AN EXPLORATORY INVESTIGATION OF THE EMBRYONIC AND LARVAL STAGES OF THE BAY MUSSEL, *Mytilus edulis* L., AS A BIOASSAY ORGANISM

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AN EXPLORATORY INVESTIGATION OF THE EMBRYONIC AND LARVAL STAGES OF THE BAY MUSSEL, Mytilus edulis L., AS A BIOASSAY ORGANISM

INTRODUCTION

The purpose of this study was to investigate the possibility of using the embryonic and larval stages of the Bay mussel, Mytilus edulis Linneaus, as a bioassay organism in measuring the toxicity of sulfite pulp-mill wastes. It was found, by W. P. Breese, assistant fishery biologist of Oregon State College, that 4 to 8 percent saltwater solutions of Kraft mill (sulfate pulp-mill) effluent readily stimulated laboratory spawning of Bay mussels during most months of the year;[1/] this quality, plus wide distribution of the species in the Northern Hemisphere, indicated the desirability of this organism as a bioassay organism. The investigation was undertaken in December 1957 at the Oregon State College Marine Laboratory at Yaquina Bay, Oregon. The final seven months of the study were conducted at the Brooklane Laboratory, Corvallis, Oregon, and the study was terminated in March 1959.

It was assumed that the embryonic and larval stages of the mussel might be more sensitive than the adult to the toxic constituents of sulfite mill wastes (the bioassays were limited to sulfite mill wastes but it may be inferred that other toxic

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1/ Personal communication in December 1957.

pulp-mill substances might produce similar effects). Unlike the adult mussel that can withdraw within its shell for prolonged periods of time, the embryo and early stage larva is without a shell or valves, and it is not until the veliger stage (some 48 hours after fertilization according to Rattenbury and Berg, 10, p. 398) that the valves are formed. Even at the 48-hour stage the velar lobe prevents the valves from completely closing and sealing the animal off from the immediate environment. The digestive tract is developed within 48 hours after fertilization, and feeding and respiration would force the organism into crucially dependent contact with the environment at this time. For these reasons, it was obvious that these early stages of development, rather than adult, are more susceptible to effects of the waste liquor and therefore the most desirable bioassay organism.

The extensive distribution of the Bay mussel would make it desirable as a bioassay organism. On the North American continent, the Bay mussel is found from the Arctic Ocean to South Carolina and from Alaska to California. It is also found in Japan and Europe. Most of the important industrial locations on seacoasts and in estuaries in the Northern Hemisphere occur in areas in which the species is present and where the need for a readily available bioassay organism is of importance.

The need for a marine bioassay organism is indicated by the expansion of the pulp industry, particularly in the Pacific

Northwest, into areas located adjacent to salt water. These pulp mills, usually located near fresh water streams, discharge great amounts of waste products into the streams and into marine areas; these products may flow on into inshore waters and could drastically alter the natural habitat of a shellfish resource.

In the manufacture of pulp by the sulfite process, the chips of wood are cooked under pressure in a solution of calcium bisulfite and sulfurous acid, according to Hopkins (5, p. 125-126), the base employed may be calcium (as in all samples used in this investigation), or ammonium, magnesium, or sodium. Over half of the wood constituents go into solution and form the waste-liquor. The specific gravity of the waste-liquor from the digester at the end of the cooking process is about 1.05 grams per liter, and is a dark reddish-brown liquid. Usually, undiluted digester liquid is not dumped into bodies of natural water, but it is diluted with water in the separation process of recovering the pulp from the digester liquid. The specific gravity of the digester or residual liquor is usually between 1.01 grams per liter and 1.02 grams per liter after dilution.

One of the first investigations of sulfite waste pollution and its toxic effect on adult oysters was undertaken in 1931 at the request of oystermen in Shelton, Washington. Hopkins (5, p. 126-129) found that sulfite liquor, from a pulp mill in Oakland Bay, Washington, when added to sea water in concentrations

from 0.67 to 10.0 parts per thousand, killed most of the Olympia oysters, Ostrea lurida Carpenter, in 2 to 29 days. Galtsoff (2, p. 162-167) found that pulp-mill waste from the same mill affected the normal activity of oyster gills, O. lurida and Crassostrea gigas Gmelin, and consequently reduced the rate of feeding. McMillin (9, p. 176-185) performed some cursory experiments with oyster larvae in one-sixth and one-tenth diluted water from Oakland Bay and found with the exception of a few larvae, complete mortality resulted within one day. Control larvae lived for nearly three weeks with water changed at bi-weekly intervals. Extensive investigation was undertaken on adult oysters but little was accomplished on embryonic and larval reactions to sulfite mill wastes.

The present study utilized the property of sulfate mill waste to induce spawning in the Bay mussel, to acquire eggs and sperm for testing the affects of sulfite mill wastes. In the manufacture of pulp from wood by the sulfate process, the chips are cooked at high temperatures and pressures in a water solution containing sodium sulfide and caustic soda as described by Galtsoff (3, p. 158-159). The chemicals dissolve the non-cellulose portion of the wood chip leaving the pulp which is then made into paper. Water that washes the pulp, and is too low in chemicals for profitable recovery, is discharged as "black liquor" into streams or inshore waters. Great quantities of steam are liberated as the cooking liquor passes from the elevated

temperature and pressure of the digester to atmospheric pressure. However, quantities of the condensed condensates also are discharged into receiving waters.

Detailed studies of normal development of the Bay mussel were made by Field (1, p. 185-189) and later by Rattenbury and Berg (10, p. 394-398). Field found that the female produced 5-12 million eggs; the male produced a continuous stream of milt for half an hour or longer.

The normal development of the embryo, when reared at a temperature of 19-22° C. (66.2° - 71.6° F.) is described by Rattenbury and Berg (10, p. 394-398). Cleavage begins about 80 minutes after fertilization, is unequal, and forms a polar lobe. There is subsequently, a withdrawing of the polar lobe into the CD cell, as the first cleavage terminates. The AB cell divides equally at the second cleavage; a second polar lobe is formed and becomes a part of the D cell at this cleavage. The third cleavage is equal in the A, B, C quadrants but unequal in the D cell. At the commencement of gastrulation the apical cells are decidedly smaller than the vegetal macromeres.

The embryo begins to rotate slowly when the first cilia appear, at about six hours. Within a short time the embryo will become very active and swim near the surface.

Gastrulation is completed in 9 or 10 hours at the vegetal pole. After invagination, the archenteron is inclined in an anterior direction. The animal pole now lies opposite the blastopore and the future dorso-posterior region is identified by the mesodermal cells and a thickening of the ectodermal layer between the mesodermal cells and the animal pole.

The embryo reaches a trochophore stage at about 24 hours, although the larva is "atypical" in having no prototroch. At this stage the larva is broad at the anterior end and narrow posteriorly. A long thick apical tuft grows at the anterior end and the surface becomes covered by short cilia. A shell gland appears as a thickening of the dorsal ectoderm, and a small transparent shell has developed on the mid-dorsal surface. On the postero-dorsal surface a non-motile bristle appears. Rapid growth alters the embryonic axes: the antero-posterior axis becomes identifiable by an apical tuft at the anterior end; and the dorso-ventral axis is marked ventrally by the stomodaeum and dorsally by the shell gland. The important features identifying the larva as a trochophore are the change in embryonic axes and the presence of the apical tuft.

A velar lobe forms at the anterior end of the body at 48 hours, and the embryo is now called a veliger. The body elongates and becomes laterally compressed. The shell becomes larger, becomes a bivalve, and an umbo is present. The valves are faintly purple in color and have transverse ridges. A large

velar lobe projects between the valves of the shell and can only be partially withdrawn. The apical tufts are lost. The digestive tract is relatively narrow and U-shaped and opens to the outside on the ventral surface. The veliger usually swims near the bottom of the container. At about 72 hours the velar lobe becomes reduced in size, and the foot develops on the ventral side of the body. Observations made at Yaquina and Corvallis on the developing eggs, embryos, and larvae agreed in general with the descriptions given by Rattenbury and Berg (10, p. 396-398).

