (size: 4.2×3.2 centimeters). Salinity of water, 33.64 parts per thousand*

[October 18, 1929, Pacific Grove, Calif.]

Time	Temperature	Concentration parts per thousand	pH	Rate of flow, cubic centimeters per hour			Remarks
				Average	Maximum	Minimum	
P-13	° C.						
11.40 a. m.	16.0	0	8.0	398	428	367	
12.08 p. m.	16.2	.5	8.0	398	408	367	
12.35 p. m.	16.8	1.0	7.9	402	410	395	
12.58 p. m.	16.8	2.0	8.0	367	424	318	
1.20 p. m.	17.0	3.0	7.9	260	301	239	
1.42 p. m.	17.6	4.0	8.0	260	327	230	
2.07 p. m.	17.8	5.0	7.9	184	239	135	
2.46 p. m.	18.0	6.0	8.0	138	230	121	
3.30 p. m.	18.5	7.0	7.9	92	176	60	Current irregular.
4.00 p. m.	17.8	9.0	7.9	30	----------	----------	Current very slow and irregular.
4.00 p. m.	(1)	(1)	(1)	(1)	(1)	(1)	
Oct. 19, 10.25 a. m.	17.8	0	8.0	337	347	327	

[1] Oyster placed in running sea water.

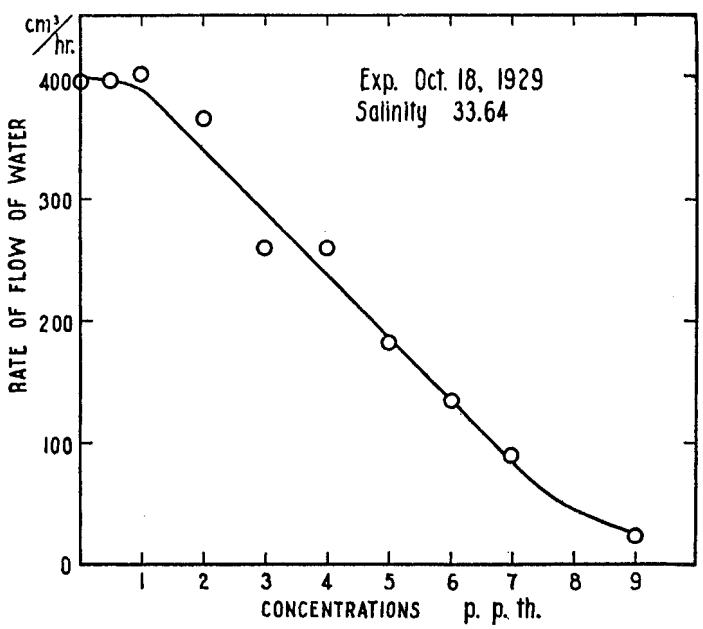

FIGURE 40.—Effect of neutral sulphite liquor on the rate of feeding of *Ostrea lurida*. Temperature, 16.0-17.8° C.; pH, 7.9-8.0

The rate of feeding of the Japanese oyster (Table 7, fig. 41) is also affected by the addition of red liquor, the decrease being noticeable at the concentration of two parts per thousand. The rate of flow continues to fall with the increase in amount of red liquor added. There is, however, a distinct difference between the two species of oysters: The Japanese oyster can sustain higher concentrations much better than can the small Olympia oyster. At the concentration of 15 parts per thousand, the rate of feeding of the Japanese oyster is still about 25 per cent of its normal rate.

TABLE 7.—*Effect of red liquor (specific gravity 1.046) on the rate of feeding of Ostrea gigas (size, 11.3×6.2 centimeters). Salinity, 33.68 parts per thousand*

[October 16, 1929, Pacific Grove, Calif.]

Time	Temperature	Concentration parts per thousand	pH	Rate of flow, cubic centimeters per hour			Remarks
				Average	Maximum	Minimum	
	° C.						
10.37 a. m.	15.8	0	7.9	3,240	3,240	3,220	
11.07 a. m.	16.0	.2	7.9	3,026	3,356	2,430	
11.40 a. m.	16.4	.4	7.9	3,143	3,356	3,130	
12.11 p. m.	16.8	1.0	7.3	3,156	3,356	3,130	
12.35 p. m.	17.0	2.0	7.0	2,223	2,365	1,944	
12.55 p. m.	17.0	3.0	6.8	1,730	2,112	1,470	Current irregular.
2.48 p. m.	17.4	4.0	6.7	2,028	2,365	1,944	Do.
3.07 p. m.	17.4	5.0	6.4	1,251	1,944	972	Do.
3.35 p. m.	17.6	8.0	6.0	771	1,276	583	Do.
3.52 p. m.	17.5	10.0	5.8	1,244	1,386	972	Do.
4.12 p. m.	17.5	15.0	5.7	816	1,076	609	Do.

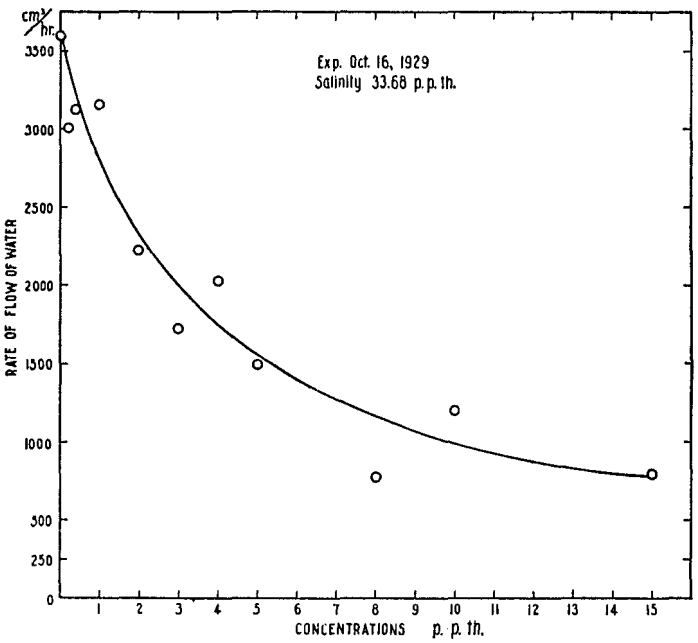

FIGURE 41.—Effect of sulphite liquor on the rate of feeding of *Ostrea gigas*. Temperature, 15.8–17.5° C.; pH, 7.9–5.7

Since red liquor is an acid solution, its effect on the activity of the ciliary epithelium could be attributed to the increase in hydrogen-ion concentration rather than to the presence of certain toxic substances. If the inhibition of ciliary activity were due to the increase in the acidity of the water, it would be a simple matter to counteract it by neutralizing the solution before it is discharged into the water. Unfortunately, this is not the case. In the experiment P13 (Table 6, fig. 40) the red liquor was neutralized by adding small amounts of NaOH until its pH was 7.1; various amounts of neutral liquor were added to the sea water, the pH of which was nearly constant (7.9 to 8.0), then the rate of flow of the water was measured. As can be seen from Table 6, neutral red liquor has a pronounced inhibitive effect on the rate of flow of water through the gills.

The conclusion seems inevitable that the liquor discharged by the pulp mill contains substances that affect the normal activity of the gill epithelium and consequently reduce the rate of feeding of oysters. It must be borne in mind that in the experiments just described, the oysters were kept in the solution of red liquor only for a brief period of time. It is quite possible that continuous exposure, even in very weak concentrations of this toxic substance, which fails to produce any visible effect immediately, may cause a general weakness of the organism, reducing its rate of metabolism and resulting in its higher mortality.

III. INVESTIGATIONS OF OYSTER MORTALITY IN OAKLAND BAY, WASH.

By H. C. McMillin

INTRODUCTION

The tide lands of lower Puget Sound have produced oysters for many generations. The springs along the shores were favorite camping sites for the Indians, and the shell piles near by bear evidence of the fondness of these people for the Puget Sound oyster. For many years after the coming of the white man the Indians continued to harvest the crop from the natural beds, but the industry passed to the white man through a system of land ownership and sale of rights.

Oysters were originally found in tide pools of the intertidal zone and on some of the low ground. In a few cases shallow channels also supported an abundant crop. At the beginning of intensive culture, the tide pools were enlarged or conditions were made more favorable for the oysters by removing the mussels and barnacles.

A new era in oyster culture in Puget Sound dates back to 1890, when the late J. Y. Waldrip leveled an area of ground, and constructed around it a dike of hand-split cedar boards. The buried ends of the pieces may still be found on Oyster Bay, where the original dike contributed to the knowledge of oyster culture. Within a short time the success of the new method was apparent. Dikes made of lumber, usually an inch thick, held in place by short stakes, appeared in almost every bay in the southern end of Puget Sound. Not all of the bottoms upon which dikes were built proved suitable for oyster culture, but successful methods of intensive culture were worked out in a short time for a large part of the intertidal zone.

