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## **Final report on effects of low oxygen tensions and high levels of hydrogen sulfide on benthic marine animals**

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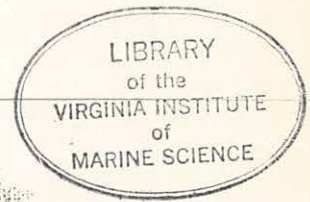
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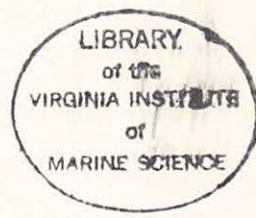
Effects of Low Oxygen Tensions and High Levels of  
Hydrogen Sulfide on Benthic Marine Animals.

by



Dexter S. Haven and Robert E. Bendl

A research project completed for the National Science  
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# ABSTRACT

This study investigated effects of low levels of dissolved oxygen (D.O.) and low levels of D.O. in combination with hydrogen sulfide ( $H_2S$ ) on the larvae and adults of oysters Crassostrea virginica and on adults of hard clams Mercenaria mercenaria. The purpose of this study was to investigate how low D.O. and low D.O. plus  $H_2S$ , which might be associated with discharges from sewerage treatment plants, could adversely influence populations of molluscs.

Field studies in Chesapeake Bay over many years have shown that low levels of oxygen may naturally be found in bay waters and values ranging between 0.0 to 0.3 ml/L commonly occur during late summer. This study indicates that these levels may be lethal to oyster larvae, adult oysters and to hard clams.

D.O. levels of 0.7 ml/L or lower in standing water cause oyster larvae to stop swimming and inhibit setting; a partial mortality occurred below 0.49 ml/L. Exposure to about 0.3 ml/L for 72 hours in flowing water resulted in 84% mortality; at 0.2 ml/L there was 100% in the same period. When  $H_2S$  was present at zero oxygen level, there was a 100% mortality in 24 hours.

Adult oysters held at 0.1 to about 0.4 ml/L for 7 to 8 days show 50% mortality; by 10 to 13 days the mortality was 100%. Low D.O. inhibits ingestion of food; at 0.3 ml/L only minimal quantities of feces are voided. Oysters exposed to zero D.O. plus  $H_2S$  ranging from 1.7 to 3.4 ml/L showed 100% mortality in 8 to 9 days.

Adult hard clams are less sensitive to low D.O. than oysters, but below about 1.7 ml/L clams begin to lose their ability to regulate oxygen consumption. Small clams survived over 38 days at D.O. levels about 0.1 ml/L with less than 5% mortality. When  $H_2S$  was present, however, (1.7 to 5.4 ml/L) 96% of the population died in 38 days.

It is concluded that if discharges from sewerage treatment plants enter estuarine systems where D.O. levels are already approaching critical levels, then the combined organic load might easily bring about levels of D.O. or  $H_2S$  which could cause extensive mortality in estuarine populations of molluscs.



## INTRODUCTION

In siting sewerage discharge systems, it is important to locate them where the added organic load will not cause dissolved oxygen values (D.O) to fall to levels which will adversely effect populations of animals especially those vulnerable to low D.O. or  $H_2S$ .

Low D.O. values (0.0 to 0.3 ml/L) often occur in the deeper waters of Chesapeake Bay and in tributary creeks in summer (Hires, Stroup and Sietz, 1966). Available evidence indicates that in recent years the problem has become more pronounced. In the lower Potomac, for example, in September 1973, oxygen became deficient at 18 feet and deeper. This resulted in a mortality of 18% of oysters in shallow water (Haven, unpublished). Other studies further indicate that in 1972, 1973 and 1974 oxygen values in the Great Wicomico River in Virginia approached 0.2 ml/L and that setting of oysters was far below average.

The literature clearly shows that low D.O. values kill or adversely influence many species of molluscs, but that the long term sub-lethal effect are poorly known (Von Brand, 1946; Morrison, 1971; Hamwi and Haskin, 1969; Hewatt, 1945-47; MacInnes and Thurberg, 1972).

$H_2S$  is associated with anaerobic conditions and an abundance of organic material. Sulfide and hydrogen sulfide are the direct result of: 1) the putrefaction of proteins under anoxic conditions, and; 2) the reduction of nitrates and nitrites in connection with the breakdown of organic materials. When nitrates and nitrites have been reduced, the reduction of sulfates occur leading to the formation

of sulfides and hydrogen sulfides (Lyche, 1956 and Gardner, 1971).

H<sub>2</sub>S is toxic to many estuarine benthic inhabitants and can cause mass mortalities especially of those organisms which are immobile (Von Brand, 1946). Two of these organisms, the American oyster Crassostrea virginica and the hard clam Mercenaria mercenaria are of economic importance and were chosen as the subjects of this study.

This project began 1 June 1972 and ended November 1974. During this period the following aspects were studied.

- I. The Effects of Low D.O. and Low D.O. Plus H<sub>2</sub>S on Survival and Biodeposition Rates of Small Oysters.
- II. Effects of Low D.O. and Low D.O. Plus H<sub>2</sub>S on Swimming and Survival of Oyster Larvae.
- III. The Effects of Low D.O. and Low D.O. Plus H<sub>2</sub>S on Survival of Hard Clams.
- IV. Effects of Low D.O. on Pumping Rates, Filtration Efficiency and Oxygen Consumption of Hard Clams.

I. The Effects of Low D.O. and Low D.O. Plus  $H_2S$  on Survival and Biodeposition Rates of Small Oysters.

MATERIALS AND METHODS

Three experiments were conducted in this phase of the study.

Sea water from the York River was drawn from the trough regulated by stop cock (C) to a nitrogen purging column (D), Figure 1. There the water was stripped of oxygen to varying levels by controlling the flow of pre-purified nitrogen with regulator (F). After being stripped, the water left the bottom of the column and was fed into flow meter (G). The oxygen regulating technique was similar to that developed by Silvers, Warren and Doudoroff (1963). Flow through the meter was controlled by stop cock (H) from which it flowed into chamber (I). The water was then siphoned from the tray into D.O. bottle (J). Samples were taken for temperature, salinity and oxygen; 5 to 8 measurements were taken daily (8 a.m. to 5 p.m.); mean values were recorded. Temperature and oxygen were measured with a YSI Model 54 oxygen meter; salinities were measured by a Beckman induction salinometer (Table 1).

During each of these studies D.O. was regulated so that four levels were run simultaneously. Mean values differed slightly in each study, but the ranges were kept within the limits shown in Table 1.

The four trays (Figure 2) were modified from a design developed by P. R. Walne (1966). These trays used baffles for flow control, and worked well since two stratified layers were created.

Twenty-one small oysters per tray ( $\bar{x}$  length 20 mm) from beak to hinge) were placed in plexi-glas "inserts" (Figure 3) that were provided with dividers. These prevented feces mixing with pseudofeces. Specimens were placed on a grooved stand and the "bill end" faced into the current. Every 72 hours, feces and pseudofeces were pipetted from the dividers, washed with distilled water, dried and weighed to the nearest 0.1 mg. These weights, for each D.O. level, were then corrected for natural sediment, collected from a control trough containing no oysters. Later calculations gave the mean weight of feces and pseudofeces produced per oyster during each study (Table 2).

### H<sub>2</sub>S Studies

H<sub>2</sub>S experiments were carried out in a closed system where the water was recycled. Sea water that was prepared as described below was forced into the chambers containing the specimens. After the chambers were completely filled and all air bubbles removed the three chambers were connected in series with tygon tubing. The tubing was placed in a peristaltic pump which circulated the water through the chambers (Figure 4).