Rattenbury and Berg (10, p. 394) obtained gametes by placing individual mussels, previously washed in fresh water, in small dishes containing 70% sea water at room temperature.

Iwata (6, p. 443) induced spawning in Bay mussels by shocking them with 20 volts of electricity over a five second duration. About one hour after the stimulation the mussel discharged the eggs or sperm. Iwata (7, p. 393) also induced spawning by injection (0.5 cubic centimeters) of an isotonic M/2 KCl aqueous solution into the visceral cavity of the mussel. The reaction time ranged from about one hour to more than five hours. In most cases, eggs or sperm were carried out of the shell by a weak current of ciliary motion of the mantle. These aspects differ somewhat from either natural spawning or the way induced by electric stimulation in which the spawning takes place about one hour after the stimulation and eggs and sperm are carried out of the shell by the strong exhalent current. However, it was not

until the use of Kraft mill effluents as a means of obtaining gametes (later described) was discovered, by W. P. Breese at the Oregon State College Marine Laboratory, Yaquina Bay, Oregon, that the mussel was considered for use as a bioassay organism.

Preliminary investigation of the embryonic and larval stages of the Bay mussel as a bioassay organism were undertaken at Yaquina Bay, February through August 1958. Some cursory experiments, prior to 1958 by W. P. Breese, of inducing spawning with a 4% by volume prepared solution of sulfate mill waste and sea water proved so encouraging that future investigation was warranted. Research by the author at Yaquina Bay attempted to determine the seasonal spawning activity of the Bay mussel. Pilot experiments of the development of the mussel embryo, larval and pelagic stages, with various sulfite concentrations covering two-week periods of testing were attempted but discontinued for lack of a proper food for the larvae.

At the commencement of investigation of the mussel larvae at the Brooklane Laboratory in Corvallis, certain practical qualifications were made in an attempt to learn if a short-term bioassay technique could be developed for assessing the toxicity of various spent sulfite concentrations. First, the actual bioassay tests were not to extend more than a forty-eight or seventy-two hour period; second, the procedure should be simple in nature and require a minimum of time and effort; and third, the results should be easily discernible, preferably by

observing development or lack of development. It was hoped that the bioassay technique developed for assessing spent sulfite toxicity could be applied to other toxic effluents or chemicals.

METHODS

In general, the technique developed for the experiments was as follows: 32 to 160 mussels were immersed in a sea water solution of 4% by volume of prepared sulfate mill waste to induce spawning. The individual mussels induced to spawn were transferred to separate dishes and allowed to continue to spawn in uncontaminated sea water. Eggs and sperm were taken from the sea water and placed in varying concentrations of sulfite liquor ranging from 0.0 to 100.0 p.p.m.; at 48 and 72 hours after fertilization, the shelled or successful larvae were counted.

The mussels used in these experiments were collected from floating log docks in Yaquina Bay and ranged from 41 to 62 mm., (1-5/8 to 2-1/2 inches) in length. During the preliminary investigation from February to August 1958, the mussels were collected approximately one hour before they were immersed in a sea water sulfate solution. On the not too frequent occasions that the mussels would not open and pump, and thereby not spawn, they were kept out of water from 24 to 48 hours to induce opening and pumping. Mussels that were transported from Yaquina Bay to Brooklane Laboratory in Corvallis were collected 24 hours before the experiments began. Before immersion in the sea water sulfate liquor, the mussels were scrubbed clean of barnacles and other organisms and any sediment that might interfere with detecting sperm or eggs.

Induction of Spawning

A 4% by volume prepared solution of sulfate black liquor and condensate in sea water was used to initiate spawning. One milliliter of black liquor and 99 milliliters of condensates were mixed and from this mixture, 40 milliliters were mixed with 960 milliliters of sea water. The sea water was obtained from Yaquina Bay in glass containers and was mixed with Oak Creek water to obtain a salinity of 23 parts per thousand. The temperature of the water at the immersion of the mussels ranged from 12° C. (53.6° F.) to 16° C. (60.8° F.) and gradually increased to the constant-temperature room maintenance of 20° C. (68.0° F.). After 1000 milliliters of the 4% prepared sulfate mill solution was poured into three or four 190-millimeter preparation dishes, 15 to 20 mussels were placed in each dish (Figure 1). Usually the mussels would begin pumping within five minutes. At this time, feces of the mussels were discharged in flat ribbon-like segments. Generally, males would begin to spawn in 30 to 45 minutes, and females in 45 to 60 minutes. The sperm were emitted in puffs or streams of white clouds and usually clouded the water. These emissions would continue for approximately half an hour. The eggs flowed out in a continuous stream and settled to the bottom. The eggs expelled while adhering together in the form of short bars (3 to 5 millimeters in length) soon fell apart and assumed the normal spherical or oval form. The spawning process continued until nearly all the

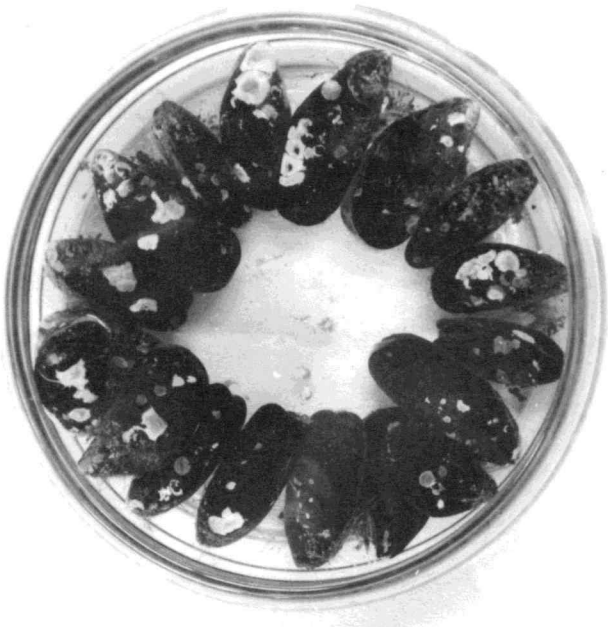

Figure 1. Bay mussels, Mytilus edulis, in 190 mm. preparation dish.

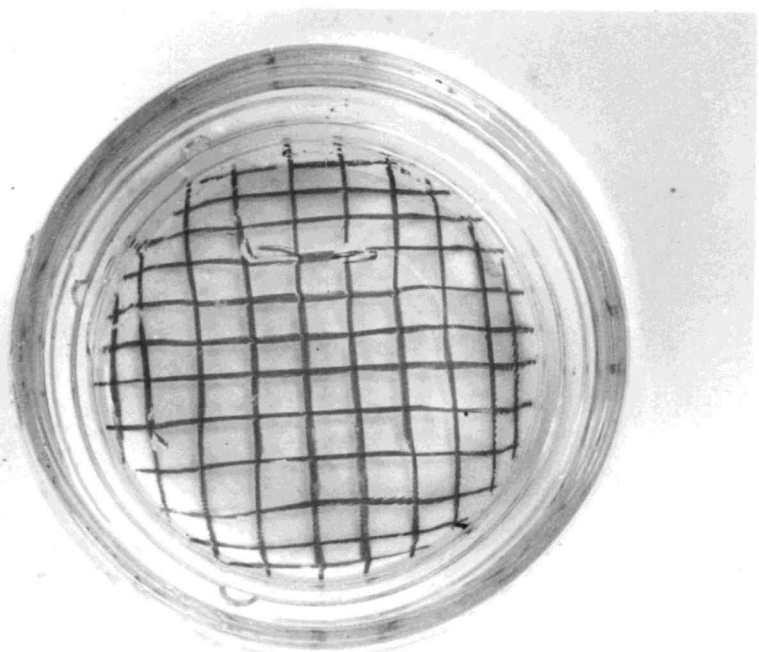

Figure 2. Preparation dish (100 mm.) showing demarkations on bottom to facilitate egg counting.

genital products were discharged. If pumping stopped before spawning began, then the 4% by volume prepared solution of black liquor and condensate in sea water was replaced.