Through several legislative acts the State has granted title to the oyster growers, and they now hold the land in fee simple. This has encouraged extensive improvement, and oyster land in Puget Sound is the most valuable of any oyster bottom in the nation, some being worth about $15,000 per acre.

Conditions in Oakland Bay are favorable to oyster culture. Tidal ranges of 8 to 18 feet cause strong currents; solid bottoms make easy the construction of permanent dikes; and gravel along the beach furnishes surfacing material. The making of new ground has continued slowly as the oystermen have spent their profits on leveling, diking, and surfacing. At present about two-thirds of the available ground is in shape to produce oysters. Each year the area is increased by new improvements. The abandoned dikes about the head of Oakland Bay and in Swindel Cove, do not represent a reduction of the industry, but are the results of early experimentation already discussed, and much of this ground will eventually be improved and cultured by modern methods. (Fig. 42.)

For many years Oakland Bay was an unfailing producer of seed oysters. A high production of adult oysters was maintained in the bay, and much seed was taken to other bays. A part of the State reserve has been improved with permanent dikes, and these beds have contributed a large sum annually to the oyster fund through the sale of seed. Privately owned areas adjacent to the "Narrows" obtained a good set of seed, which was an important source of income to the owner. There were, in reality, two oyster industries in Oakland Bay: One, the production of seed, which was carried on largely by the State; and the other, the raising of adult oysters, which engaged private enterprise. The adult oyster thrived well and the set was regular, although subject to annual variations. Cold weather during low tides has twice exacted a heavy toll of oysters. In 1916 it appeared that complete destruction had resulted. A few oysters, however, on the lower beds and in the channels survived, and the following summer a good crop of seed was obtained. This fact shows that a few adult oysters can, under favorable conditions, produce a good set of seed oysters.

Within the last three years conditions have changed radically. At the request of the oystermen a preliminary survey of the beds was made in May, 1929, which brought to light a few very definite facts. Many of the oysters were dying, and a majority on the lower beds were already dead. In the channels and on the undiked ground careful search revealed the presence of a few medium-sized oysters which were in the early stages of decomposition. Clams were working out of the ground, and they were in such a weakened conditions that one could pull the shell open with the fingers to examine the watery decomposing body within, which still showed signs of life. In the dikes of medium height a few large oysters were alive, but they showed no signs of the recent growth which would be expected. When these oysters were shucked they soon lost water from the body, and there remained a thin flabby piece of meat which had a decidedly bitter taste. On the higher beds the condition of the oysters was the same, but only a few had died recently.

This condition was not what one would expect from freezing, because, in that case, the higher and more exposed oysters would have suffered the most. In the present case, oysters on high, exposed ground showed no abnormal death rate.

No seed was obtained in 1927 and 1928, and judging from the condition of the oysters in May, 1929, it appeared doubtful, due to their emaciated condition, that any would develop reproductive material in that year. The oysters, apparently, did not remain open and feeding, as did those in adjacent bays. As soon as gray larvæ could be found in the mantle cavity of oysters in Totten Inlet, samples of water taken from Oakland Bay were introduced into normal sea water in which larvæ were living to determine if any reaction would follow. A few experiments, which will be discussed later, showed that larvæ were noticeably affected by Oakland Bay water.

SURVEY OF THE BEDS

An effort was made to determine the exact condition of the beds at the time of the investigation. (Figs. 44 and 45.) The area inside of each dike was examined and samples taken. A frame, inclosing a square yard, was placed on the bed, and all shells and oysters in the inclosure were taken up in a box for later examination. The samples were taken at random if the bed appeared to have an even distribution of oysters over its entire area. On low ground, where the bottom was uneven, or on beds then being worked, an effort was made to get adequate samples. The number ranged from 1 to 3 square yards in each dike. A number of records w re checked

FIGURE 42.—Taking up seed oysters on State reserve beds in Oakland Bay

by retaking, and no significant variation was found. A series of samples was taken at one time, depending upon the location and upon the height of the tide. The height of the surface of the bed above the lowest oyster-bearing level was measured and three figures were obtained from each sample: The volume of shells, the number of live oysters, and the number of dead oysters.

The first two determintions were easily made, but the number of dead oysters was more difficult to ascertain. There is no way of knowing how long any one oyster has been on the bed, but within a short time after death, the two valves, or halves, of an oyster shell break apart. We therefore, counted the number of specimens of

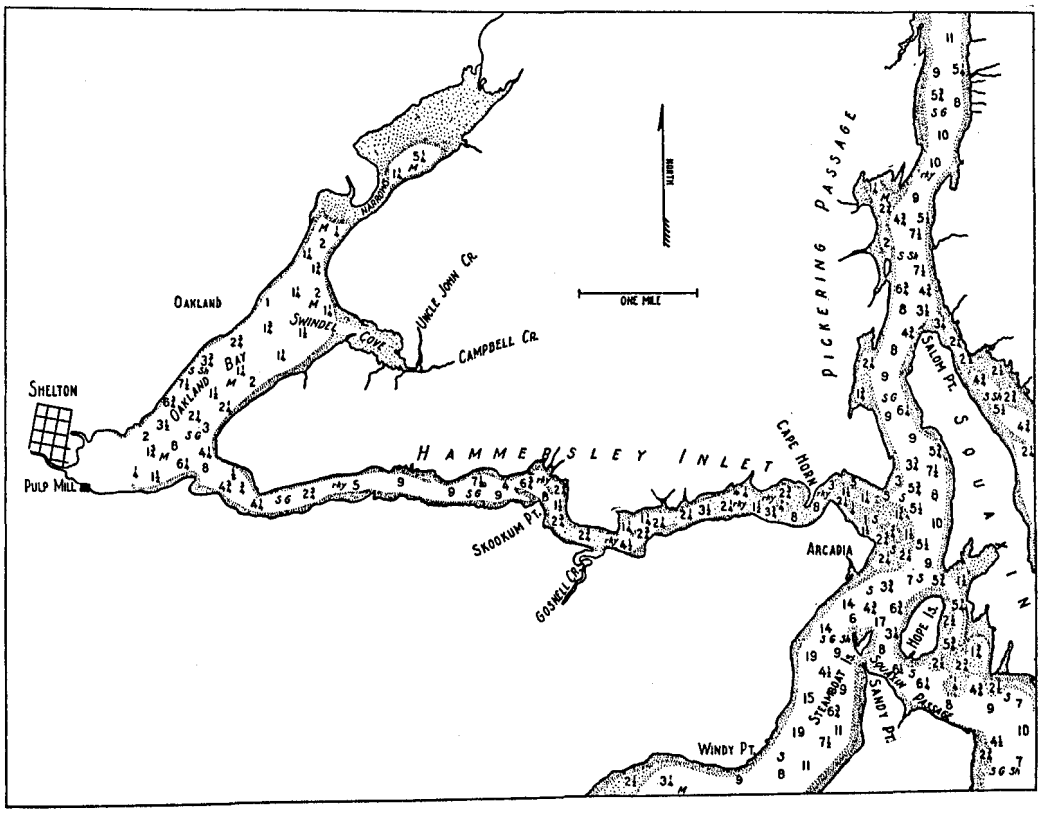

FIGURE 43.—General map of Oakland Bay, Hammersley Inlet, and Pickering Passage

which the two valves remained attached, and considered them as respresentative of the recently dead oysters. Doubtless, a number of valves were separated in handling, but this method appeared to be the only one available and by exercising due care a fair idea of the condition of the beds was obtained. The ratio between the number of dead and live oysters does not give as complete a picture of the loss that has occurred over the oyster beds as one would desire, nor is it equally reliable for all areas from which samples were taken. Where the current is swift, empty shells are carried away quite rapidly, making the apparent percentage of dead oysters very much lower than actually is the case. However, the results show to a satisfactory degree the general conditions as they existed at the time of the survey. (Fig. 46.)

Table 8 indicates that, in most cases, the mortality was proportional to the height of the bed. On all low ground the oysters have experienced a heavy mortality, while the higher beds show no abnormal death rate, except where the currents are slow. Beds, Nos. 86 to 93, in Swindel Cove are relatively high; but suffered a heavy death rate. These beds are behind the large gravel bar which partially closes the mouth of the cove, and are covered by a slowly moving eddy when the tide is high. On the northwest side of the creek which crosses the oyster beds in Oakland

FIGURE 44.—Outline of oyster beds at head of Oakland Bay with number and height in inches corresponding with Table 1. Bed No. 40 is lowest diked area, surface of which was chosen as zero level

Bay, the private and State reserve beds have suffered a greater loss than those of the same level elsewhere. Here again the current is sluggish and the casualty high.