Water with high H<sub>2</sub>S levels was prepared several days in advance. Large 18 liter bottles were filled with anoxic sea water, then anaerobic sediments from the York River were added to cover the bottom of the bottle to a depth of 5 to 6 cm. During the holding period H<sub>2</sub>S developed in the water at levels ranging from 1.7 to 3.4 ml/L; D.O. levels were 0.0 or in one instance 0.1 ml/L. Water used for D.O studies was



stored in 18 liter bottles for the same period, but no sediments were added.

Water was changed and dead specimens removed every five days. During the change, measurements were taken for pH, S<sup>0</sup>/oo, D.O. and H<sub>2</sub>S. These measurements were taken for old water being discarded as well as for water being replaced from the supply bottle. Measurements are referred to as "before and after" measurements (Table 3). This demonstrated that when the water was changed the test animals were not subject to any great change in D.O. or H<sub>2</sub>S levels.

Feces and pseudofeces measurements were not made in the H<sub>2</sub>S studies because they were conducted in a closed system where there were few if any particles to be removed by the oyster.

D.O. concentrations and temperature for all oxygen experiments were measured with a YSI polarigraphic probe. Salinities were measured on a Beckman induction salinometer. H<sub>2</sub>S measurements were taken as described by Strickland and Parsons (1968). D.O. concentrations for H<sub>2</sub>S experiments were measured with a modified Winkler method. Hydrogen-ion concentration was monitored with a Coleman pH meter with glass reference electrode.

## RESULTS

### Low D.O.

The percent mortality is expressed as the ratio of live to dead oysters; oysters were removed as dead when they gaped and would not

respond to tactile stimulation (Table 1). Data for experiments 1, 2 and 3 showed that at D.O. levels ranging from 0.1 to 0.4 ml/L about half of the oysters died in about 8 days and 100% mortality occurred between 10 and 13 days. At D.O. levels ranging from a mean of 0.3 - 0.4 ml/L survival time was much longer and mortality ranged from 0 to 14% by the 10th day. Controls kept in well oxygenated water showed zero mortality. The data shown in Table 1 for each of the low D.O. values in experiments 1, 2 and 3 are shown graphically in Figure 5.

While levels of 0.3 - 0.4 to 0.9 ml/L caused zero or only minor mortalities, these levels or lower had a decided influence on bio-deposition rates. At about 0.8 ml/L fecal production was less than half of that shown by controls, in contrast, pseudofeces production was not influenced until levels of about 0.3 ml/L were reached (Table 2). At 0.3 ml/L fecal production was zero or just measurable; pseudofeces was produced at about 10% of the level shown by controls.

#### High H<sub>2</sub>S

Studies on effects of low D.O. plus H<sub>2</sub>S were done in recycled water in contrast to those involving low D.O. alone where water was continuously changed; therefore, the results of the two studies are not strickly comparable.

When H S was present at D.O. values of 0.0, mortality was rapid. Two studies conducted at a H<sub>2</sub>S range from 1.7 to 3.4 mg/L showed 100%

mortality in 8 to 9 days; a third study showed 98% mortality in 8 days. In contrast, oysters held at 0.0 to 0.2 mg/L showed low mortalities at 8 days ranging from 17 to 22%. These mortalities are much lower than those obtained in the preceding study conducted in a flow through system. The reason for this lowered mortality may be associated with the fact that in the recycled system oysters were not open as often as in the flow through study (Tables 1 and 3).

## II. The Effects of Low D.O. and Low D.O. Plus H<sub>2</sub>S on Swimming and Survival of Oyster Larvae.

### INTRODUCTION

These experiments determined: 1) the physiological response of larvae to low D.O. values; and, 2) mortality patterns in respect to low D.O. or low D.O. plus H<sub>2</sub>S. These aspects are covered below in three sections: 1) standing water studies testing effects of low D.O.; 2) flow through studies utilizing various D.O. levels; and, 3) studies using recycled water in which larvae were subject to low D.O. plus H<sub>2</sub>S.

D.O., pH, salinity and H<sub>2</sub>S were measured as outlined in Section I.

#### Standing Water Studies

##### I. Immature Larvae

Two similar studies were conducted with immature larvae. One exposed immature larvae for 24 hours to D.O. at 4 levels (Table 4). Larvae were held in glass containers each holding about 2 liters; jars were fitted with gas tight lids. Larval density was about 10 per ml. Each oxygen level, except the control, was made in triplicate.

After 24 hours exposure to various D.O. concentrations a strong beam of light was directed against the side of the jars to determine if larvae were swimming in the water. Those swimming in the water showed up as specks of light. To confirm the presence or absence

of swimming larvae, water was collected from 5 locations above the bottom. The larvae in suspension were counted with a Coulter Electronic Particle Counter. Next, samples of larvae were collected with a pipette from the bottom of each jar (live and dead), placed under a compound microscope, and 100 examined. They were classed as those showing movement of the swimming organ (the vellum) or those showing no movement. After the initial 24 hour examination all larvae were sieved from the jars and placed in aerated sea water. All samples were again examined after 24 hours. Oyster larvae which showed no movement of the vellum or those with no tissue in the shell after this second holding period were considered dead.

The second study involving immature larvae duplicated the first except that the initial holding period was for 48 hours and not 24.

The results of both studies showed that low D.O. had an adverse effect on larvae.

During the initial 24 and 48 hour holding period there was only a slight decline in D.O. from the initial 0.21 and 0.35 ml/L levels (about 0.1 ml/L). However, values which were initially 0.70 ml/L declined by about half (0.35 to 0.42 ml/L), Table 4. Therefore, in evaluating the results of this study, exposure levels are based on those observed at the end of each experiment.

The 24 and 48 hour study showed that with one exception, D.O. values below 0.42 inhibited swimming of larvae; the exception being in the 24 hour study (0.70 ml/L initial D.O. level) when larvae were observed all swimming at the surface. These visual observations



were confirmed by counts with a Coulter Electronic Particle Counter which showed that the water in the jars above the bottom contained about the same number of particles as did filtered sea water (Table 4).

The final 24 hour examination (24 hours in aerated water) indicates that 24 hour exposure to 0.21 or 0.49 ml/L produced only light mortalities ranging from 0 to 16%. In contrast, 48 hour exposure to values below 0.28 ml/L resulted in 42 to 96% mortality. Final values ranging from 0.35 to 0.42 ml/L gave mortalities ranging from only 16 to 27%.

## II. Mature Larvae

Effects of low D.O. were evaluated on mature larvae (ready to set). The technique of holding the larvae, levels of exposure, and time intervals were the same as those for the immature larvae. However, in these experiments there was no estimate of mortality based on microscopic examination and a Coulter Counter was not used to evaluate larval density. Instead effects were evaluated on the basis of numbers of larvae setting on shell placed in the containers.

Again as in the preceding study there was a slight drop in D.O. during the 24 and 48 hour holding period from the original levels of 0.21 and 0.35 ml/L levels. Those originally established at 0.70 ml/L showed a similar concentration at 24 hours; at 48 hours, levels declined to about half (Table 5).

This study confirmed the one conducted with immature larvae in respect to effects of low D.O. on swimming ability of oyster larvae. Values below 0.35 ml/L inhibited swimming; moreover, the 24 hour study

indicated that values as high as 0.70 ml/L also inhibited swimming. In all cases only a very few of the mature larvae set in the 24 or 48 hours they were held in water with D.O. values less than 0.70 ml/L.

While values lower than 0.70 ml/L inhibited swimming and setting and caused a partial mortality of larvae, many survived and set. That is, numbers of spat per shell after 24 hours aeration was about as high on shells exposed to low D.O. as it was for the controls.