When spawning commenced, the mussels were removed from the 190 mm. preparation dishes containing the 4% sulfate liquor and sea water and placed in individual 100 mm. preparation dishes containing 250 ml. of sea water at a salinity of 23 p.p.t. and a temperature of 20° C. (68.0° F.). Spawning continued in the fresh sea water (i.e., water without the sulfate waste liquor).

Egg Count

For the study of fertilized eggs under the influence of varying concentrations of sulfite waste liquor, it was necessary to begin each concentration with approximately the same number of eggs per preparation dish. A slide with sperm and eggs was observed at the beginning of spawning and periodic checks on early cleavage of embryos gave indications of successful or unsuccessful fertilization. If fertilization was extremely poor (less than one half the embryos developing normally), a different male and female were used. Only the sperm from one male and the eggs from one female were used in any one bioassay. The number of eggs spawned into the 100 mm. preparation dishes was estimated by counting a fraction of the 250 ml. volume. The female mussel was removed and the contents of eggs and water were poured back and forth into large containers until the eggs were evenly

dispersed. Immediately, a sample was taken with a pipette and a 1 ml. aliquot then measured into a 50 ml. beaker from which a count of eggs was accomplished. The count can easily be done under a magnification of 10x on a dissecting microscope. The 1 ml. count will enable one to calculate the dilution required to obtain 500 eggs per 100 mm. preparation dish.

Ten 100 mm. preparation dishes were needed for the following 48- and 72-hour sulfite concentrations: control, 25 p.p.m., 50 p.p.m., 75 p.p.m., and 100 p.p.m. Two additional controls and two 100 p.p.m. preparations were also needed for pH and Winkler dissolved-oxygen tests at the beginning and termination of experiments. The preparation dishes were thoroughly cleaned with borax and hot water. The dishes were allowed to drip dry. The underside of the dishes was marked off in squares (Figure 2) with a grease pencil and a straight edge. This checkered design, under a magnification of 10x on a dissecting microscope, facilitated counting of the eggs that settled on the bottom of the dish.

Approximately 500 eggs were pipetted into 100 mm. preparation dishes that contained 20 ml. of sea water for receiving the eggs. The eggs were then counted by moving the preparation dish from one square to the next under the 10x dissecting microscope.

Five ml. of sea water containing sperm was used in each experiment. Later, it was found that considerably less was needed to insure fertilization. One ml. of sea water containing

sperm was found to be sufficient, but since a standard procedure using 5 ml. was originally defined, it was carried through in all experiments.

Sulfite Liquor Concentrations

The sulfite liquor, known as "hot blown liquor", collected from the blow pits (which usually contained more than 10% solids), was adjusted to 10% solids before each bioassay experiment by adding water before any dilutions were made. One ml. of the sulfite solution was added to 999 ml. of the sea water. This gave one part of sulfite to 1000 parts of water. This solution was then diluted further with sea water to 25 p.p.m., 50 p.p.m., 75 p.p.m., and 100 p.p.m. to obtain the total bioassay solutions of 250 ml. The sulfite samples used in these experiments were from mills using calcium-base cooking liquors.

Termination of Experiments

At 48 hours and 72 hours after fertilization, the larvae in each series were killed in different preparation dishes with 5 ml. of 10% formalin. The addition of two drops of 1% Rose Bengal stained the larvae adequately in 1 to 2 hours. Most of the liquid was decanted out of the 100 ml. preparation dishes to aid in counting the dead larvae.

The successful or normal larvae at 48 and 72 hours had well-developed valves and a large velar lobe. The velar lobe in the 72-hour series was slightly reduced in size in comparison to the

48-hour series. There were some gradients of shelling in the 48-hour series but fewer in the 72-hour series. In questionable instances of incomplete shelling, the final criterion was the development of the digestive tract. If the digestive tract indicated normal organization and structure, it was assumed that the larva would have become a normal larva and was counted as such.

A standard Winkler test for dissolved oxygen was performed on the extra series of control and 100 p.p.m. sulfite concentrations at the beginning and termination of experiments. The dissolved oxygen at the beginning of the experiment was approximately 9 milligrams per liter. At the conclusion of the experiment, the dissolved oxygen never dropped below 4.0 milligrams per liter in either the control or 100 p.p.m. concentration.

The pH was taken at the start and termination of the experiments. The pH ranged from 7.92 to 7.68.

The statistical analysis of the data shows the larvae as either success or failure; that is, shelled or nonshelled. The Chi-square value with 4 degrees of freedom is broken down into two components; namely, linear regression with 1 degree of freedom, and the deviation from linearity with 3 degrees of freedom. In each case the regression coefficient is given. This coefficient is expressed as the rate of decrease of percentage of successful development per p.p.m. of sulfite waste.

RESULTS

The Effect of Pulp Mill A Sulfite Waste Liquor on Mussel Larvae

The results of the five experiments on the Bay mussel larvae were in general agreement; in that mortality increased as the concentration of sulfite liquor increased from 25 p.p.m. to 100 p.p.m. Experiments 1, 3, and 5 were terminated at 48 hours after fertilization; Experiments 2 and 4 at 72 hours after fertilization.

Experiment 1 (Appendix Table 1), terminated at 48 hours after fertilization, shows an increase of mortality as the sulfite waste concentration increased (Figure 3). The control sample had 147 larvae that developed normally out of 518 eggs or 28.3%, and the other extreme, the test sample at 100 p.p.m. had 100% mortality. If the 5% significance level is used, the Chi-square value of 256.57 indicates the percentage of successful development of larvae decreases with the increase in sulfite waste concentration. The Chi-square value of 18.26 indicates the rate of decline is linear. The average rate of decrease was 0.29 percent per p.p.m. In terms of the concentrations, the development of normal larvae decreases as the concentration increases and the decrease is proportionate from one concentration to another.

Experiment 2 (Appendix Table 2) is in agreement with Experiment 1 in that mortality increased as the sulfite concentration increased. Again, there was 100% mortality in the 100 p.p.m.