Records taken in Oyster Bay (Totton Inlet) in the same manner as the census of Oakland Bay are also shown in Table 10. In material that had been culled recently less than 18 per cent was found to be dead. One year after being worked, between 0.8 per cent and 12.5 per cent of the oysters may normally be found to have died as a result of handling, the percentage varying according to the number of seed oysters broken. In all cases where the loss is greater than 12.5 per cent the oysters have been recently handled and a large number of the small ones broken in process of removing the marketable adults.

TABLE 8.—*Census of oyster beds in Oakland Bay*

Dike number	Volume of shells	Area	Live oysters	Dead oysters	Dead	Height [1]	Dike number	Volume of shells	Area	Live oysters	Dead oysters	Dead	Height [1]
	Bushels	*Sq. yds.*			*Per cent*	*Inches*		*Bushels*	*Sq. yds.*			*Per cent*	*Inches*
1	0.43	3	131	56	30	58	49	.95	1	203	97	32	0
2	.19	2	32	52	62	54	50	.48	2	87	106	55	0
3	.28	2	60	5	8	62	51	.16	4	1	3	75	0
4	.53	2	314	35	1	58	52	.20	1	53	81	60	0
5	.38	2	85	13	15	54	53	.61	1	362	267	43	4
6	.36	2	132	40	28	49	54	.54	1	119	156	57	17
7	.65	1	210	44	17	35	55						21
8	1.21	2	555	89	14	40	56	.38	1	172	142	45	21
9	.65	1	155	122	44	48	57	.40	1	85	88	51	6
10	.40	1	309	26	8	47	58	.50	2	83	239	65	8
11	.76	2	439	54	11	63	59	.64	1	162	76	32	13
12	.74	2	64	32	33	30	60	.29	1	66	166	72	29
13	.65	1	219	69	25	35	61	.47	1	192	74	28	40
14	.83	2	506	73	13	40	62	.61	1	460	95	17	34
15	.74	1	352	58	14	43	63	.28	1	203	176	46	30
16	.77	1	248	21	8	54	64	.24	1	116	104	47	6
17	.68	1	318	236	43	43	65	.38	1	95	96	51	14
18	.35	1	216	60	22	40	66	.57	1	153	146	49	9
19	.40	1	22	164	88	35	67	.53	1	61	122	67	18
20						22	68	.63	1	48	64	57	25
21	.67	2	52	272	84	28	69	.52	1	55	65	54	18
22						17	70	.29	1	10	40	80	22
23	.40	1	18	88	83	12	71	.52	1	81	81	50	12
24	.57	2	34	42	55	40	72	.24	1	35	28	44	22
25	1.04	3	69	132	66	35	73	.18	1	22	87	80	36
26	.83	2	114	288	72	29	74	.54	1	42	78	65	22
27						25	75	.59	1	82	133	62	42
28						15	76	.14	1	17	71	81	28
29						14	77	.24	1	48	66	58	24
30	.28	4	45	120	74	21	78	.24	1	35	108	75	32
31						9	79	.82	1	141	459	76	33
32	.38	2	0	80	100	0	80	.67	1	428	101	19	34
33	.39	1	218	292	57	29	81	.12	2	8	92	92	34
34	.08	2	7	38	85	14	82	.64	1	102	183	64	13
35						2	83	.35	1	195	87	31	12
36	.20	1	91	86	49	1	84	.38	1	81	176	68	20
37	.47	1	42	31	42	36	85	.42	1	166	145	47	35
38						29	86	.05	3	5	28	85	29
39	.76	2	134	254	65	20	87	.35	1	18	99	85	25
40	.16	1	18	19	52	0	88	.06	1	80	58	42	31
41	.73	2	279	289	51	42	89	.13	2	22	127	85	32
42	.81	1	207	189	48	31	90	.13	1	9	56	86	31
43	.52	1	196	181	48	25	91	.30	1	25	104	81	28
44						37	92	.14	1	2	67	97	32
45	.70	1	171	386	69	20	93	.24	1	4	58	94	33
46						0	94	.30	1	168	143	46	0
47	.95	1	99	127	56	4	95	.19	2	15	16	51	0
48	.68	2	232	660	74	18							

[1] Above lowest oyster bearing level.

In order to obtain a mathematical expression for the relation of the height of the beds to the number of adult oysters and percentage of mortality in Oakland Bay, the coefficient of correlation was calculated. Samples were taken on a line extending from high water to low water directly across the beds. (Table 9.) The results of three such series were combined, making a total of 21 stations. Standard methods of computations gave a correlation coefficient ("r") between height and per cent of dead oysters of -0.6996 ± 0.075, showing that the loss is very closely associated with the height of the beds, the largest number of dead oysters being on the low ground. Between height of bed and number of living oysters the coefficient of correlation was 0.5866 ± 0.096, showing that the number of live oysters increased directly with the height of the bed. The values thus obtained are relatively high for biological data. They are respectively 9 and 6 times their probable error, which shows that the correlation is significant. Similar treatment of figures obtained from beds in Totten Inlet (Oyster Bay) showed no correlation between the number of dead oysters and position of beds from which the sample was taken ($r = -0.3316 \pm 0.226$) but a high positive correlation between the number of live oysters and position ($r = 0.8166 \pm$

0.005). (Table 10.) The actual number of living oysters on the high beds were similar but the lower beds of Oyster Bay were more heavily populated. A total of 84

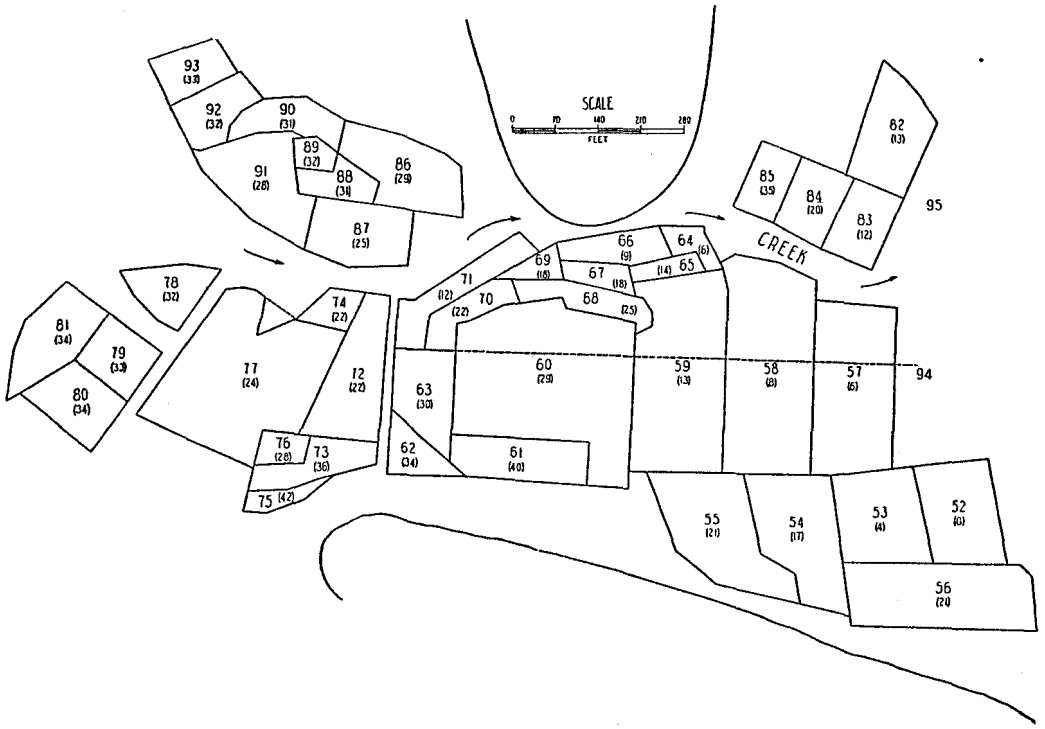

FIGURE 45.—Outline of oyster beds in Swindel Cove. Number of bed with height in inches in parenthesis corresponding with Table 1. Bed No. 52 is lowest diked area, surface of which was chosen as zero level. Beds Nos. 52 and 40 (fig. 44) are at the same level

samples taken in Oakland Bay showed an average volume of 0.538 bushels per square yard, while in Totten Inlet (Oyster Bay) 10 samples gave an average of 0.423 bushels.

TABLE 9.—*Correlation table of 21 station in Oakland Bay showing relations between height of bed and per cent of dead oysters and between height of bed and number of live oysters*

Dike No.	Height [1]	Dead	Live oysters	Dike No.	Height [1]	Dead	Live oysters
	Inches	*Per cent*			*Inches*	*Per cent*	
40	0	52	52	7	35	17	210
94	0	46	168	13	35	25	219
36	1	49	91	37	36	42	31
57	6	51	85	14	40	13	253
58	8	65	119	8	40	14	278
59	13	32	162	15	43	14	352
34	14	85	7	10	47	8	309
39	20	65	65	16	54	8	248
33	29	57	218	11	63	11	439
60	29	72	66				
63	30	46	203	"r" on height		−0. 6996±0. 075	+0. 5866±0. 096
62	34	17	460				

[1] Above lowest oyster-bearing level.