We conclude that D.O. values less than about 0.70 ml/L inhibit swimming and setting and may cause partial mortalities. It is suggested, however, that if larvae in the natural environment were to settle to the bottom as they did in the glass jars that few would survive since they would be preyed on by many species of invertebrates.

#### Flow Through System - D.O. Studies

Studies conducted in standing water were not conclusive in respect to mortality since larvae were not fed during the study and there was the probability that accumulated metabolites may have influenced the results. Therefore, additional studies were conducted. In one series, larvae were held in a system which allowed the water to be changed constantly and where larvae were exposed to several D.O. levels. Larvae were retained inside gas tight plexiglas chambers in nitex screen bags. Incurrent water was fed into the bag through a glass



tube, and exited through a second tube into a small bottle where D.O., salinity, temperature and other measurements made. Three separate studies were made. During each, 3 test and 3 control chambers were established, and similar numbers of straight hinged larvae placed in the nitex bag. Oysters were subjected to 0.2, 0.3, 0.8 ml/L and the controls. Fluctuations from the means, ~~shown in the tabular~~ material were of the same order of magnitude as that outlined in Section I. Later, at intervals of 24, 48 and 72 hours, the chambers were opened, and larvae examined. After these initial exposure periods the larvae were placed in aerated water for 24 hours and re-examined.

Mortalities of straight hinged larvae after 24 hours aeration were based on numbers of the larvae showing empty shells plus those showing no movement of the vellum or those filled with protozoans as contrasted to those shells showing movement of the vellum (Table 6).

Levels of 0.8 ml/L of D.O. produced only 14% mortality by 48 hours, but by 72 hours, 54% had died. At 0.3 ml/L 84% died by 72 hours; at 0.2 ml/L there was 100% mortality by 72 hours (Table 6 and Figure 6).

#### Cycled System - D.O. Plus H<sub>2</sub>S

Tests were run to determine the lethality of low D.O. plus H<sub>2</sub>S on oyster larvae. In this study the same apparatus was used for holding larvae as in the previous test, but the water was recycled through the chambers with a peristaltic pump (Harvard Apparatus Co.).

A source of water high in  $H_2S$  was prepared several days in advance of each study by filling 18 liter bottles with anoxic sea water and anaerobic estuarine sediments as previously described.

Controls were maintained under 0 to 0.1 ml/L D.O. Each study ran for 24 hours. Larvae were examined at the end of this period as outlined for those in the preceding studies. They were then placed in aerated water for 24 hours and re-examined.

When  $H_2S$  was present at zero D.O. levels the combination was highly lethal to larvae. Concentrations of  $H_2S$  ranging from 5.2 to 7.0 mg/L caused 100% mortality in 24 hours (Table 7; Figure 6). In contrast mortalities ranged from 7 to 14% in controls held at D.O. levels of 5.2 to 5.8 ml/L, and from 20 to 29% in groups held at zero D.O. levels.

### III. The Effects of Low D.O. and Low D.O. Plus H<sub>2</sub>S on Survival of Hard Clams.

#### INTRODUCTION

Three studies were conducted to determine the chronic effects of low D.O. and low D.O. plus H<sub>2</sub>S on hard clams.

#### MATERIALS AND METHODS

Small hard clams ranging in width from 20 to 25 mm were subjected to low D.O. and low D.O. plus H<sub>2</sub>S for periods ranging up to 38 days. Mean values used for D.O. alone ranged from 0.0 to 0.2 ml/L. In studies for D.O. plus H<sub>2</sub>S, D.O. was 0 ml/L; H<sub>2</sub>S ranged from a mean of 1.8 to 5.4 ml/L (Table 9). Techniques to hold small hard clams and measurements of temperature, salinity, pH, D.O. and H<sub>2</sub>S were the same as outlined in Section I. Variation in D.O. about the mean values shown were also the same as was outlined in the first section.

#### RESULTS

The three experiments indicated that hard clams have a much higher tolerance to low D.O. and D.O. plus H<sub>2</sub>S than oysters.

In the groups subject to a mean D.O. range from 0.0 to 0.1 ml/L, mortality in the 5 day study was 1%; in the 11 day study 5% died; in the 38 day experiment mortalities were zero (Table 8).



In contrast, the clams exposed to 0.0 ml/L D.O. and a range of  $H_2S$  from 1.8 to 5.4 ml/L showed much higher mortalities. In the 5 day study mortality was 1%; in the 11 day study 3% died. In the experiment which ran 38 days, mortality was only 4% by 27 days, but by 38 days 96% had died.

During the  $H_2S$  study over the first 27 days, up to 44% of the hard clams gaped with the siphons extended. They responded readily to stimuli; many retracted their siphons or closed when the chambers were jarred (Figure 7). After 27 days opening was erratic and the clams began to die.

IV. Effects of Low Oxygen on Pumping Rates, Filtration Efficiency and Oxygen Consumption of Hard Clams.

INTRODUCTION

There follows a summary of the MS thesis written by Mr. Dennis Walsh. These studies showed that D.O. levels below about 1.7 ml/L adversely influence hard clams.

The oxygen consumption, pumping rate and filtration efficiency of Mercenaria mercenaria, from the York River, Virginia, were measured at low oxygen tensions and compared to the same measurements taken at high oxygen tensions. All experiments were conducted under naturally fluctuating conditions of salinity, temperature, turbidity, and food levels. Analysis of results from 30 clams indicated Mercenaria could maintain a constant oxygen consumption in declining oxygen tension, but the critical oxygen tension ( $P_c$ ) at which this respiratory regulation ceased appeared to depend on the clam's size and sex. Gravid females, dry tissue weight 1.20 - 5.03 g, displayed a  $P_c$  near an oxygen tension of 40 mm Hg (25% sat. or about 1.7 ml/L\*). Male clams with gametes, whose dry tissue weight was less than 3.0 g had a  $P_c$  near 80 mm Hg oxygen tension (50% sat. or 2.8 ml/L\*). Some evidence is offered that larger males have a  $P_c$  similar to females. Three modes of respiratory regulation were observed. In the first, oxygen utilization and pumping rate remained unchanged at all oxygen levels above  $P_c$  such that the

\* Assumes 16‰ and 23°C.

oxygen consumption, the product of these two variables, remained unchanged. In the second mode a decrease in pumping rate was compensated by a sufficient increase in oxygen utilization to give a constant oxygen consumption. In an anomalous third mode, an increase in pumping rate was not fully compensated by a decrease in oxygen utilization, but oxygen consumption remained constant. The efficiency with which the gill's cilia were able to remove particles in the 3-20 m range was found to be independent of the pumping rate, oxygen consumption and the oxygen tensions. Multiple linear regression analysis showed that the observed values of turbidity and food level showed little effect on either male or female Mercenaria at high or low oxygen tensions. Sexual differences were again evident at high versus low oxygen tensions when size of the clam was analyzed by MLR analysis.

## CONCLUSIONS

### Sections I - IV

This study has shown that low D.O. values similar to those which occur naturally in Chesapeake Bay may adversely influence populations of oysters and hard clams.

A major adverse effect would be on larvae of oysters which are planktonic for about two weeks before they attach to shell substrate. During this period the larvae may be transported by tidal currents many miles from their point of origin (Wood and Hargis, 1971). Often, this transport occurs in the lower regions of the estuaries, similar

to Chesapeake Bay, where D.O. levels are lowest. If during this transport, the water becomes deficient in D.O., or if the larvae (during periods of vertical migration) enter layers where D.O. levels are critical, there will be a good probability that many will die.