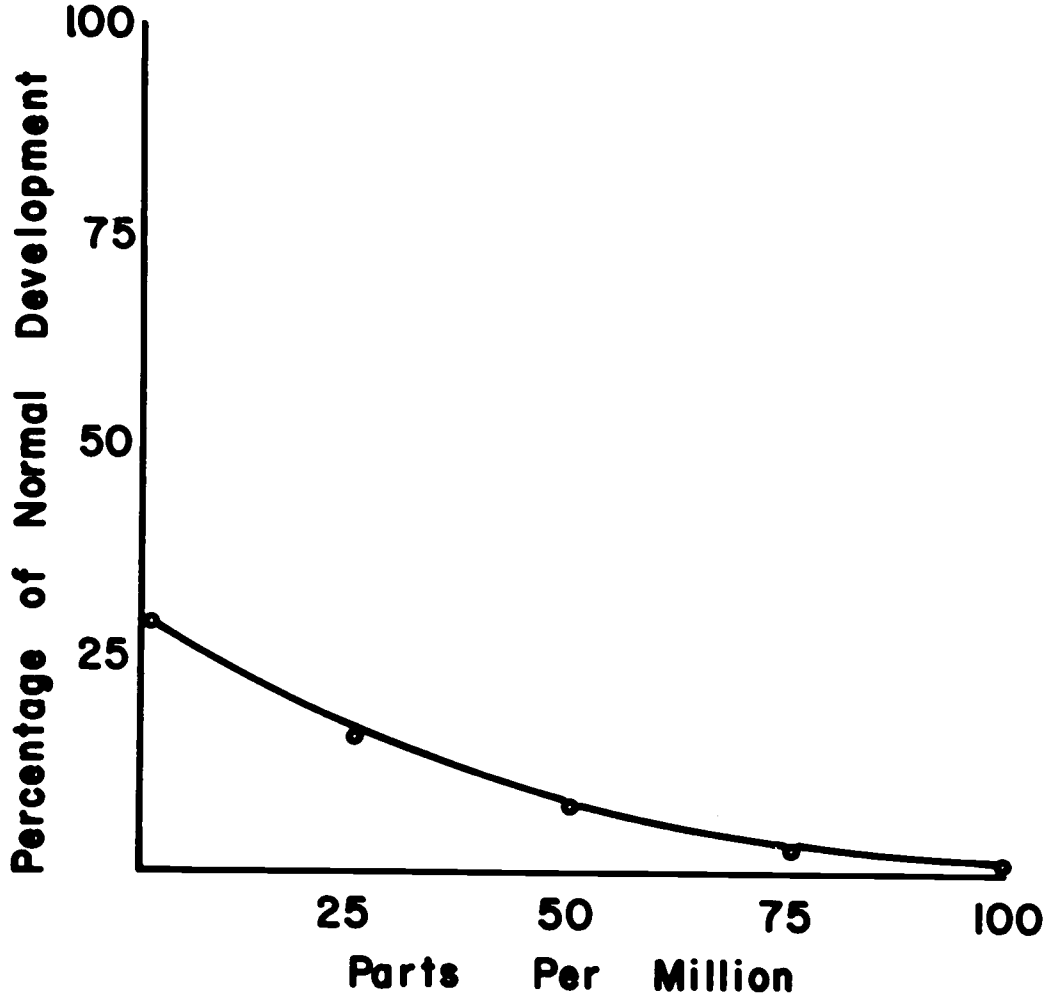

Figure 3. Typical results for Pulp Mill A sulfite waste experiments (1-5) are shown by Experiment 1.

concentration; 113 larvae out of 609 eggs or 18.5% developed normally in the control sample. If the 5% significance level is used, the Chi-square value of 192.56 indicates that the percentage of successful larvae decreases with the increase of sulfite waste concentration. The Chi-square value of 23.90 indicates that the rate of decline is linear. The average rate of decrease was 0.18 percent per p.p.m. Again the development of normal larvae decreases as the concentration increases and the decrease is proportionate from one concentration to another.

Experiment 3 (Appendix Table 3), terminated at 48 hours after fertilization, departs from the two previous experiments in that there was not 100% mortality in the 100 p.p.m. concentration. A total of 34 normal larvae out of 477 eggs were identified at the termination of the experiment, or 92.9% mortality. In the control group, 339 larvae developed normally out of 581 eggs at the end of the 48-hour period, or 58.3%. The Chi-square value of 335.07 indicates that the percentage of successful larvae development decreases with the increase of sulfite waste concentration. The value of 24.91 indicates that the rate of decline is linear. The average rate of decrease is 0.47 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases and the decrease is proportionate from one concentration to another.

Experiment 4 (Appendix Table 4), terminated at 72 hours after fertilization, agrees generally with Experiment 3 in that

the 100 p.p.m. concentration did not have 100% mortality as did Experiments 1 and 2. Fifteen larvae developed normally out of 445 eggs, 96.7% mortality. In control, 293 larvae developed normally out of 544 eggs, or 53.8%. The Chi-square value of 376.06 indicates the percentage of successful larvae decreases with the increase of the sulfite waste concentration. The Chi-square value of 18.59 indicates that the rate of decline is linear. The average rate of decrease is 0.53 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases and the decrease is proportionate from one concentration to another.

Experiment 5 (Appendix Table 5), terminated at 48 hours after fertilization, does show an increase of mortality as the concentration increases, but the decline in successful larvae development is not linear. Only 55 larvae developed normally out of 501 eggs in the control group, or 10.9%. Thirteen larvae developed normally out of 499 eggs in the 100 p.p.m. concentration, or 2.6%. The Chi-square value of 25.52 indicates that the percentage of successful development decreases with the increase in sulfite waste concentration. The Chi-square value of 3.74 indicates that the rate of decrease is not linear. The average rate of decrease is 0.07 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases, but that the decrease is not proportionate from one concentration to another.

The Effect of Pulp Mill B Sulfite Waste Liquor on Mussel Larvae

The results of the five experiments on the Bay mussel larvae were in general agreement with the experiments with the Pulp Mill A sulfite waste. The mortality increased as the concentration of sulfite liquor increased from 25 p.p.m. to 100 p.p.m.

Experiment 6 (Appendix Table 6), terminated at 48 hours after fertilization, shows that there was an increase of mortality as the sulfite waste concentration increased. Seventy-two larvae out of a total of 402 eggs or 17.9%, developed successfully in 100 p.p.m.; 299 larvae developed normally out of 389 eggs in control or 74.2%. If the 5% significance level is used, the Chi-square value of 423.37 indicates that the percentage of successful development of larvae decreases with the increase of sulfite waste concentration. The Chi-square value of 37.23 indicates the rate of decline is linear. The average rate of decrease is 0.47 percent per p.p.m. In terms of the concentration the development of normal larvae decreases as the concentration increases, and the decrease is proportionate from one concentration to another.

Experiment 7 (Appendix Table 7), terminated at 72 hours after fertilization, shows that there was an increase of mortality as the sulfite waste concentration increased. Thirty-three larvae out of a total of 493 eggs developed successfully in 100 p.p.m. or 7.6%; 162 larvae developed normally out of 425 eggs in the control group or 38.1%. If the 5% significance

level is used, the Chi-square value of 166.48 indicates that the percentage of successful development of larvae decreases with the increase of sulfite waste concentration. The Chi-square value of 2.96 indicates that the rate of decline is not linear. The average rate of decrease is 0.31 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases, and the decrease is not proportionate from one concentration to another.

Experiment 8 (Appendix Table 8), terminated at 48 hours after fertilization, again shows that there was an increase of mortality as the sulfite waste concentration increased (Figure 4). Only 11 normal larvae developed out of 437 eggs in the 100 p.p.m. group or 2.5%; 77 larvae developed normally out of 561 eggs in the control group or 13.7%. If the 5% significance level is used, the Chi-square value of 50.25 indicates the percentage of successful development of larvae decreases with the increase of sulfite waste concentration. The Chi-square value of 0.92 indicates that the rate of decline is not linear. The average rate of decrease is 0.11 percent per p.p.m. In terms of the concentration the development of normal larvae is not proportionate from one concentration to another.