TABLE 10.—*Census of oyster beds in Totten Inlet (Oyster Bay)*

Dike No.	Volume of shells	Area	Live oysters	Dead oysters	Dead	Height[1]	Dike No.	Volume of shells	Area	Live oysters	Dead oysters	Dead	Height[1]
	Bushel	*Sq. yd.*			*Per cent*	*Inches*		*Bushel*	*Sq. yd.*			*Per cent*	*Inches*
O1	0.68	1	367	3	0.8	63	O3	.23	1	280	40	12.5	10
O5	.35	1	405	23	5.4	51	B1	.47	1	150	6	5.1	10
B5	.69	1	410	29	6.6	41	B6	.35	1	132	8	5.7	0
B4	.57	1	292	31	9.6	30							
B3[1]	.43	1	140	25	17.8	22	"r" on height			0.8166±0.085		−0.3316±0.226	
O4[1]	.18	1	232	48	17.1	18							
B2[1]	.28	1	164	32	16.3	15							

[1] Above lowest oyster bearing level.

The total population of adult oysters in a few areas in Oakland Bay was greater than on any bed in the other bays. In Swindel Cove two beds averaged more than

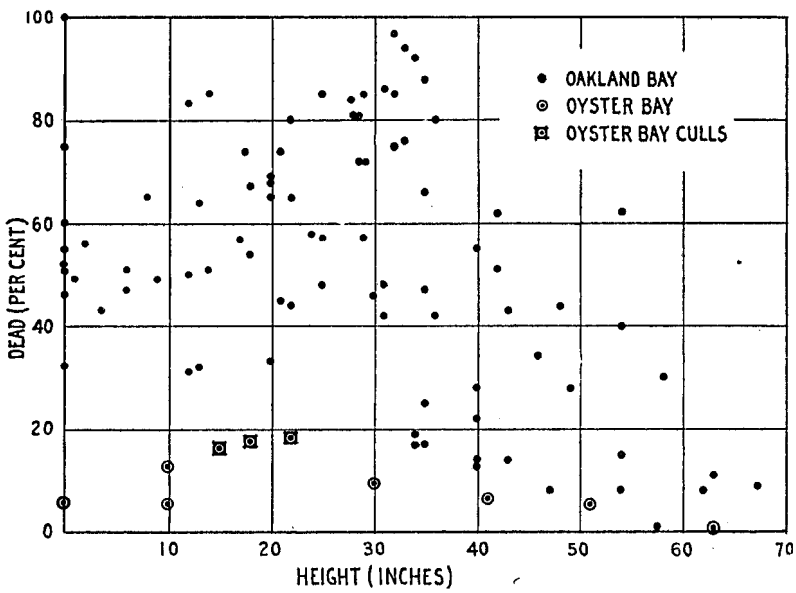

FIGURE 46.—The height of the oyster beds in Oakland Bay, Swindel Cove, and Oyster Bay in relation to the proportion of dead oysters

400 adult oysters per square yard, and numerous small areas outside of the dikes were as densely populated.

Further comparisons might be made with improved beds in other bays, but the conditions which now exist in Oakland Bay are peculiar to that locality, and unique in the history of oyster culture of Puget Sound. No similar set of conditions has ever been known in the vicinity. Various reports have been investigated regarding abnormal losses of oysters in a limited locality, but investigation showed they were in no way parallel cases with the one now under consideration. Where abnormal death rate has occurred in the past, it has invariably been when the oysters were outwardly in good condition. In a small section of Skookum Inlet the oysters died very rapidly for a short time. At the beginning of the time of high death rate, the oysters were in excellent condition and the growers were receiving a special bonus above the regular price for their catch. In no other case has the oyster appeared to remain closed, become thin, and ceased to grow before dying. Nor has any condition been so prolonged as that existing now in Oakland Bay.

ECOLOGICAL OBSERVATIONS

SPAWNING

No set of oysters has been obtained in Oakland Bay for three years, and at present, the live oysters are in an abnormal physical condition. During the summer of 1929 reproductive products were formed only in a very few individuals. Three specimens containing eggs in the segmentation stage were found, and although a great many oysters were examined during the course of the summer, none was found which contained shelled larvæ (commonly called "black spawn") in the mantle cavity.

Failure of set has been experienced in other bays. Eld Inlet (Mud Bay), prior to 1928, was considered to be poor setting ground. The set in Hood Canal and North Bay was also very light for several years. However, in each case outside of Oakland Bay, the oysters developed sexual products, and spawned normally. Either natural water conditions made the mortality of larvæ high, or proper cultch was lacking. At any rate, in all cases a few seed were found each year.

In May, 1929, over 200 bushels of adult oysters were moved from North Bay to Oakland Bay. They were in good condition and spawned; the larvæ were carried the proper time, and liberated apparently in good condition. So far as is known, these were the only oyster larvæ in Oakland Bay during the season, and not a single seed oyster has been found on shells on the beds. Special spat collectors developed by the Bureau of Fisheries were placed on various parts of the bed and caught 25 seed oysters on 2½ bushels of shells, an average of 1 spat to 1,000 shells.

The failure of the set was probably due to two or more related factors. The adult oysters failed to develop sexual products, which precluded any chance of spawning, and the conditions which affected the adult oysters probably prevented normal development of the larvæ from introduced oysters.

Water samples, taken in April from Oakland Bay and held in tightly stoppered bottles, were examined to determine their affect on oyster larvæ. Dilutions of one-sixth and one-tenth were tested with normal sea water for control. Samples of water taken near the southern end of Oakland Bay caused an instantaneous reaction in which all larvæ closed their shells and sank to the bottom. A sample taken over the oyster beds produced a less sharp reaction. A few larvæ were swimming about after one day in the weaker solution, but later observations showed no movement. In normal sea water the larvæ continued to live and swam about the dish continually. The water was changed at bi-weekly intervals, and no dead larvæ were noted for nearly three weeks.

It is probable, therefore, that water conditions in Oakland Bay were unfavorable to the larval oyster and resulted in the death of nearly every one.

GROWTH OF BARNACLES

Not only the oysters in Oakland Bay have been affected, but other organisms as well. Some forms have, apparently, been stimulated; others suppressed. To illustrate this point we might refer to the floating equipment used about the oyster beds. It is a universal practice to scrape and repaint floating equipment at regular intervals, for, under normal conditions those parts of objects which are immersed in sea water are quickly covered with plant or animal growth. Boats and scows used on the oyster beds have always been cleaned and repainted at least once a year, but in the last three years no growths have appeared, and the work of reconditioning has

been found unnecessary. Since these pieces of apparatus do not leave the oyster beds, these facts indicate that abnormal conditions exist in the water over the oyster beds.

Barnacles, mussels, and hydroids normally found on floating euqipment used about the oyster beds are not noticeably affected by normal fluctuations in the physical factors in the environment. They are found where the salinity is higher or lower than that ever found in Oakland Bay. They occur to the north and to the south and thrive in water which is both warmer and colder than near the oyster beds. We can assume, therefore, that fluctuations in temperature and salinity do not account for their absence from the floating equipment referred to; domestic sewage does not appear to affect these organisms seriously, and one looks upon the annual crop as a matter of course. Their absence, therefore, from the oyster beds indicates that abnormal factors, other than temperature, salinity, or sewage, must be considered in relation to the condition in Oakland Bay at the present time.

PLANT GROWTH

It is evident that the growth of some plants was stimulated by the conditions existing in Oakland Bay during the winter of 1928–29, and the spring and summer of 1929. The chain diatom *Melosira borreri*, Greville, grow in dense masses over some sections of the oyster beds. Where the current was slow over beds that were between 2 and 4 feet above the lowest oyster dikes, it formed a mat that completely covered the oysters and shells. This diatom is present at all times in small amounts on oyster beds of the same level in near-by bays, but it is evident that the growth was greatly stimulated in this particular place.

The distribution of this plant growth apparently bore no relation to the mortality of the oysters. Many beds were free from it, and had a high death rate of oysters. The lower grounds, which suffered the most in the recent disturbance, were not affected by *Melosira* at all. Some beds that were covered with a heavy mat experienced almost a total loss of oysters, while others showed nothing abnormal.

It is concluded, therefore, that *Melosira* does not injure the oysters, but, that its dense growth indicates a disturbed condition of the water in which it normally grows in small amounts.