The adults of oysters and hard clams are especially vulnerable to low D.O. levels since they are sedentary. In Chesapeake Bay both species of molluscs occur in regions where D.O. values may become low each summer due to natural causes.

It is clear that if sewerage with its high BOD levels were added to a system which was only slightly above the critical level that the added impact on the ecosystem might have extremely adverse effects on molluscan populations.

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Table 1

Mortality of oysters exposed to varying levels of D.O.

EXPERIMENT 1

Days	$\bar{x}$ O <sub>2</sub> Level ml/L	O <sub>2</sub> ml/L Range	S‰	T°C	% Open	Daily Mortalities	Cumulative % Mortality
1	0.2	0.1 - 0.3	16.8	23	7	0	0
	0.3	0.1 - 0.3	16.8	23	40	0	0
	0.6	0.3 - 1.0	16.8	23	52	0	0
	4.3	3.6 - 4.6	16.8	23	80	0	0
2	0.1	SAME AS ABOVE	17.2	23	9	0	0
	0.3		17.2	23	44	0	0
	0.8		17.2	23	39	0	0
	4.4		17.2	23	57	0	0
3	0.1		17.2	23	0	0	0
	0.3		17.2	23	28	0	0
	0.8		17.2	23	91	0	0
	4.4		17.2	23	83	0	0
4	0.1		17.3	24	4	1	5
	0.3		17.3	24	20	0	0
	0.8		17.3	24	90	0	0
	4.4		17.3	24	91	0	0
5	0.2		17.3	23	21	2	14
	0.3		17.3	23	35	0	0
	0.9		17.3	23	42	0	0
	4.7		17.3	23	52	0	0
7	0.2		16.8	24	0	4	33
	0.3		16.8	24	0	0	0
	0.9		16.8	24	0	0	0
	4.7		16.8	24	33	0	0
8	0.2		16.8	24	37	4	52
	0.3		16.8	24	69	0	0
	0.9		16.8	24	41	0	0
	4.7		16.8	24	64	0	0
9	0.2		18.9	23	44	3	66
	0.3		18.9	23	29	0	0
	0.9		18.9	23	55	0	0
	4.7		18.9	23	30	0	0

Table 1 (Contd.)

## Experiment 1 (Contd.)

Days	$\bar{x}$ O <sub>2</sub> Level ml/L	O <sub>2</sub> ml/L Range	S <sup>o</sup> /oo	T <sup>o</sup> C	% Open	Daily Mortalities	Cumulative % Mortality
10	0.2	SAME AS ABOVE	18.6	23	44	2	71
	0.3		18.6	23	29	0	0
	0.6		18.6	23	55	0	0
	4.4		18.6	23	70	0	0
11	0.2	SAME AS ABOVE	18.9	23	52	3	90
	0.3		18.9	23	26	0	0
	0.7		18.9	23	61	0	0
	4.3		18.9	23	70	0	0
12	0.2	SAME AS ABOVE	18.7	23	--	2	100
	0.4		18.7	23	28	0	0
	0.7		18.7	23	68	0	0
	4.6		18.7	23	70	0	0

Table 1 (Contd.)

EXPERIMENT 2

Days	$\bar{x}$ O <sub>2</sub> Level ml/L	O <sub>2</sub> ml/L Range	S <sup>o</sup> /oo	T <sup>o</sup> C	% Open	Daily Mortalities	Cumulative % Mortality
1	0.4	0.1 - 0.5	15.5	22	0	0	0
	0.5	0.3 - 0.7	15.5	22	26	0	0
	1.1	0.3 - 1.2	15.5	22	57	0	0
	4.6	3.1 - 5.8	15.5	22	49	0	0
2	0.2	SAME AS ABOVE	14.0	22	52	0	0
	0.4		14.0	22	40	0	0
	0.7		14.0	22	55	0	0
	5.3		14.0	22	80	0	0
3	0.2		14.4	22	57	0	0
	0.4		14.4	22	71	0	0
	0.8		14.4	22	75	0	0
	4.3		14.4	22	85	0	0
4	0.2		13.5	21	61	0	0
	0.4		13.5	21	66	0	0
	0.8		13.5	21	55	0	0
	4.1		13.5	21	55	0	0
5	0.2		13.7	22	59	2	9
	0.4		13.7	22	61	0	0
	0.8		13.7	22	50	0	0
	4.8		13.7	22	71	1	0
7	0.2		13.0	22	62	3	22
	0.4		13.0	22	47	0	0
	0.8		13.0	22	47	0	0
	4.0		13.0	22	71	0	0
8	0.2		12.1	22	83	5	47
	0.7		12.1	22	65	1	4
	1.0		12.1	22	75	0	0
	5.8		12.1	22	66	0	0
9	0.2		12.8	22	60	6	76
	0.4		12.8	22	34	0	4
	0.7		12.8	22	65	0	0
	4.4		12.8	22	80	0	0
10	0.4		14.5	22	--	5	100
	0.4		14.5	22	21	1	8
	0.9		14.5	22	36	0	0
	4.1		14.5	22	47	0	0

Table 1 (Contd.)

EXPERIMENT 3							
Days	$\bar{x}$ O <sub>2</sub> Level ml/L	O <sub>2</sub> ml/L Range	S <sup>o</sup> /oo	T <sup>o</sup> C	% Open	Daily Mortalities	Cumulative % Mortality
1	0.2	0.1 - 0.5	21.2	23	19	0	0
	0.4	0.3 - 0.5	21.2	23	23	0	0
	0.9	0.5 - 1.0	21.2	23	38	0	0
	4.3	2.7 - 5.3	21.2	23	47	0	0
2	0.1		21.5	23	14	0	0
	0.4		21.5	23	33	0	0
	0.9		21.5	23	28	0	0
	4.3		21.5	23	42	0	0
3	0.1		21.6	23	57	0	0
	0.4		21.6	23	0	0	0
	0.8		21.6	23	23	0	0
	4.4		21.6	23	47	0	0
4	0.2		21.0	23	14	1	4
	0.4		21.0	23	18	0	0
	0.9		21.0	23	22	0	0
	4.3		21.0	23	23	0	0
5	0.1		21.2	23	20	5	28
	0.3		21.2	23	22	1	4
	0.8		21.2	23	33	0	0
	4.2		21.2	23	47	0	0
6	0.1		22.3	22	20	0	28
	0.3		22.3	22	14	0	4
	0.8		22.3	22	34	0	0
	4.4		22.3	22	48	0	0
7	0.1		22.5	23	10	1	33
	0.3		22.5	23	20	0	4
	0.8		22.5	23	23	0	0
	4.6		22.5	23	36	0	0
8	0.1		21.3	23	5	1	38
	0.3		21.3	23	10	0	4
	0.8		21.3	23	13	0	0
	4.6		21.3	23	46	0	0
9	0.1		20.0	22	7	0	38
	0.3		20.0	22	20	1	8
	0.9		20.0	22	15	0	0
	4.6		20.0	22	48	0	0
10	0.1		20.1	22	4	3	52
	0.3		20.1	22	15	0	14
	0.9		20.1	22	16	0	0
	4.3		20.1	22	36	0	0



Table 1 (Contd.)