Experiment 9 (Appendix Table 9), terminated at 72 hours after fertilization, shows that there was an increase of mortality as the sulfite waste concentration increased. Only 1 larva developed normally out of 353 fertilized eggs in the

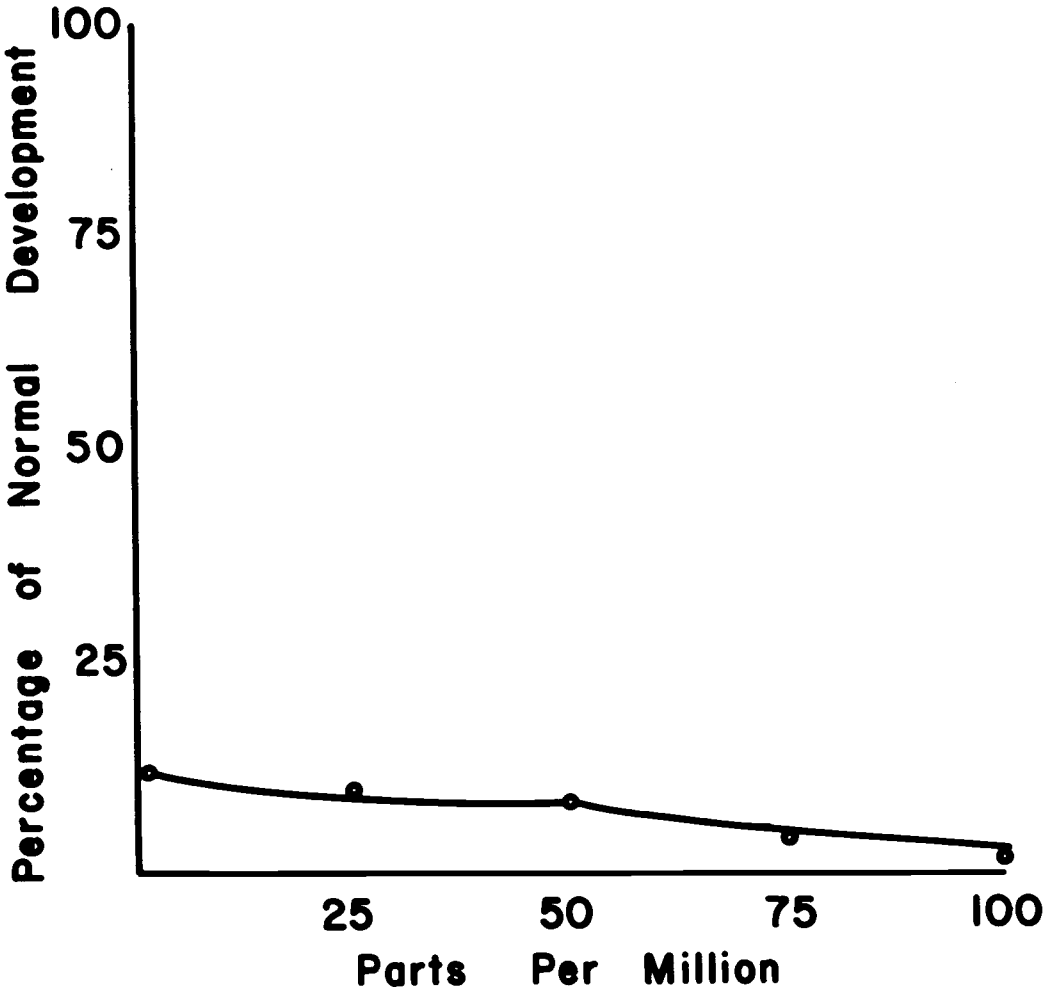

Figure 4. Typical results for Pulp Mill B sulfite waste experiments (6-10) are shown by Experiment 8.

100 p.p.m. group or 0.3%; 60 larvae developed normally out of 475 fertilized eggs in the control group or 12.6%. If the 5% significance level is used, the Chi-square value of 181.31 indicates that the percentage of successful development of larvae decreases with the increase in the sulfite waste concentration. The Chi-square value of 14.01 indicates that the rate of decline is linear. The average rate of decrease was 0.13 percent per p.p.m. In terms of the concentrations, the development of normal larvae decreases as the concentration increases, and the decrease is proportionate from one concentration to another.

Experiment 10 (Appendix Table 10), terminated at 48 hours after fertilization, shows that there was an increase of mortality as the sulfite waste concentration increased. Six larvae developed normally out of 468 fertilized eggs in the 100 p.p.m. group or 1.2%; 38 larvae developed normally out of 446 eggs in the control group or 8.5%. If the 5% significance level is used, the Chi-square value of 17.09 indicates the percentage of successful development of larvae decreases with the increase of sulfite waste concentration. The Chi-square value of 9.31 indicates that the rate of decline is not linear. The average rate of decrease is 0.06 percent per p.p.m. In terms of the concentration the development of normal larvae decreases as the concentration increases, and the decrease is proportionate from one concentration to another.

The Effect of Pulp Mill C Sulfite Waste Liquor on Mussel Larvae

The Pulp Mill C sulfite waste causes generally the same results as the wastes used in the previous experiments. Mortality increased as the concentration of sulfite liquor increased from 25 p.p.m. to 100 p.p.m.

Experiment 11 (Appendix Table 11), terminated at 48 hours after fertilization, shows an increase in mortality as the sulfite waste concentration increased. No larvae developed normally in the 100 p.p.m. group; but 370 larvae out of 408 eggs developed normally in the control group or 90.7%. If the 5% significance level is used, the Chi-square value of 1015.13 indicates the percentage of successful development of larvae decreases with the increase in sulfite waste concentration. The Chi-square value of 23.09 indicates that the rate of decline is linear. The average rate of decrease was 0.90 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases, and that the decrease is proportionate from one concentration to another.

Experiment 12 (Appendix Table 12), terminated at 72 hours after fertilization, shows that there was an increase of mortality as the sulfite waste concentration increased. Only 9 larvae developed normally out of 506 eggs in 100 p.p.m. concentration or 1.8%; 426 larvae developed normally out of 534 eggs in the control group or 79.8%. If the 5% significance level is used, the Chi-square value of 826.57 indicates that the

percentage of successful development of larvae decreases with the increase in sulfite waste concentration. The Chi-square value of 19.13 indicates that the rate of decline is linear. The average rate of decrease was 0.78 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases, and the decrease is proportionate from one concentration to another.

Experiment 13 (Appendix Table 13), terminated at 48 hours after fertilization, shows that there was an increase of mortality as the sulfite waste concentration increased (Figure 5). Only 4 larvae developed normally out of 463 fertilized eggs in the 100 p.p.m. concentration or 0.9%; 126 larvae developed normally out of 517 eggs in the control group or 24.4%. If the 5% significance level is used, the Chi-square value of 107.53 indicates that the percentage of successful development of larvae decreases with the increase in sulfite waste concentration. The Chi-square value of 16.70 indicates that the rate of decline is linear. The average rate of decrease was 0.20 percent per p.p.m. In terms of the concentrations, the development, and the decrease is proportionate from one concentration to another.

Experiment 14 (Appendix Table 14), terminated at 72 hours after fertilization, shows that there was an increase in mortality as the sulfite waste concentration increased. Only 1 larva developed normally out of 572 eggs in the 100 p.p.m. concentration or 0.2%; 54 larvae developed normally out of 518 eggs