POSSIBLE EFFECTS OF LOG STORAGE

The effect of logging operations and sawmill waste upon oysters is a much debated question. In Oakland Bay sawdust is absent, and can not be considered as affecting the oysters. There are, however, at all times, many log booms in the bay, and they have been blamed for oyster mortality. Certain chemicals in the logs are supposed to pass into solution and to affect the oysters. In Eld Inlet the log storage is proportionally greater than in Oakland Bay; new logs are constantly being added and any chemicals that leach out of the timber would be in high concentration in this place. However, the water is normal in color and the oysters which live in the bay prove that no harmful chemicals are present. In coves and lagoons where the bottom is covered with decaying vegetation and many tree trunks are strewn about, one would expect the wood chemicals in solution to be at a maximum. Such places are known to produce a natural set of oysters which grow and mature normally. Nothing about their condition suggests any effect of wood chemicals.

The oyster larva will set upon pieces of hemlock bark recently stripped from a tree. In this case the oyster comes in direct contact with the phloem tissue which

carries in a soluble state the tannins, resins, and other chemicals which might leach out into the water. If injurious chemicals pass into solution from logs or bark, one would expect to find evidence of a negative reaction of oyster larvæ toward bark.

Since no direct evidence has been found to show that logs or logging operations injure oysters, and observations indicate that no injury is probable, it can be safely assumed that the serious condition now existing in Oakland Bay is in no way related to the presence of log booms in the adjacent waters.

POSSIBLE EFFECTS OF DOMESTIC SEWAGE

Within the last few years Shelton has had a fourfold increase in population. Not all of the new residence areas have been connected with the sewer system emptying into Oakland Bay, and the disposal of sewage has not increased proportionally with the population. Dr. W. M. Beach, health officer of Shelton, states that domestic sewage disposal has increased less than 20 per cent in the last four years. The oyster beds are at the opposite end of the bay from the sewer outlet, and the State sanitary engineer was unable to find evidence of domestic sewage pollution over the oyster beds.

On the mud flats near the city of Olympia oysters thrive in water that is heavily polluted with domestic sewage. Also in Liberty Bay and Sinclair Inlet, they live in contaminated water. Legal restrictions are necessary to prevent the marketing of oysters from such beds, but reproduction and growth are apparently normal. Therefore, sewage is evidently not a factor for consideration in the present problem. At any rate, it is improbable that the small increase has a bearing on the recently developed condition of the oysters.

SULPHITE WASTE LIQUOR POLLUTION

As a result of the survey of conditions in Oakland Bay some definite conclusions are forced upon us: (1) The oysters are dying at an alarming rate; no set has been obtained in three years, and consequently, the oyster industry is at a standstill. (2) The growth of at least one marine plant is greatly stimulated, and that of some animals is prevented. (3) Barnacles, clams, mussels, and hydroids which tolerate a wide range of physical conditions have apparently not reproduced in Oakland Bay in the last three years. (4) There is no indication that this condition has been caused by abnormal temperature, or by a varying salt content of water. (5) Log storage, sawdust, or domestic sewage probably could not have caused this great, sudden, and unique change in the fauna and flora of the vicinity. (6) There remains one factor to examine; that of the sulphite waste liquor added to the water of Oakland Bay by the Rainier Pulp and Paper Co.

During the construction of this mill an examination of Oakland Bay waters was made, and the *unpublished* report of H. W. Nightingale, State sanitary engineer, of January 21, 1927, states:

> From this preliminary investigation it is concluded that the mill is so located with respect to the shell-fish growing areas that the discharge of its waste will create a potential danger. From the standpoint of the chemical determinations on the sea water in Oakland Bay and Hammersley Inlet the conditions now appear to be normal for the support of the marine life.

As a result of investigations it was recommended that a portion of the waste liquor be taken out of Oakland Bay either by scow or by pipe line, and a pipe line was constructed before the mill began operations. Some of the waste liquor was pumped out of the bay, and some was dumped into the bay at the mill.

There is no way of determining the volume of liquor discharged at the mill or pumped to the discharge tanks. The following quotations are taken from the unpublished daily reports of State Fishery Inspectors C. C. Rice and E. Hart:

> On March 14, 1929, we visited the discharge tanks at the end of the pipe line and found them empty, and no evidence of them having been used recently. On March 15, 1929, we visited the discharge tanks at the end of the pipe line and found them about three-fourths full of red liquor (175,000 gals.), which had been pumped into the discharge tanks during the night before, and which was released into Hammersley Inlet at high tide (8.34 a. m.). We visited the discharge tanks again at 7.00 p. m., and found them empty, nothing having been pumped into the tanks during the day.

On the morning of March 16 the same inspectors found the tanks one-third full of red liquor (70,000 gallons), and the same amount at 6.40 p. m. the same day. No liquor was pumped on March 17, and only a little (not over 20,000 gallons) on March 18. Thus we see that, according to the reports of State inspectors, in a period of 5 days, during which the plant was under constant observation and produced about 1,400,000 gallons of waste liquor, less than 20 per cent of the total was pumped out of Oakland Bay; and during 2 days out of the 5 very little, if any, was disposed of through the pipe line.

Conditions on the oyster beds changed rapidly after the mill started operations. Heretofore, the water was clear, and the oystermen were able to spread shells without the aid of marking stakes. Soon it was impossible to see bottom except in very shallow water, and the normal green color of the water was replaced by the coffee-brown shade characteristic of dilute sulphite waste liquor. During the summer and autumn of 1929 a large proportion of the liquor was disposed of through the pipe line, and the oysters resumed growth, and a very few seed were obtained. During these observations (December 9, 1929) a sudden appearance of deeply discolored water at the oyster beds led to inquiry at the pulp mill concerning discharges of concentrated liquor into the bay at the plant. The foreman stated that about 6,000 to 7,000 gallons of concentrated liquor had been released through the sewer about 36 hours before the appearance of the brown coloration on the oyster beds.

Since the pulp mill was located at one end of Oakland Bay and the oyster beds at the other, with the outlet on one side of the bay between the two, a study of the tidal currents was necessary to determine whether the liquor discharged at the mill might reach the oyster beds. (Fig. 48.) The currents in the bay were traced by means of an Eckman current meter which registers the direction and velocity of the current at any depth to which it is lowered.

It was learned that the flood tide causes a rapid flow of water into Oakland Bay from Hammersley Inlet, and that the reverse movement takes place at nearly the same velocity, that is about 2.2 feet per second on the surface. The main stream of the current passes across the southern end of Oakland Bay, and along the northwest shore toward and through the "Narrows" where the oyster beds are located. The out-going tide is at the first a general surface movement toward the head of Hammersley Inlet, but gradually the flow of water from the head of the bay causes a strong current down the center of the bay past the mouth of Hammersley Inlet, and then back along the south shore before it leaves Oakland Bay. (Fig. 47.) These currents are easily observed by the movement of drift and are well known to the boatmen of the vicinity.

The bottom currents differ in direction and velocity from the surface currents. At the entrance to Oakland Bay the water is about 50 feet deep at low water. A deep channel crosses the lower end of the bay, follows the northeast shore, and runs

out about halfway up the bay. As the water enters Oakland Bay the current is confined to the surface by flowing over a shallow stretch of bottom in the upper end of Hammersley Inlet. (Table 12.) After the surface water has attained a velocity of about 2 feet per second, the lower strata slowly begins to move, but the velocity never exceeds 0.6 foot per second at the entrance of Oakland Bay. Farther along

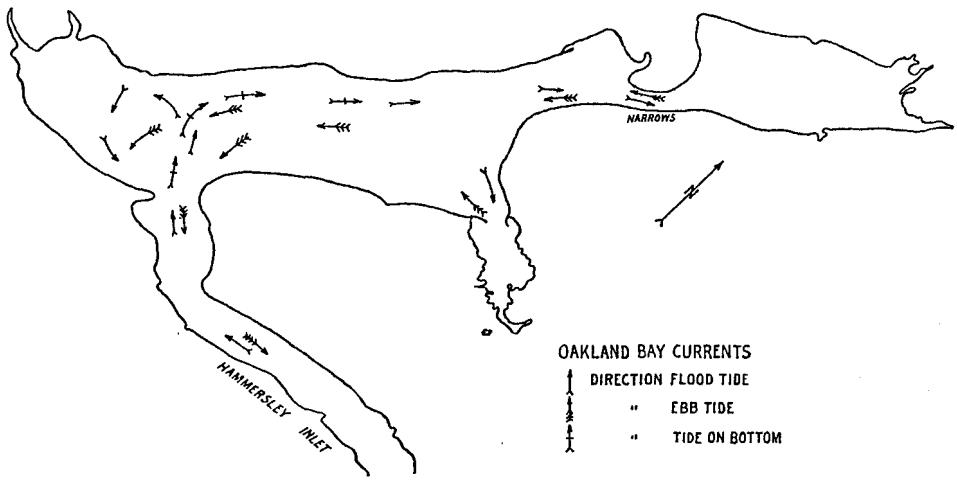

FIGURE 47.—Direction of currents in Oakland Bay

the channel the current of moving water becomes deeper and in front of the Union Oil Co. in over 35 feet of water the flood tide current has an equal velocity from surface to bottom. The flood current continues much longer on the bottom than on the top, and there is little or no reverse in the direction of the current with the ebb tide. (Fig. 49, Table 11.) In other words, the currents caused by the ebb tide