## Experiment 3 (Contd.)

Days	$\bar{x}$ O <sub>2</sub> Level ml/L	O <sub>2</sub> ml/L Range	S <sup>o</sup> /oo	T <sup>o</sup> C	% Open	Daily Mortalities	Cumulative % Mortality
11	0.1	SAME AS ABOVE	20.3	22	5	3	66
	0.3		20.3	22	9	0	14
	0.9		20.3	22	12	0	0
	4.3		20.3	22	40	0	0
12	0.1	SAME AS ABOVE	22.5	23	10	5	90
	0.3		22.5	23	25	0	14
	0.8		22.5	23	21	0	0
	4.3		22.5	23	48	0	0
13	0.1	SAME AS ABOVE	22.2	22	1	2	100
	0.4		22.2	22	10	1	14
	0.9		22.2	22	15	0	0
	4.3		22.2	22	48	0	0

Table 2

Effect of Various Dissolved Oxygen Concentrations on Production of Feces and Pseudofeces (Biodeposition Rates) by Oysters.  
Quantity shown is mg dry weight per oyster for 72 hours.

Mean O <sub>2</sub> * Level ml/L		Exp. # 1 Hours Exposed			Exp. # 2 Hours Exposed			Exp. # 3 Hours Exposed		
		72	144	216	72	144	216	72	144	216
4.3	Feces	58.6	69.4	55.5	16.7	42.3	32.6	22.4	16.2	19.6
	P/Feces	96.4	124.6	95.8	29.6	28.8	28.8	25.4	37.3	22.8
0.8	Feces	23.6	62.0	11.9	3.4	3.0	2.9	12.5	6.1	13.5
	P/Feces	78.5	124.9	72.8	24.7	25.8	24.5	15.6	9.9	14.2
0.3	Feces	0	14.0	0	0	0	0	8.8	2.8	4.5
	P/Feces	15.1	46.5	38.9	5.4	19.2	8.5	6.2	11.4	8.3
0.2	Feces	0	0	1.3	0	0	0	0.8	0	0
	P/Feces	3.7	15.0	27.9	3.8	2.5	2.5	3.2	1.2	1.4

\* Values for individual experiments are shown in Table 1.

Table 3  
Effects of H<sub>2</sub>S and low Dissolved Oxygen Concentrations on Oysters

EXPERIMENT 1										EXPERIMENT 2									
Days	Chamber	Temp	S‰	pH	H <sub>2</sub> S ml/L (MTP)	ug-at S/L	O <sub>2</sub> ml/L	% Open	% Mortality	Days	Chamber	Temp	S‰	pH	H <sub>2</sub> S ml/L (MTP)	ug-at S/L	O <sub>2</sub> ml/L	% Open	% Mortality
1	Test	22	18.4	7.2	2.8	123	0.0	4	0	1	Test	24	18.2	7.4	1.7	78	0.0	6	0
	Control	22	18.4	7.6	0.0	0	0.2	8	0		Control	24	18.2	7.6	0.0	0	0.2	15	0
2	Test	23	18.4	---	---	---	---	2	0	2	Test	24	18.2	---	---	---	---	8	5
	Control	23	18.4	---	---	---	---	3	0		Control	24	18.2	---	---	---	---	12	0
3	Test	23	18.4	---	---	---	---	3	5	3	Test	24	18.2	---	---	---	---	3	25
	Control	23	18.4	---	---	---	---	10	0		Control	24	18.2	---	---	---	---	6	1
4	Test	23	18.4	---	---	---	---	6	25	4	Test	24	18.2	---	---	---	---	0	38
	Control	23	18.4	---	---	---	---	12	0		Control	24	18.2	---	---	---	---	10	5
BEFORE										BEFORE									
5	Test	23	18.4	7.2	2.9	130	0.0	2	38	5	Test	24	18.2	7.3	1.9	86	0.0	4	46
	Control	23	18.4	7.5	0.0	0	0.0	6	13		Control	24	18.2	7.6	0.0	0.0	0.0	15	8
AFTER										AFTER									
	Test	24	18.8	7.2	3.3	148	0.0	0	---		Test	24	18.5	7.3	2.4	110	0.0	---	---
	Control	24	18.8	7.6	0.0	0	0.1	8	---		Control	24	18.5	7.5	0.0	0.0	0.2	---	---
6	Test	24	18.8	---	---	---	---	2	68	6	Test	24	18.5	---	---	---	---	2	62
	Control	24	18.8	---	---	---	---	12	14		Control	24	18.5	---	---	---	---	15	10
7	Test	24	18.8	---	---	---	---	5	76	7	Test	24	18.5	---	---	---	---	0	78
	Control	24	18.8	---	---	---	---	15	16		Control	24	18.5	---	---	---	---	20	15
8	Test	24	18.8	7.3	3.4	156	0.0	6	100	8	Test	24	18.5	---	---	---	---	0	85
	Control	24	18.8	7.4	0.0	0	0.0	10	16		Control	24	18.5	---	---	---	---	10	20

Table 3 (Contd.)

## EXPERIMENT 3

Days	Chamber	Temp	pH	B <sub>2</sub> S ml/L (NTP)	Wt -at S/L	O <sub>2</sub> ml/L	% Open	% Mortality
1	Test	24	17.8	7.2	3.0	136	0.0	
	Control	24	17.8	7.7	0.0	0.0	3	0
2	Test	24	17.8	---	---	---	10	0
	Control	24	17.8	---	---	---	12	5
3	Test	24	17.8	---	---	---	0	28
	Control	24	17.8	---	---	---	16	3
4	Test	24	17.8	---	---	---	1	42
	Control	24	17.8	---	---	---	8	8
5	Test	24	17.8	7.2	3.2	146	0.0	58
	Control	24	17.8	7.6	0.0	0.0	6	8
	Test	24	18.0	7.4	3.0	138	0.0	---
	Control	24	18.0	7.8	0.0	0.0	0.1	---
6	Test	24	18.0	---	---	---	0	76
	Control	24	18.0	---	---	---	10	10
7	Test	24	18.0	---	---	---	2	85
	Control	24	18.0	---	---	---	8	12
8	Test	24	18.0	7.4	3.1	142	0.0	98
	Control	24	18.0	7.8	0.0	0.0	9	17

## Effects of Low Oxygen on Oyster Larvae (Straight Hinge)

Initial O <sub>2</sub> Level Ml/Liter	T°C		Final O <sub>2</sub> Level Ml/Liter	T°C	Swimming with Light	Coulter Counts Larvae/ Ml	% Vellum Moving	O <sub>2</sub> Ml/L	T°C	Swimming with Light	% Vellum Moving	% Mortality	
0.21	19	24 Hours	0.21	19	None	0.62	82	5.46	19	Yes	84	16	
0.21	19		0.21	19	None	0.87	94	5.53	19	Yes	98	2	
0.21	19		0.21	19	None	0.62	94	5.46	19	Yes	98	2	
0.35	19		0.28	19	None	0.68	94	5.04	19	Yes	96	4	
0.35	19		0.49	19	None	0.75	96	5.46	19	Yes	100	0	
0.35	19		0.35	19	None	0.63	90	5.32	19	Yes	98	2	
0.70	19		0.35	19	At	--	96	5.60	19	Yes	100	0	
0.70	19	48 Hours	0.35	19	Top	--	100	5.04	19	Yes	100	0	
0.70	19		0.35	19	--	96	5.04	19	Yes	98	2		
5.04	19		4.76	19	All	10.3	100	4.80	19	Yes	100	0	
NATURAL BACK GROUND COUNT													
FILTERED SEA WATER (1u)			0.70										
0.21	19		48 Hours	0.28	19	None	1.4	52	5.46	19	Yes	42	58
0.21	19			0.28	19	None	1.2	0	5.04	19	Yes	4	96
0.21	19	0.28		19	None	1.6	41	5.32	19	Yes	58	42	
0.35	19	0.28		19	None	2.6	30	5.04	19	None	51	49	
0.35	19	0.21		19	None	0.1	0	5.60	19	Slight	36	74	
0.35	19	0.28		19	None	1.8	50	5.30	19	Yes	38	72	
0.70	19	0.35		19	None	1.8	66	5.32	19	Yes	84	16	
0.70	19	48 Hours	0.42	19	None	1.8	32	5.04	19	Yes	63	27	
0.70	19		0.35	19	None	1.3	78	5.32	19	Yes	84	16	
5.11	19		3.9	19	Yes	12.1	100	4.0	19	Yes	100	0	
NATURAL BACKGROUND COUNT													
FILTERED SEA WATER (1u)			1.2										
			S°/oo 16.90										