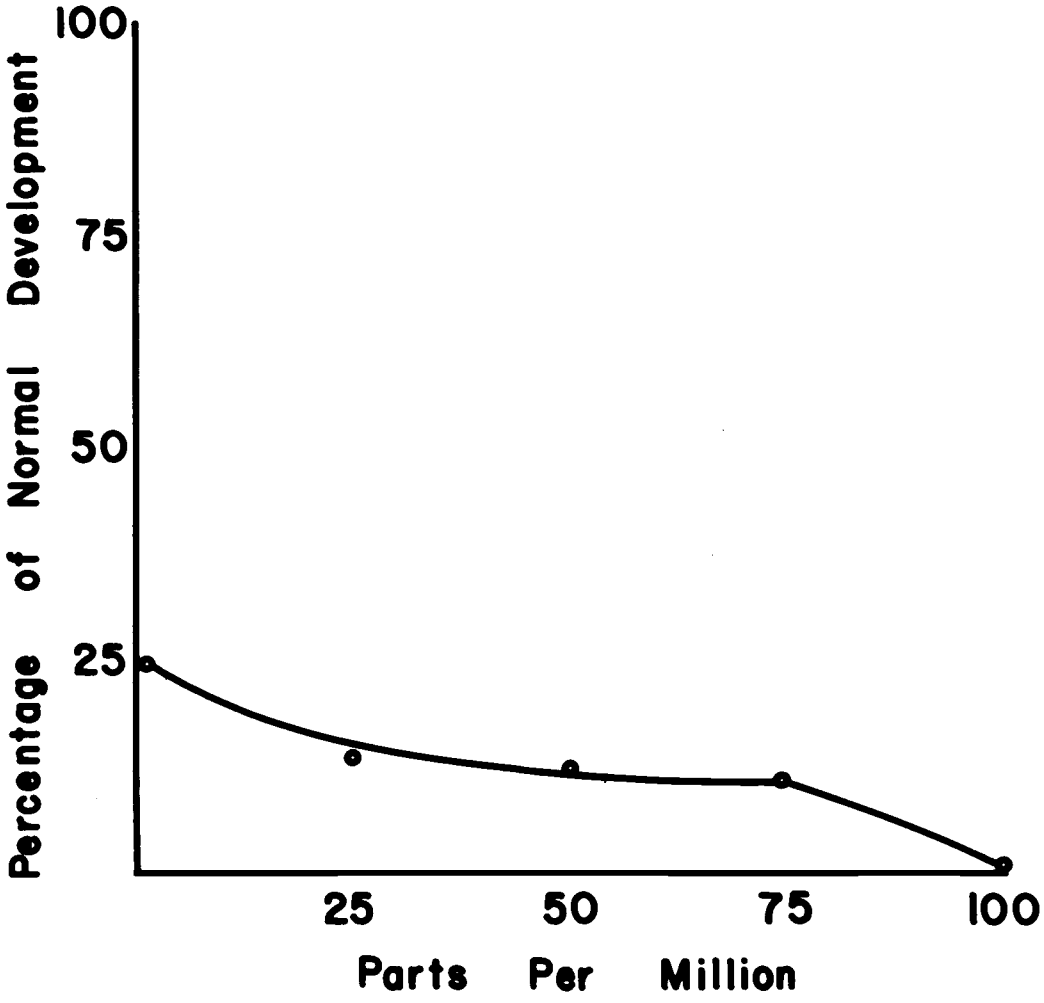

Figure 5. Typical results for Pulp Mill C sulfite waste experiments (11-15) are shown by Experiment 13.

in the control group or 10.4%. If the 5% significance level is used, the Chi-square value of 66.19 indicates that the percentage of successful development of larvae decreases with the increase in sulfite waste concentration. The Chi-square value of 8.00 indicates that the rate of decline is not linear. The average rate of decrease was 0.11 percent per p.p.m. In terms of the concentrations, the development of normal larvae decreases as the concentration increases, but the decrease is not proportionate from one concentration to another.

Experiment 15 (Appendix Table 15), terminated at 48 hours after fertilization, shows that there was an increase in mortality as the sulfite waste concentration increased. Only 11 larvae developed normally out of 470 eggs in the 100 p.p.m. concentration or 2.3%; 43 larvae developed normally out of 376 eggs in the control group or 11.4%. If the 5% significance level is used, the Chi-square value of 31.68 indicates that the percentage of successful development of larvae decreases with the increase in sulfite waste concentration. The Chi-square value of 4.08 indicates that the rate of decline is not linear. The average rate of decrease was 0.09 percent per p.p.m. In terms of the concentration, the development of normal larvae decreases as the concentration increases, and the decrease is not proportionate from one concentration to another.

Induced Spawning Occurrences for the Bay Mussel

Spawning was attempted 63 times with a 4% by volume prepared solution of sulfate black liquor and condensate in sea water, from December 1957 through March 1959, (Table 16). The males failed to spawn 12 times in 63 attempts and the females failed to spawn 18 times in 63 attempts. There was a total of 26 times that either male or female failed to spawn and hence made fertilization impossible. In only 4 out of 63 attempts was there no spawning by either males and females.

The most mussels spawned in the 4% by volume prepared sulfate solution occurred July 6, 1958, when 23 mussels (14 males and 9 females) out of 120 mussels spawned. In 1958, spawning of either sex did not occur once in 6 attempts in April and in 7 attempts in June, and twice in 11 attempts in July. In 1958 female mussels failed to spawn once in 6 attempts during April, twice in 6 attempts in June, twice in 11 attempts in July, twice in 9 attempts in August, once in 2 attempts in September, once in 2 attempts in October, four times in 5 attempts in November, and 5 times in 6 attempts in December (once in 1957 and four times in 1958). In 1958, male mussels failed to spawn once in 3 attempts in March, twice in 6 attempts in April, twice in 6 attempts in June, 3 times in 11 attempts in July, twice in 9 attempts in August, and once in 5 attempts in November. The males also failed to spawn once in 4 attempts in January of 1959. The results indicate that both male and female mussels do spawn,

in a 4% by volume prepared solution of sulfate black liquor and condensate in sea water, at almost any season of the year.

DISCUSSION

The fifteen experiments on the development of the Bay mussel eggs indicate that with the lowest concentration of sulfite liquor, 25 p.p.m., the numbers of larvae that developed were less than control in all bioassay experiments (see Appendix Tables 17, 18, and 19). Further, the highest concentration, 100 p.p.m., produced 100% mortality in three experiments (Experiments 1, 2, and 11).

In the five experiments with Pulp Mill A sulfite waste solutions, not more than 7.2% (as compared with 58.3% for control) larvae developed normally in the 100 p.p.m. concentration. In the five experiments with Pulp Mill B sulfite waste solution, not more than 18.0% (as compared with 76.9% for control) larvae developed normally in the 100 p.p.m. concentration. In the five experiments with Pulp Mill C sulfite waste solution, not more than 2.4% (as compared with 10.5% for control) larvae developed normally in the 100 p.p.m. concentration. At 100 p.p.m. concentration there is little possibility of obtaining normal larvae in either the 48-hour or 72-hour bioassays. The Chi-square values indicate that even though the regression is not always linear, the trend is predominately linear.

There appears to be some difference in effects on larval development among the sulfite wastes from the three mills. Mill A produced 100% mortality in two experiments (Experiments 1 and 2); however, in Experiment 3 the mortality was 92.8%. Mill B

produced a wider range in percent mortality but never 100% mortality; in Experiment 6 the mortality was 82.0%, and the closest to 100% mortality occurred in Experiment 9 with 99.7% mortality. Mill C appears to have produced the highest consistent mortality; Experiment 11 resulted in 100% mortality and in the five experiments never produced less than 97.6% mortality. As anticipated, a difference in toxicity between the effluents from the three mills is indicated, with evidence that egg and larval mortality approaches 100% at 100 p.p.m. concentrations of all three effluents.

There also appears to be some difference in effects on larval development among the sulfite wastes with respect to the duration of experiments (i.e., 48 hour or 72 hour). In Experiments 1 and 2, 3 and 4, 6 and 7, 8 and 9, 11 and 12, 13 and 14 (see Appendix Tables 17, 18, and 19) in which the eggs and sperm for each pair were taken from the same sample, the trend in four out of six cases is toward a higher mortality in the 72-hour bioassays than in the 48-hour bioassays. In one case the mortality was the same. However, with the exception of Experiment 6 (82.0% mortality) and 7 (92.3%), a difference of slightly over 10%, the results in the 48-hour and 72-hour experiments are in sufficient agreement and, therefore, there appears to be little reason to extend the bioassays over 48 hours.

The larvae in the control group developed as previously described by Rattenbury and Berg. Cleavage began approximately

80 minutes after fertilization. Most of the eggs in all experiments appeared to have been fertilized and underwent cleavage. It was during cleavage that mortality was most noticeable in the control and the four experimental concentrations. The eggs would apparently divide a few times and then stop dividing or divide erratically. Within a short time (2-4 hours), many of the abnormally developing embryos would disintegrate.

The embryos began to rotate approximately 6 hours after fertilization; in one instance the embryos began to rotate within 4 hours after fertilization, or approximately 2 hours sooner than expected. The velar lobe formed at the anterior end of the body, and the U-shaped digestive tract became evident after 48 hours. At 72 hours after fertilization the velar lobe became reduced in size, and the foot developed on the ventral side of the body.