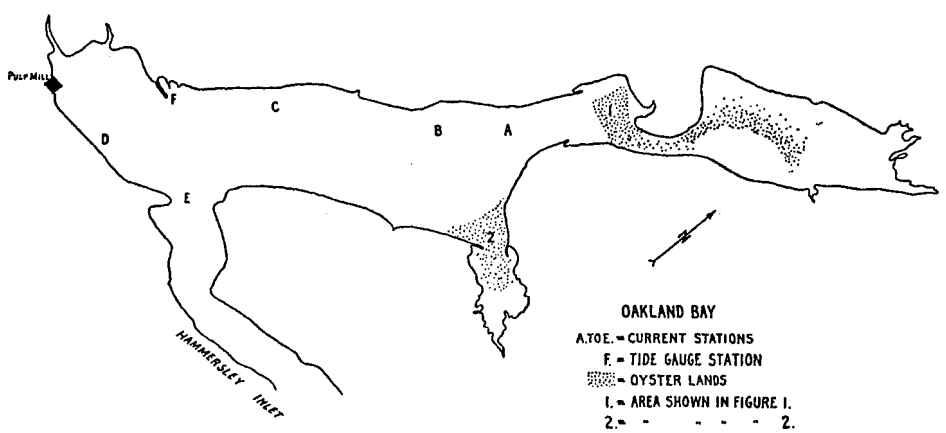

FIGURE 48.—Principal stations for current observations and tide recording station in Oakland Bay

are more closely confined to the surface than flood tide currents. The surface current of the flood tide crosses the bay on a diagonal line near the head, and continues through the "Narrows." The bottom current reaches the end of the channel and spreads out on the bottom, but continues directly up the bay to the oyster beds at the head of Oakland Bay.

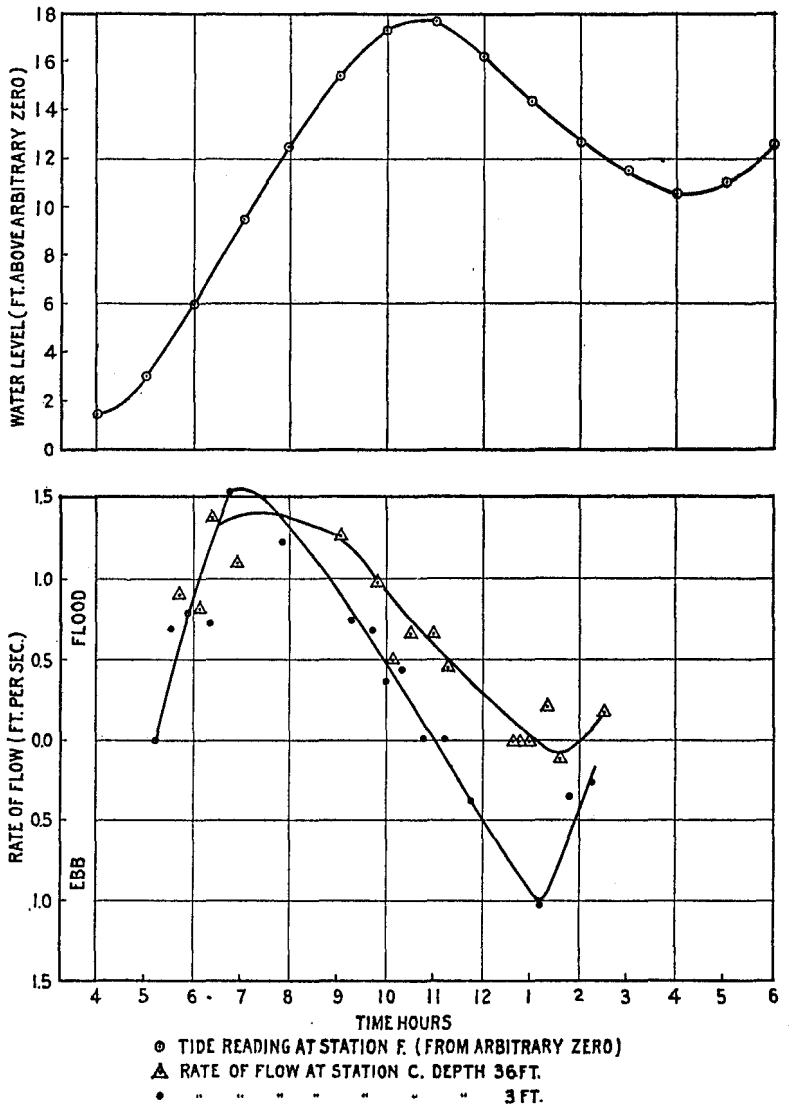

FIGURE 49.—Current velocities at station C and height of tide at station F. November 21, 1929

TABLE 11.—*Currents at station "C," Oakland Bay, November 21, 1929*

Time	36 feet	Direction	Time	36 feet	Direction	Height of tide[1]
	Feet per second			*Feet per second*		*Feet*
5.15 a. m.	(2)	Flood.	5.30 a. m.	0.69	Flood	4.2
5.40 a. m.	0.90	Do.	5.55 a. m.	.79	do	5.3
6.05 a. m.	.81	Do.	6.20 a. m.	.72	do	7.0
6.25 a. m.	1.39	Do.	6.45 a. m.	1.54	do	8.5
6.55 a. m.	1.10	Do.	7.55 a. m.	1.23	do	12.3
9.00 a. m.	1.25	Do.	9.15 a. m.	.74	do	16.0
9.25 a. m.	1.07	Do.	9.40 a. m.	.68	do	16.8
9.50 a. m.	.98	Do.	10.00 a. m.	.36	do	17.3
10.05 a. m.	.50	Do.	10.20 a. m.	.43	do	17.6
10.30 a. m.	.66	Do.	10.45 a. m.	(2)	do	17.8
10.55 a. m.	.66	Do.	11.07 a. m.	(2)	do	17.5
11.15 a. m.	.44	Do.	11.45 a. m.	.39	Ebb	16.8
11.35 a. m.	.39	Do.	12.17 p. m.	.65	do	15.7
11.55 a. m.	(2)	Do.	1.10 p. m.	1.04	do	14.0
12.35 p. m.	0	Do.	1.50 p. m.	.35	do	12.9
12.45 p. m.	0	Do.	2.15 p. m.	.27	do	12.3
12.53 p. m.	0	Do.				12.0
1.20 p. m.	.20	Do.				
1.35 p. m.	.12	Ebb.				
2.00 p. m.	0	Flood.				
2.30 p. m.	.18	Do.				

[1] Above arbitrary zero.

[2] Too slow to measure rate.

TABLE 12.—*Current velocities December 1, 1929, at station E* [1]

[Total depth 48 feet]

Time	Height of tide [2]	36 feet	Surface	12 feet	Direction	Time	Height of tide [2]	36 feet	Surface	12 feet	Direction
	Feet						*Feet*				
10.45 a. m.	13.1		0.59		Ebb.	3.15 p. m.	13.7			0.36	Flood.
11.00 a. m.	12.7	[3] 0.05			Do.	3.30 p. m.	14.1			.32	Do.
11.15 a. m.	12.3	.25			Do.	3.45 p. m.	14.6		2.23		Do.
11.30 a. m.	11.9	[3].05			Do.	4.00 p. m.	15.0		1.89		Do.
11.45 a. m.	11.6	.05			[4]	4.15 p. m.	15.5		1.00		Do.
12.00 m.	11.3	[3].03			[4]	4.30 p. m.	15.9		1.67		Do.
12.15 p. m.	11.1	.28			Ebb.	4.45 p. m.	16.2		1.47		Do.
12.30 p. m.	10.9	[3].13			Do.	5.00 p. m.	16.4		1.98		Do.
12.45 p. m.	10.7	[3].02			Do.	5.15 p. m.	16.5		1.47		Do.
1.00 p. m.	10.6	.15			[4]	5.30 p. m.	16.6		1.83		Do.
1.15 p. m.	10.7	.33			Ebb.	5.45 p. m.	16.6		1.54		Do.
1.30 p. m.	10.8	.25			Do.	6.00 p. m.	16.5		.90		Do.
1.45 p. m.	11.0	.32			Do.	6.20 p. m.	16.2		.68		Ebb.
2.00 p. m.	11.4		.73		Flood.	6.35 p. m.	15.8	0.20			Do.
2.15 p. m.	11.9	[3].02			[4]	6.50 p. m.	15.4		1.01		Do.
2.30 p. m.	12.3	.02			Ebb.	7.00 p. m.	15.0		1.66		Do.
2.45 p. m.	12.8	.23			Do.	7.15 p. m.	14.6		1.66		Do.
2.50 p. m.	12.9	.23			Do.	7.30 p. m.	14.0		1.12		Do.
2.55 p. m.	13.0	.28			Flood.	7.45 p. m.	13.6		1.16		Do.
3.00 p. m.	13.2		2.18		Do.						