Table 5

(Standing Water)

Effects of Low Oxygen on Oyster Setting  
(Mature Larvae)

O <sub>2</sub> Level Ml/Liter	Temp. °C		O <sub>2</sub> Level Ml/Liter	Temp. °C	Swimming with Light	Spat Strikes		O <sub>2</sub> Level Ml/Liter	Temp. °C	Swimming with Light	Spat Set No/Shell
0.21	21	24 Hours	0.14	21	None	0	24 Hours Aeration	4.06	21	Yes	1000
0.21	21		0.21	21	None	0		4.20	21	Yes	860
0.14	21		0.17	21	None	0		3.36	21	Yes	1000
0.35	21		0.14	21	None	0		4.06	21	Yes	800
0.35	21		0.14	21	None	0		3.85	21	Yes	800
0.35	21		0.70	21	None	0		4.06	21	Yes	750
0.70	21		0.70	21	None	7		3.92	21	Yes	700
0.70	21		0.70	21	None	3		3.71	21	Yes	700
0.70	21		0.70	21	None	6		4.20	21	Yes	800
4.55	21		4.02	21	Yes	83		4.41	21	Yes	500
0.14	21	48 Hours	0.14	21	None	0	24 Hours Aeration	4.55	21	Yes	203
0.21	21		0.21	21	None	0		4.34	21	Yes	750
0.21	21		0.21	21	None	0		3.85	21	Yes	800
0.35	21		0.35	21	None	0		4.34	21	Yes	250
0.35	21		0.35	21	None	0		4.55	21	Yes	1500
0.35	21		0.35	21	None	0		3.71	21	Yes	150
0.70	21		0.35	21	None	0		3.85	21	Yes	600
0.70	21		0.35	21	None	0		3.71	21	Yes	500
0.70	21		0.07	21	None	0		4.55	21	Yes	150
4.60	21		4.30	21	Yes	500		4.55	21	Yes	1000
S <sup>o</sup> /oo 16.02											

\* To the nearest 100

Table 6

Physiological Characteristics of Oyster Larvae at Varied O<sub>2</sub> Levels  
(Straight Hinge)

		Ex- posed Hrs	Temp °C	% O <sub>2</sub> ml/L	S‰	% Velum Extended	% Velum Not Extended	% Velum Moving	% Velum Stopped	% Shell Full	% Shell Empty	% Shell Protozoa	% Velum Extended	% Velum Not Extended	% Velum Moving	% Velum Stopped	% Shell Full	% Shell Empty	% Shell Protozoa	% Mortality*
Experiment 1	Test	24	26	0.2	18.6	21	79	12	88	100	0	0	74	16	93	7	100	0	0	7
	Control	24	26	4.2	18.6	56	44	86	14	100	0	0	58	42	94	6	100	0	0	6
	Test	48	26	0.2	18.6	4	96	4	96	100	0	0	28	72	30	70	100	0	0	70
	Control	48	26	4.5	18.6	30	70	90	10	100	0	0	40	60	90	10	100	0	0	10
	Test	72	26	0.2	18.2	12	88	0	100	100	0	0	0	30	0	30	30	38	32	100
	Control	72	26	4.3	18.2	52	48	86	14	100	0	0	47	53	92	8	100	0	0	8
Experiment 2	Test	24	26	0.3	18.1	33	67	15	85	100	0	0	63	37	89	11	100	0	0	11
	Control	24	26	4.2	18.1	68	32	92	8	100	0	0	72	28	93	7	100	0	0	7
	Test	48	26	0.3	18.1	21	79	38	62	100	0	0	32	48	50	30	80	20	0	50
	Control	48	26	4.3	18.1	84	16	100	0	100	0	0	28	72	96	4	100	0	0	4
	Test	72	26	0.3	18.1	4	78	0	82	82	14	4	14	22	16	20	36	40	24	84
	Control	72	26	4.4	18.1	35	65	84	16	100	0	0	50	44	84	10	94	4	2	16
24 Hrs Aeration																				
Experiment 3	Test	24	26	0.8	18.1	52	48	84	16	100	0	0	64	36	86	14	100	0	0	14
	Control	24	26	4.2	18.1	64	36	92	8	100	0	0	72	28	90	10	100	0	0	10
	Test	48	26	0.8	18.2	36	64	90	10	100	0	0	20	80	86	4	90	8	2	14
	Control	48	26	4.0	18.2	43	57	80	20	100	0	0	26	70	80	16	96	4	0	20
	Test	72	26	0.8	18.8	14	86	24	76	100	0	0	26	74	46	54	100	0	0	54
	Control	72	26	4.4	18.8	56	44	46	54	100	0	0	38	62	87	13	100	0	0	13

\* % shell with protozoan + % shells empty + % velum stopped





Table 8

Three Studies Showing Effects of Low Dissolved Oxygen and Low  
Dissolved Oxygen Plus Hydrogen Sulfide on Hard Clams.

Experiment 1

Days	Chamber	T°C	S/∞	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	ix Open	% Mortality
1	Test	23	17.0	7.3	91	2.0	0.0	3	0
	Control	23	17.0	7.8	0.0	0.0	0.1	10	0
2	Test	22	17.0	---	---	---	---	2	0
	Control	22	17.0	---	---	---	---	8	0
3	Test	22	17.0	---	---	---	---	1	1
	Control	22	17.0	---	---	---	---	13	1
4	Test	22	17.0	---	---	---	---	4	1
	Control	22	17.0	---	---	---	---	34	1
5	Test	22	17.0	7.1	86	1.8	0.0	2	1
	Control	22	17.0	7.6	0.0	0.0	0.0	6	1

Experiment 2

Days	Chamber	T°C	S/∞	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	ix Open	% Mortality
1	Test	22	17.6	7.2	179.2	3.9	0.0	9	0
	Control	22	17.6	7.8	0.0	0.0	0.0	10	0
2	Test	22	17.6	---	---	---	---	3	0
	Control	22	17.6	---	---	---	---	7	0
3	Test	22	17.6	---	---	---	---	6	0
	Control	22	17.6	---	---	---	---	13	0
4	Test	22	17.6	---	---	---	---	2	0
	Control	22	17.6	---	---	---	---	12	3
5	Test	22	17.6	---	---	---	---	5	0
	Control	22	17.6	---	---	---	---	10	3

Table 8 (Contd.)

Experiment 2 (Contd.)									
Days	Chamber	T°C	S/∞	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	$\bar{x}$ Open	% Mortality
BEFORE									
7	Test Control	22	17.6	7.0	125.0	2.8	0.0	5	1
		22	17.6	7.6	0.0	0.0	0.0	3	3
AFTER									
	Test Control	22	18.1	7.2	139.5	3.1	0.0	--	1
		22	18.1	7.7	0.0	0.0	0.0	--	--
8	Test Control	22	18.1	---	---	---	---	6	1
		22	18.1	---	---	---	---	10	4
9	Test Control	22	18.1	---	---	---	---	3	1
		22	18.1	---	---	---	---	4	4
10	Test Control	22	18.1	---	---	---	---	3	3
		22	18.1	---	---	---	---	5	5
11	Test Control	22	18.1	7.1	129.6	2.9	0.0	3	3
		22	18.1	7.6	0.0	0.0	0.0	6	5

Experiment 3

Days	Chamber	T°C	S/∞	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	$\bar{x}$ Open	% Mortality
1	Test Control	24	18.0	7.2	17.79	3.9	0.0	3	0
		24	18.0	7.6	0.0	0.0	0.0	13	0
2	Test Control	24	18.0	---	---	---	---	1	0
		24	18.0	---	---	---	---	3	0
3	Test Control	23	18.0	---	---	---	---	1	0
		23	18.0	---	---	---	---	1	0

Table 8 (Contd.)