Although precautions were taken to obtain 50% fertilization by observing cleavage in a sample of the eggs, the average control mortality at the termination of the 15 experiments was 64.1%. This indicates that no less than 50% fertilization of the eggs at the beginning of the experiments be obtained for the bioassays.

The months of November and December are critical periods for obtaining females that can be induced to spawn. It is possible that this critical period is a recovery time from the late summer or fall spawning. The problem of a supply of eggs and sperm during these months may be resolved by a method devised by

Loosanoff (8, p. 611), i.e., removing mollusks from Long Island Sound early in the summer before spawning and replanting in colder waters, such as those of Boothbay Harbor, Maine. The water temperature of the latter area permits a slow development of the gonads but is low enough to prevent the transplanted mollusks from spawning. Thus, by the time the mollusks in Long Island Sound have spawned and are spent, those kept in Maine waters are still ripe and may be induced to spawn in the laboratory during the late summer and fall. The period March through July, and possibly August, is favorable for acquiring females for bioassay purposes. However, it seems that some Bay mussels, both male and female, have physiologically-ripe sex cells throughout the twelve months of the year.

More investigation should be made on the constituents of sulfate liquor that initiate spawning in the mussels. Incomplete results, however, suggest that black liquor alone, condensate alone, or black liquor and condensate together, might induce spawning in Bay mussels. The triggering effect induced by the sulfate liquor may be due to the presence of stilbene compounds with estrogenic and androgenic properties. These compounds are known to be present in wood and may be extracted during the cooking process.[1/]

1/ Letter from Isaiah Gellman, Regional Engineer for National Council for Stream Improvement, to R. E. Dimick, quoting Dr. Stein of the Rayonier Marine Biological Laboratory, Hoodsport, Washington, December 6, 1957.

The Winkler tests for dissolved oxygen at both the beginning and termination of experiments indicate that probably sufficient dissolved oxygen was present for the larvae during the experiments, and indicate that a lack of dissolved oxygen did not contribute to larval mortality. Although the oxygen tolerance level is not known for the larvae, the minimum observation of 4.0 milligrams per liter of oxygen appeared adequate for normal development. The sulfite waste, although a strong reducing agent, apparently was not strong enough in concentrations employed to diminish the oxygen supply.

There is reason to believe that bacteria increased in the full and stoppered bottles of sea water (Harvey, 4, p. 64-68) and contributed to consumption of oxygen. More important, it is not known what effect bacteria (their enzymes and metabolites) have on developing larvae in a sulfite concentration containing sugars and related substances. It is possible that antibiotics could control the bacterial populations and thus minimize probable inimical effects in testing containers.

The conditions in the laboratory are very different from those found in natural areas in which dumping of such wastes into bays and estuaries occurs. Although the tidal currents do not readily eliminate pollution, the mussel larvae in bays and estuaries would be subjected to fluctuating conditions. For example, the mussel larvae (in contrast to those held under laboratory conditions), could be subjected to varying

concentrations, as the mill wastes are not discharged at a uniform rate. The temperature, oxygen and silting effects would be quite variable in nature in the receiving waters.

The extent of the damage to the Bay mussel (and perhaps other mollusks) might be predicted should the concentrations studied in the laboratory be duplicated in bays and estuaries. Theoretically, this might seldom occur, but even in very weak concentrations--much below these experimental concentrations, which fail to produce any effect immediately--the pollutants may reduce the vitality of the organism and result in higher mortality weeks or months later. It must be emphasized that the lethal effects observed in these laboratory tests occurred within 48 to 72 hours; the effects of long-period exposure to sulfite liquor concentrations lower than those employed in these tests awaits further study.

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APPENDIX

Appendix Table 1. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 1.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	147	99	31	13	0	290	.113060
failure	371	514	377	577	436	2275	.886939
n	518	613	408	590	436	2565	

Source of Variation	SS	DF	χ^2
Treatment	27.5589	4	274.83
Linear regression	25.7281	1	256.57
Deviation from linearity	1.8308	3	18.26
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.1003		

The average rate of decrease is .29 percent per p.p.m.

Appendix Table 2. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 2.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	113	49	31	8	0	290	.066402
failure	496	543	552	669	566	2826	.933597
n	609	592	583	677	566	3027	

Source of Variation	SS	DF	χ^2
Treatment	13.4188	4	216.46
Linear regression	11.9372	1	192.56
Deviation from linearity	1.4816	3	23.90
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.0620		

The average rate of decrease is .18 percent per p.p.m.

Appendix Table 3. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 3.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	339	213	175	102	34	863	.323827
failure	242	388	316	413	443	1802	.676172
n	581	601	491	515	477	2665	

Source of Variation	SS	DF	χ^2
Treatment	78.8229	4	359.98
Linear regression	73.3674	1	335.07
Deviation from linearity	5.4555	3	24.91
$\bar{\bar{y}}$ $(1-\bar{\bar{y}})$	.2190		

The average rate of decrease is .47 percent per p.p.m.

Appendix Table 4. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 4.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	293	217	169	91	15	785	.332345
failure	251	190	337	369	430	1577	.667654
n	544	407	506	460	445	2362	

Source of Variation	SS	DF	χ^2
Treatment	87.5695	4	394.65
Linear regression	83.4453	1	376.06
Deviation from linearity	4.1242	3	18.59
$\bar{\bar{y}}$ $(1-\bar{\bar{y}})$	.2219		

The average rate of decrease is .53 percent per p.p.m.

Appendix Table 5. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 5.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	55	34	41	31	13	174	.068938
failure	446	443	473	502	486	2350	.931061
n	501	477	514	533	499	2524	

Source of Variation	SS	DF	χ^2
Treatment	1.8783	4	29.26
Linear regression	1.6382	1	25.52
Deviation from linearity	.2401	3	3.74
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.0642		

The average rate of decrease is .07 percent per p.p.m.

Appendix Table 6. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 6.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	299	337	139	99	72	946	.433944
failure	90	195	300	319	330	1234	.566055
n	389	532	439	418	402	2180	

Source of Variation	SS	DF	χ^2
Treatment	113.1403	4	460.60
Linear regression	103.9960	1	423.37
Deviation from linearity	9.1443	3	37.23
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.2456		

The average rate of decrease is .64 percent per p.p.m.

Appendix Table 7. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 7.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	162	129	85	57	33	466	.210099
failure	263	305	374	350	460	1752	.789900
n	425	434	459	407	493	2218	

Source of Variation	SS	DF	χ^2
Treatment	28.1200	4	169.44
Linear regression	27.6284	1	166.48
Deviation from linearity	.4916	3	2.96
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.1660		

The average rate of decrease is .31 percent per p.p.m.

Appendix Table 8. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 8.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	77	46	39	17	11	190	.080508
failure	484	433	455	372	426	2170	.919491
n	561	479	494	389	437	2360	

Source of Variation	SS	DF	χ^2
Treatment	3.7881	4	51.17
Linear regression	3.7200	1	50.25
Deviation from linearity	.0681	3	.92
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.0740		

The average rate of decrease is .11 percent per p.p.m.

Appendix Table 9. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 9.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	60	49	21	75	1	146	.062876
failure	415	451	535	423	352	2176	.937123
n	475	500	556	438	353	2322	

Source of Variation	SS	DF	χ^2
Treatment	4.5104	4	195.32
Linear regression	4.1868	1	181.31
Deviation from linearity	.3236	3	14.01
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.0231		

The average rate of decrease is .13 percent per p.p.m.