[1] Observations by member State fisheries staff.
[2] Above arbitrary zero.
[3] Approximate, below accurate range of current meter.
[4] No direction recorded by instrument.

The concentrated liquor is heavier than sea water (specific gravity 1.05). When it is released, as on December 9, it sinks to the bottom as it cools and probably accumulates in the deep hole near the entrance to Oakland Bay. From there it must pass slowly up the bay with the bottom currents, and reach the upper end of the bay before any great amount of it has had a chance to become mixed with the water which leaves Oakland Bay on the ebb tide. For this reason it was possible to notice changes in the water over the oyster beds in a short time after a small amount (6,000 to 7,000 gallons) had been discharged at the mill.

CONCENTRATION OF LIQUOR IN OAKLAND BAY

Continued addition of waste liquor to the bay would cause a gradual increase in concentration until an equilibrium is reached. The concentration of liquor at equilibrium, or the maximum amount of liquor which would remain in the bay, is proportional to two factors: (1) The amount added daily, and (2) the proportion of water lost daily by tidal action. To illustrate: If 1 acre-foot of liquor be discharged daily at the mill, and one-twentieth of the water in the bay be renewed each 24 hours by tidal action, at equilibrium 20 acre-feet of liquor would be present in the bay. Assuming complete mixing of liquor and water, 1 acre-foot of liquor (1/20 of 20) would be lost each day, and the same amount would be added. Therefore, an approximation of the amount of water lost each day from Oakland Bay will give us an index to the possible concentration of liquor in the bay.

It was therefore necessary to determine the amount of water in Oakland Bay, and the amount lost on each tide. The volume of water in the bay at low tide was calculated from figures given by United States Coast and Geodetic Survey chart and tidal records. The areas of nine parallel cross sections were determined from figures given in Table 13 by reproducing the contour of the bottom of the bay between the shores and calculating the area of water in each plane. (Fig. 50, Table 13.) The volume was determined in a similar way using the areas of the cross sections and the distance from the lower end of the bay as coordinates and calculating the

area under the curve. The results indicated that there were 11,672.6 acre-feet of water in Oakland Bay at low tide. By measuring the number of acres in the bay and calculating the average depth, a less reliable figure was obtained for the volume of the bay. It was 11,427 acre-feet and serves as a check on the first calculation. It was also found that 718 acre-feet of water remained in the deep hole above the

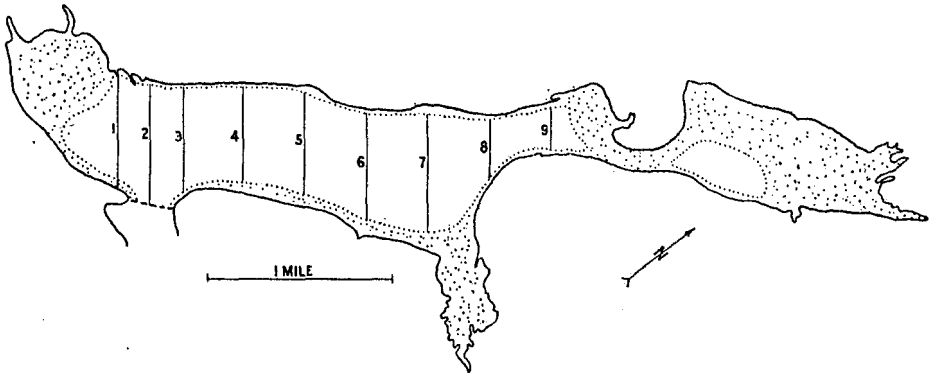

FIGURE 50.—Locations of cross sections used in volume determinations

"Narrows" in what is locally called "the head of the bay." We have therefore an indicated volume of 12,390 acre-feet of water in the bay at low tide. (Table 15.)

TABLE 13.—*Depths and distances of cross sections of Oakland Bay. Locations shown in Figure 50*

[Distance calculated from northwest shore at zero tide level. All measurements calculated in feet]

Section 1		Section 2		Section 3		Section 4		Section 5		Section 6		Section 7		Section 8		Section 9	
Distance	Depth	Distance	Depth	Distance	Depth	Distance	Depth	Distance	Depth	Distance	Depth	Distance	Depth	Distance	Depth	Distance	Depth
1,000	10.3	1,000	18.4	516	30.5	300	39.0	580	18.6	775	10.8	1,650	9.0	1,000	9.9	1,060	4.5
2,000	17.5	2,000	48.0	1,200	24.2	1,800	9.0	2,000	7.2	2,000	8.5						
3,000	25.5	3,000	48.0	2,450	19.3	2,660	10.5	3,000	10.0	3,000	4.5						
3,100	0	3,440	0	3,040	0	2,760	0	3,100	0	3,110	0	3,414	0	1,840	0	1,200	0

TABLE 14.—*Area of cross sections of Oakland Bay and distance calculated from southwest end of bay at zero tide level, calculated from Table 13*

Section No.	Area	Distance	Section No.	Area	Distance
	Square feet	*Feet*		*Square feet*	*Feet*
1	41,775	2,000	6	22,754	10,000
2	111,620	3,000	7	15,363	12,000
3	59,450	4,000	8	9,108	14,000
4	50,760	6,000	9	2,835	16,000
5	32,812	8,000	10	0	17,000

NOTE.—Volume 508,421,100 cubic feet or 11,671.7 acre-feet.

TABLE 15.—*Area of surface and volume of water in Oakland Bay*

Portion	Area at high tide	Volume	
		Low tide	High tide
	Acres	*Acre-feet*	*Acre-feet*
Main bay	935	11,672	21,583
Shelton boom	141	(1)	747
Swindel Cove	91	(1)	485
Tide flats head of bay	381	(1)	2,030
Deep above narrows	80	718	1,566
Total	1,608	12,390	26,411

[1] Bare at low tide.

The average tidal range at the Shelton Dock is 10.6 feet. To calculate the volume of water at high tide, it was assumed that the tide land of the bay would have an average depth, between zero tide level and high tide, of one-half this range, or 5.3 feet. The 613 acres of tide land, therefore, would be covered with 3,259 acre-feet of water. The 1,015 acres not bare at low tide would have an additional volume of 10,759 acre-feet at high tide and a total of 23,149 acre-feet. There is, therefore, an indicated volume of 26,411 acre-feet in the bay at high tide. Since there are, of necessity, irregularities in the bottom which can not be measured in calculations, the volume at high tide is considered to be 26,000 acre-feet and 12,000 acre-feet at low tide. Therefore, on the average, 14,000 acre-feet of water leave Oakland Bay at each tide. All of this water could be contained in Hammersley Inlet and might return with the tide, but such is not the case. On the smaller tides most of the water returns, only a small portion being lost by mixing with the waters in the eddies. (Fig. 43.)

An effort was made to estimate the volume of water which is lost from Oakland Bay by tidal action. Hammersley Inlet receives an average of 14,000 acre-feet of water from Oakland Bay on the ebb tide and returns a similar amount on the flood tide. If the same water which leaves Oakland Bay does not return, it is lost by mixing with Hammersley Inlet water or by entering Pickering Passage where it is carried into Case Inlet and does not return on the ebb tide, the most of which may come through Squaxin Passage. (Fig. 43.) In other words, the tidal action in Hammersley Inlet is an oscillating movement in which water moves from Oakland Bay into Hammersley Inlet and back again. Some consideration must be given to the loss of Oakland Bay water by mixing in Hammersley Inlet. Each cove and stream mouth was observed on two to five occasions, and it was estimated that they retained less than 50 acre-feet of water at high tide which may have been derived from Oakland Bay. Exclusive of this amount, Oakland Bay water which does not leave Hammersley Inlet returns on the ebb tide to the bay. We must also bear in mind that the principal discharge of the sulphite liquor is at the lower end of Hammersley Inlet; hence the water which replaces that lost into Hammersley Inlet is itself polluted.

An attempt was made to determine when ebb tide currents would carry water from Oakland Bay through Hammersley Inlet into Pickering Passage. It was found that on a normal ebb tide preceding low water recorded as −1.3 feet at Seattle, water from Oakland Bay may reach Pickering Passage. By drifting in a skiff down Hammersley Inlet and maintaining a position as nearly as possible in the swiftest current, it was found that the boat did not reach the passage before low water. It is recognized that a certain inaccuracy is introduced by the use of a boat for this purpose. However, the work was carried out on a calm day when air resistance would be at a minimum and the drift was with the prevailing air currents. These errors were more than counterbalanced by keeping the boat in the swift current and out of the eddies. Floating débris drifting at random did not cover an equal distance. There are usually about 50 tides a year which are lower than −1.3 feet (reference station, Seattle); these average −2.5 feet in height. On such a tide it was found that by starting at the entrance of Oakland Bay with the beginning of the ebb and drifting in the strongest part of the surface current, one could arrive at the lower end of Pickering Passage two hours before low water.