Experiment 3 (Contd.)

Days	Chamber	T°C	S‰	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	x̄ Open	% Mortality
4	Test	23	18.0	---	---	---	---	3	0
	Control	23	18.0	---	---	---	---	2	0
BEFORE									
5	Test	23	18.0	7.1	161.0	3.6	0.0	8	0
	Control	23	18.0	7.6	0.0	0.0	0.0	5	0
AFTER									
	Test	23	17.8	7.1	110.0	2.4	0.0	--	--
	Control	23	17.8	7.7	0.0	0.0	0.1	--	--
6	Test	24	17.8	---	---	---	---	2	2
	Control	24	17.8	---	---	---	---	3	0
9	Test	24	17.8	---	---	---	---	8	2
	Control	24	17.8	---	---	---	---	10	0
BEFORE									
10	Test	24	17.8	7.2	93.6	2.0	0.0	7	2
	Control	24	17.8	7.7	0.0	0.0	0.0	6	0
AFTER									
	Test	24	18.0	7.1	245	5.4	0.0	--	--
	Control	24	18.0	7.6	0.0	0.0	0.0	--	--
11	Test	23	18.0	---	---	---	---	1	2
	Control	23	18.0	---	---	---	---	0	0
12	Test	23	18.0	---	---	---	---	4	2
	Control	23	18.0	---	---	---	---	5	0
13	Test	23	18.0	---	---	---	---	7	2
	Control	23	18.0	---	---	---	---	6	0

Table 8 (Contd.)

Experiment 3 (Contd.)

Days	Chamber	T <sup>o</sup> C	S <sup>o</sup> oo	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	lx Open	% Mortality
14	Test	23	18.0	---	---	---	---	14	4
	Control	23	18.0	---	---	---	---	21	0
BEFORE									
15	Test	23	18.0	7.1	220	4.9	0.0	0	4
	Control	23	18.5	---	---	---	---	30	0
AFTER									
	Test	23	18.5	7.2	156	3.4	0.0	--	--
	Control	23	18.5	7.7	0.0	0.0	0.0	--	--
18	Test	23	18.5	---	---	---	---	24	4
	Control	23	18.5	---	---	---	---	16	0
19	Test	23	18.5	---	---	---	---	44	4
	Control	23	18.5	---	---	---	---	30	0
BEFORE									
20	Test	23	18.5	7.1	125	38	0.0	3	4
	Control	23	18.5	7.5	0.0	0.0	0.0	0	0
AFTER									
	Test	23	18.8	7.2	136	3.0	0.0	--	--
	Control	23	18.8	7.5	0.0	0.0	0.0	--	--
21	Test	23	18.8	---	---	---	---	4	4
	Control	23	18.8	---	---	---	---	0	0
22	Test	23	18.8	---	---	---	---	12	4
	Control	23	18.8	---	---	---	---	0	0
23	Test	23	18.8	---	---	---	---	11	4
	Control	23	18.8	---	---	---	---	3	0
24	Test	23	18.8	---	---	---	---	1	4
	Control	23	18.8	---	---	---	---	3	0

Table 8 (Contd.)

Experiment 3 (Contd.)

Days	Chamber	T°C	S <sup>o</sup> /oo	pH	ug -at S/L	ML (H <sub>2</sub> S)/L (NTP)	O <sub>2</sub>	̄x Open	% Mortality
25	Test	23	18.8	7.3	110	2.4	0.0	10	4
	Control	23	18.8	7.8	0.0	0.0	0.0	4	0
26	Test	23	17.9	---	---	---	---	10	4
	Control	23	17.9	---	---	---	---	5	0
27	Test	23	17.9	---	---	---	---	12	4
	Control	23	17.9	---	---	---	---	4	0
30	Test	24	17.9	7.2	140	3.1	0.0	6	11
	Control	24	17.9	7.2	0.0	0.0	0.0	4	0
	Test	24	18.4	7.3	165	3.6	0.0	--	--
	Control	24	18.4	7.8	0.0	0.0	0.1	--	--
31	Test	24	18.4	---	---	---	---	12	11
	Control	24	18.4	---	---	---	---	12	0
32	Test	24	18.4	---	---	---	---	19	11
	Control	24	18.4	---	---	---	---	15	0
33	Test	24	18.4	---	---	---	---	8	22
	Control	24	18.4	---	---	---	---	4	0
34	Test	24	18.4	---	---	---	---	13	32
	Control	24	18.4	---	---	---	---	17	0
BEFORE									
35	Test	24	18.4	7.2	145	3.2	0.0	15	35
	Control	24	18.4	7.7	0.0	0.0	0.0	9	0
	Test	24	18.4	7.3	229	5.1	0.0	--	--
	Control	24	18.4	7.8	0.0	0.0	0.0	--	--
36	Test	24	18.4	---	---	---	---	2	61
	Control	24	18.4	---	---	---	---	1	--
37	Test	24	18.4	---	---	---	---	1	73
	Control	24	18.4	---	---	---	---	0	0
38	Test	24	18.4	7.2	211	4.7	0.0	0	96
	Control	24	18.4	7.6	0.0	0.0	0.0	0	0

Figure 1.

Shows basic design of apparatus used to hold oysters, oyster larvae and hard clams.