Appendix Table 10. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 10.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	38	36	34	29	6	143	.058415
failure	408	561	424	450	462	2305	.941584
n	446	597	458	479	468	2448	

Source of Variation	SS	DF	χ^2
Treatment	1.4520	4	26.40
Linear regression	.9400	1	17.09
Deviation from linearity	.5120	3	9.31
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.0550		

The average rate of decrease is .06 percent per p.p.m.

Appendix Table 11. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 11.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	370	320	329	108	0	1127	.442307
failure	38	169	260	343	611	1421	.557692
n	408	489	589	451	611	2548	

Source of Variation	SS	DF	χ^2
Treatment	256.0986	4	1038.22
Linear regression	250.4024	1	1015.13
Deviation from linearity	5.6962	3	23.09
$\bar{\bar{y}}$ $(1-\bar{\bar{y}})$	.2467		

The average rate of decrease is .90 percent per p.p.m.

Appendix Table 12. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 12.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	426	429	216	166	9	1246	.452268
failure	108	193	312	399	497	1509	.547731
n	534	622	528	565	506	2755	

Source of Variation	SS	DF	χ^2
Treatment	209.4970	4	845.70
Linear regression	204.7580	1	826.57
Deviation from Linearity	4.7390	3	19.13
$\bar{\bar{y}}$ $(1-\bar{\bar{y}})$	.2477		

The average rate of decrease is .78 percent per p.p.m.

Appendix Table 13. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 13.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	126	79	68	50	4	327	.126303
failure	391	512	495	405	459	2262	.873696
n	517	591	563	455	463	2589	

Source of Variation	SS	DF	χ^2
Treatment	13.7088	4	124.23
Linear regression	11.8656	1	107.53
Deviation from linearity	1.8432	3	16.70
$\bar{\bar{y}}$ $(1-\bar{\bar{y}})$	.1103		

The average rate of decrease is .20 percent per p.p.m.

Appendix Table 14. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 14.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	54	48	42	12	1	157	.063537
failure	464	444	431	404	571	2314	.936462
n	518	492	473	416	572	2471	

Source of Variation	SS	DF	χ^2
Treatment	4.4140	4	74.19
Linear regression	3.9383	1	66.19
Deviation from Linearity	.4757	3	8.00
$\bar{\bar{y}}$ $(1-\bar{\bar{y}})$	.0595		

The average rate of decrease is .11 percent per p.p.m.

Appendix Table 15. Comparison of the number of Bay mussel larvae that developed normally (success) with those that failed to develop normally (failure) in Experiment 15.

	SWL concentration p.p.m.						
	0.0	25	50	75	100	Total	Ratio
success	43	41	19	21	11	135	.063890
failure	333	433	402	351	459	1978	.936109
n	376	474	421	372	470	2113	

Source of Variation	SS	DF	χ^2
Treatment	2.1390	4	35.76
Linear regression	1.8950	1	31.68
Deviation from linearity	.2440	3	4.08
$\bar{\bar{y}}(1-\bar{\bar{y}})$	.0598		

The average rate of decrease is .09 percent per p.p.m.

Appendix Table 16. Spawning occurrences for the Bay mussel over a 15-month period, December 1957 to March 1959.

Date	Number of mussels in sample	Total spawned	Sex	
			Male	Female
12/26/57	20	1	1	
3/ 1/58	60	1		1
3/26/58	24	9	8	1
3/27/58	24	7	4	3
4/ 5/58	19			
4/12/58	28	1		1
4/13/58	36	7	5	2
4/19/58	24	4	2	2
4/20/58	24	9	4	5
4/26/58	24	3	1	2
5/11/58	24	5	2	3
6/20/58	24	1	1	
6/21/58	28			
6/21/58	40	4		4
6/22/58	48	3	2	1
6/27/58	40	6	2	4
6/28/58	40	5	3	2
7/ 5/58	122	5	3	2
7/ 6/58	120	23	14	9
7/11/58	120	21	15	6
7/12/58	40	8	3	5
7/13/58	40	12	8	4
7/14/58	40	18	15	3
7/18/58	40			
7/19/58	72			
7/20/58	88	4		4
7/25/58	40	2	1	1
7/27/58	40	2	1	1
8/ 2/58	80	7	3	4
8/10/58	30	2	2	
8/12/58	54	1		1
8/14/58	56	4	1	3
8/16/58	40	1	1	
8/17/58	40	1		1
8/18/58	88	7	6	1
8/26/58	42	4	1	3
8/30/58	35	7	5	2
9/ 6/58	40	3	2	1
9/13/58	40	1	1	
10/12/58	40	3	2	1
10/31/58	40	3	3	

Appendix Table 16. (continued)

Date	Number of mussels in sample	Total spawned	Sex	
			Male	Female
11/ 2/58	49	1		1
11/ 8/58	40	1	1	
11/ 9/58	160	1	1	
11/14/58	100	1	1	
11/19/58	50	1	1	
12/20/58	64	1	1	
12/22/58	70	2	2	
12/26/58	96	5	5	
12/29/58	77	1	1	
12/30/58	84	4	3	1
1/13/59	53	2	1	1
1/21/59	69	3	1	2
1/27/59	63	2	1	1
1/31/59	75	1		1
2/ 5/59	33	2	1	1
2/11/59	32	4	3	1
2/12/59	33	3	2	1
2/20/59	36	2	1	1
2/21/59	36	6	5	1
2/28/59	69	9	7	2
3/ 1/59	70	3	1	2
3/ 8/59	70	8	5	3

Total number of times spawning attempted	63
Number of times neither male nor female spawned	4
Number of times female mussel failed to spawn	18
Number of times male mussel failed to spawn	12
Number of times either male or female failed to spawn	26

Appendix Table 17. Mill A sulfite waste mortality on eggs and larvae of Experiments 1-5.

Experiment	Concentration p.p.m.	Time hours	Percent mortality
1	0.0	48	71.6
	25	48	83.8
	50	48	92.4
	75	48	97.8
	100	48	100.0
2	0.0	72	81.4
	25	72	91.7
	50	72	94.6
	75	72	98.8
	100	72	100.0
3	0.0	48	41.6
	25	48	64.5
	50	48	64.3
	75	48	80.1
	100	48	92.8
4	0.0	72	46.1
	25	72	46.6
	50	72	66.6
	75	72	80.2
	100	72	96.6
5	0.0	48	89.0
	25	48	92.8
	50	48	92.0
	75	48	94.1
	100	48	97.3

Appendix Table 18. Mill B sulfite waste mortality on eggs and larvae of Experiments 6-10.

Experiment	Concentration p.p.m.	Time hours	Percent mortality
6	0.0	48	23.1
	25	48	36.6
	50	48	68.3
	75	48	76.3
	100	48	82.0
7	0.0	72	61.8
	25	72	70.2
	50	72	81.4
	75	72	85.9
	100	72	93.3
8	0.0	48	86.2
	25	48	90.3
	50	48	92.1
	75	48	95.6
	100	48	97.4
9	0.0	72	87.3
	25	72	90.2
	50	72	96.2
	75	72	96.5
	100	72	99.7
10	0.0	48	91.4
	25	48	93.9
	50	48	92.5
	75	48	93.9
	100	48	98.7

Appendix Table 19. Mill C sulfite waste mortality on eggs and larvae of Experiments 11-15.

Experiment	Concentration p.p.m.	Time hours	Percent mortality
11	0.0	48	9.3
	25	48	34.5
	50	48	44.1
	75	48	76.0
	100	48	100.0
12	0.0	72	20.2
	25	72	31.0
	50	72	59.0
	75	72	70.6
	100	72	98.2
13	0.0	48	75.6
	25	48	86.6
	50	48	87.9
	75	48	89.0
	100	48	99.1
14	0.0	72	89.5
	25	72	90.2
	50	72	91.1
	75	72	97.1
	100	72	99.8
15	0.0	48	88.5
	25	48	91.3
	50	48	95.4
	75	48	94.3
	100	48	97.6