At approximately the time when the water from Oakland Bay could have reached Pickering Passage, as estimated in the manner just described, the rate of the flow of

the current at Cape Horn was measured by means of drifting floats. The speed of the current was 1.6 feet per second. Previous to low slack water, not over 5,400 linear feet of current passed the cape; the channel has a cross sectional area of 4,000 square feet; therefore, not over 21,600,000 cubic feet or 496 (approximately 500) acre-feet of water could have been lost. Once again we must bear in mind that the water which replaced that lost into Pickering Passage was necessarily polluted with sulphite waste liquor, due to the discharge of the pulp liquor at the lower end of Hammersley Inlet and its constant presence in the vicinity.

We have, therefore, an indicated loss from Oakland Bay of 50 acre-feet of water per tide, or about 700 acre-feet per week for normal tides, and an additional 500 acre-feet for one extreme tide, a total of 1,200 acre-feet per week. In order to use even numbers we can consider 1,400 acre-feet per week, or 200 acre-feet per day as the maximum average loss. Therefore, the volume of water contained in the bay is 130 times the amount lost daily. With this figure we can estimate the equilibrium concentration of liquor in Oakland Bay following the continued dumping of any amount of liquor. (Table 16.) The continued dumping of 75,000 gallons of liquor per day at the mill would result in a concentration of 1 part of liquor to 870 of water at equilibrium. Discharge of 280,000 gallons daily at the mill would result in a concentration of 1 to 233 in the bay.

TABLE 16.—*Accumulation of sulphite waste liquor in Oakland Bay*

Assumed daily discharge	Assumed daily discharge	Theoretical accumulation at equilibrium	Equilibrium concentration, liquor to sea water	Assumed daily discharge	Assumed daily discharge	Theoretical accumulation at equilibrium	Equilibrium concentration, liquor to sea water
Gallons	*Acre-feet*	*Acre-feet*		*Gallons*	*Acre-feet*	*Acre-feet*	
50,000	0.15	19.48	1–1300	200,00	.61	79.06	1–329
75,000	.23	29.86	1–870	250,000	.77	99.71	1–261
100,000	.31	40.23	1–646	280,000	.86	111.32	1–233
150,000	.46	59.66	1–436	320,000	.98	126.77	1–205

The concentration of liquor in the bay at any one time after operations started may be calculated by standard mathematical processes:

If a = the number of acre-feet of liquor discharged at the mill per day, b, the amount of water changed per day by tidal action, and V, the total volume of the bay, then

$$\frac{V-a-b}{V} \quad \text{---------- (1)}$$

represents the proportion of original water left at the end of the first day, and

$$\left(\frac{V-a-b}{V}\right)^t \quad \text{---------- (2)}$$

that left at the end of t days. Since

$$\left(\frac{V-a-b}{V}\right)^t = \left(1-\frac{a+b}{V}\right)^t,$$

therefore the proportion of pulp liquor plus the amount of new water is

$$1-\left(1-\frac{a+b}{V}\right)^t \quad \text{---------- (3)}$$

Since the amount of pulp liquor in the bay bears the constant ratio of

$$\frac{a}{a+b} \qquad (4)$$

to the value of pulp liquor plus new water, therefore the proportion of pulp liquor (p) to the total volume of the bay may be expresses as

$$p=\frac{a}{a+b}\left[1-\left(1-\frac{a+b}{V}\right)^{t}\right] \qquad (5)$$

NOTE.—The writer wishes to acknowledge the assistance of Prof. Harold Hotelling of Stanford University in mathmatical problems considered above.

The assumptions made in the derivation of this formula are the source of a small error, but do not rob the results of their significance. In this case it is assumed that the liquor mixed instantaneously and completely with the water and that the inflow of both liquor and water is a continuous process rather than a discontinuous one. Over a long period of time the error due to these two assumptions is negligible although for a period of one or two days the error may be significant. Any error due to lack of mixing of the pulp liquor with the water would tend to increase the amount of liquor in the bay.

By the use of equation 5, the increase in concentration of liquor in Oakland Bay has been calculated, assuming that 70,000 gallons of liquor per day were discharged at the mill. (Table 10.) At first the concentration rises rapidly but at a constantly decreasing rate as equilibrium is approached. As the time factor increases the value of expression (5) approaches the value (4) which expresses the equilibrium concentration, being in this case 1 part of liquor to 931 parts of sea water.

CONCLUSIONS

The effect of sulphite liquor in various dilutions upon oysters is not within the scope of field observations. From data herein given it is concluded that any sulphite waste liquor dumped into Oakland Bay at the Rainier Pulp & Paper Co. plant of necessity must reach the oyster beds. From the physical character of the liquor and from observations, it is apparent that when concentrated liquor is discharged at the mill the accumulation over the oyster beds is greatest near the bottom. Due to the variety of currents the water above the bottom is not comparable to that in which the oysters live, and examinations of water taken at arbitrary locations about the bay can not indicate the conditions with which the oysters must contend. Chemical analysis of the water is not a complete index to the concentration of sulphite waste liquor because the chemical nature of the substances contained therein is unknown. The "oxygen balance" test for the detection of sulphite liquor can not be considered in this problem because no work has ever shown either that the toxicity of sulphite liquor to oysters is in any way proportional to its oxygen demand or that such liquor may not exist unchanged in a toxic state in the presence of dissolved oxygen.

From these facts the following conclusions are drawn:

(1) Conditions in Oakland Bay are unique in the history of oyster culture in Puget Sound. The adult oysters have experienced an abnormally high death-rate for some time, the living oysters spawn little if any, and no set has been obtained in three years.

(2) Other animals, clams, barnacles, mussels, and hydroids, and at least one marine plant, *Melosira borreri*, living on the oyster beds, have been affected in a peculiar manner. Boats, scows, and other floating equipment which have never left the vicinity of the oyster beds do not become covered with barnacles, mussels, and hydroids, which grow upon such equipment under normal conditions. These animals thrive under a wide range of physical conditions, and their absence can not be explained by abnormal temperature or salinity of the water.

(3) There is no evidence by which sewage, log storage, or sawdust could be reasonably considered as an agent of destruction.

(4) The diatom, *Melosira borreri*, normally found in small amounts on oyster beds throughout the lower end of Puget Sound, has changed the character of its growth in Oakland Bay. Apparently this plant is able to use to advantage the chemicals now found in abnormal concentrations in the waters of Oakland Bay. Dense masses of it grow in places where the current is slow, but the area upon which it is found is not the place of highest mortality of the oysters. Some beds which contain the greatest percentage of living oysters have been continually covered with masses of *Melosira*.

(5) Sulphite waste liquor reaches the oyster beds. Its characteristic color has been constantly present in the water over the oyster beds since the mill started operations.

(6) Since the chemical nature of lignin is unknown and since its oxidation products are likewise obscure, no chemical means can be relied upon to demonstrate its presence or absence, in contradiction to visual evidence and observations on the currents.

(7) The "oxygen balance" test is only a measure of stability of dissolved materials, therefore the oxygen demand of any dilution of sulphite liquor is not a reliable index to its toxicity to oysters.

(8) Due to the configuration of Oakland Bay and adjoining bodies of salt water, a small proportion of polluted water escaped each day, and continuous dumping of liquor at the mill gradually builds up a high concentration in the bay.

(9) The dumping of 70,000 gallons of sulphite liquor daily would build up a concentration of 1 part liquor to 931 of water in Oakland Bay. Hopkins has shown (see accompanying report) that the important abnormal conditions of the oysters in Oakland Bay can be reproduced under controlled conditions in the laboratory by subjecting oysters to treatment by mixtures of liquor and sea water of the same strength as shown to be present in Oakland Bay.

Increase in concentration of sulphite liquor in Oakland Bay caused by discharge of 70,000 gallons per day at the mill, calculated from formula 5

Number of days	Parts sea water per 1 part liquor	Number of days	Parts sea water per 1 part liquor
1	102, 940	200	1, 183
10	12, 596	250	1, 086
50	2, 905	300	1, 033
100	1, 730	350	1, 002
150	1, 356	∞	931

REFERENCES

NIGHTINGALE, H. W.

1927. Trade waste investigations. A preliminary report on the physical, chemical, and biological conditions of sea water in the vicinity of Shelton which will have a bearing upon the probable effect of sulphite pulp waste on adjacent oyster-growing areas. Special report of the Washington Director of Health, January 27, 1927. Thirty-sixth and Thirty-seventh Annual Reports of Washington Department of Fisheries and Game, Division of Fisheries, pp. 51–61. Olympia.