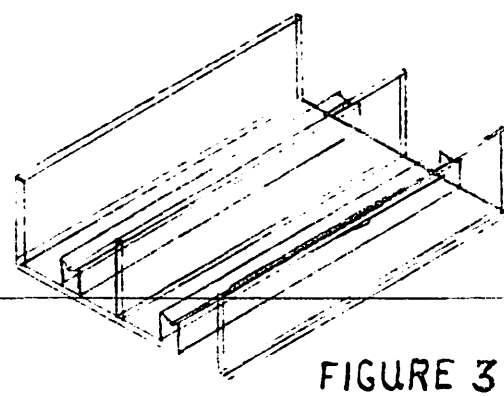
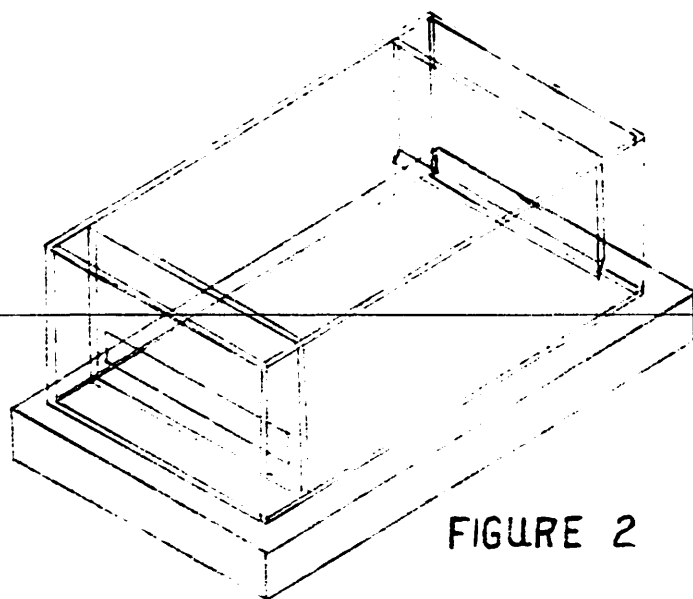
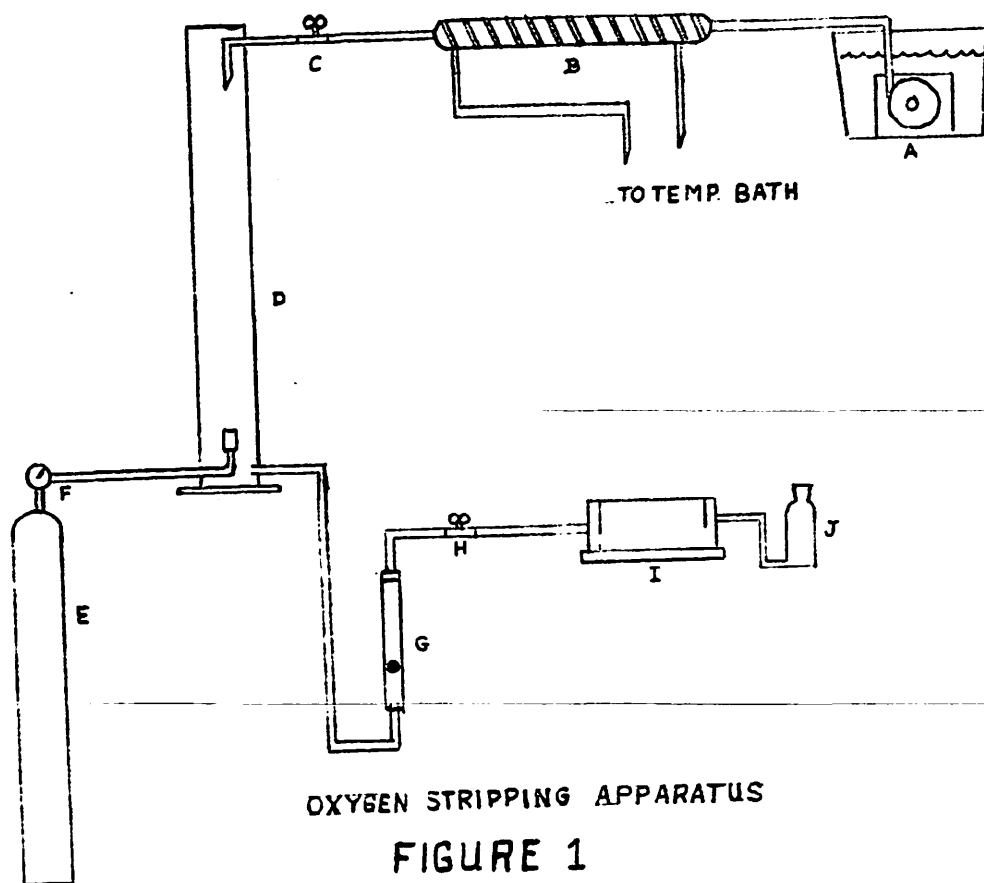
- A. Submerged pump
- B. Constant temperature coil
- C. Stop cock
- D.  $N_2$  purging column
- E.  $N_2$  cylinder
- F. Valve to regulate  $N_2$  flow
- G. Flow meter
- H. Stop cock to regulate flows
- I. Chamber to hold test animals
- J. Sample D.O. bottle

Figure 2.

Details of holding chamber (I) which could be fitted with a gas tight cover. This was used for larval studies and for  $H_2S$  experiments.

Figure 3.

Details of plexiglas inserts used to test chronic effects of low D.O. on hard clams and oysters.



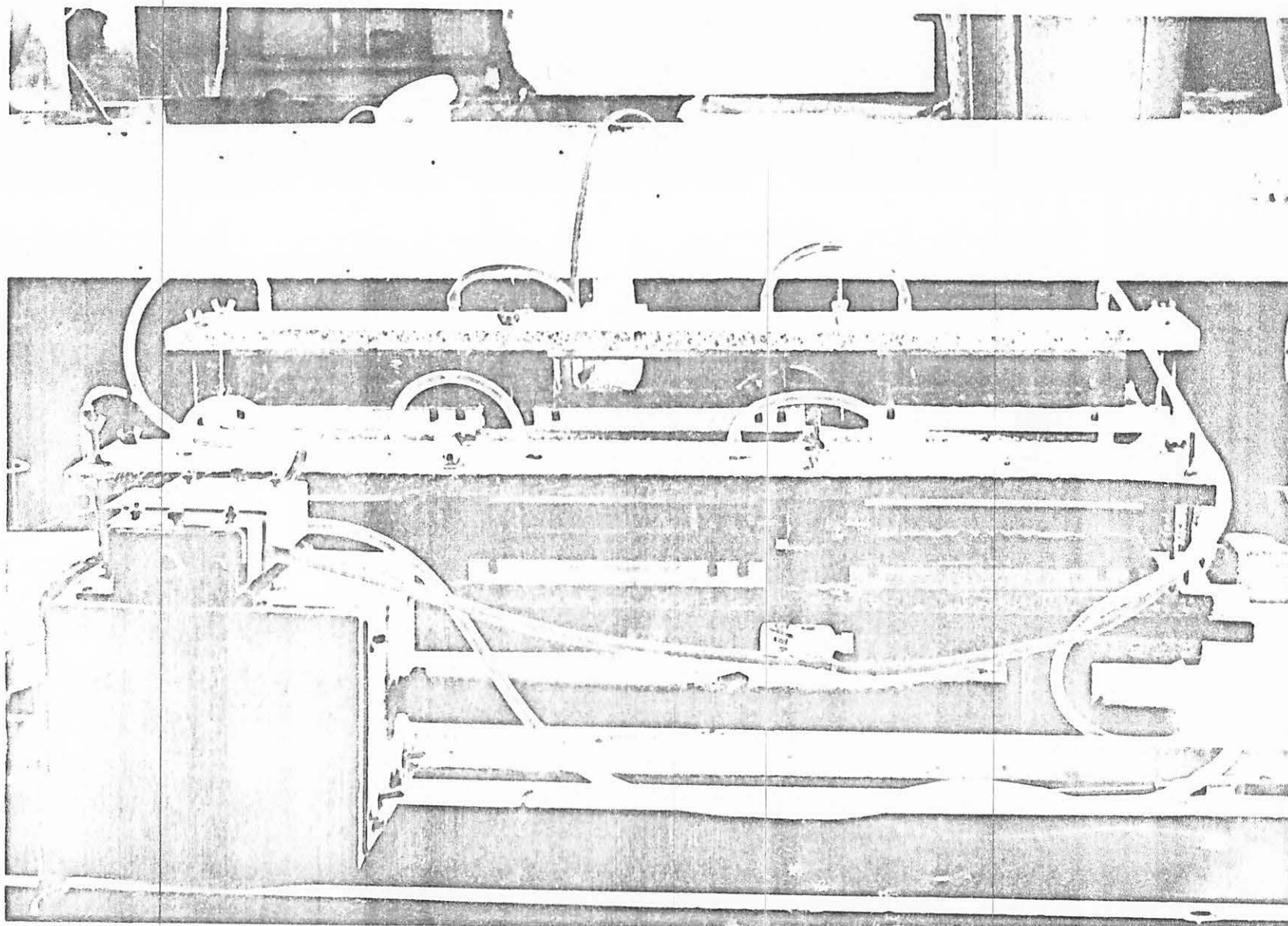
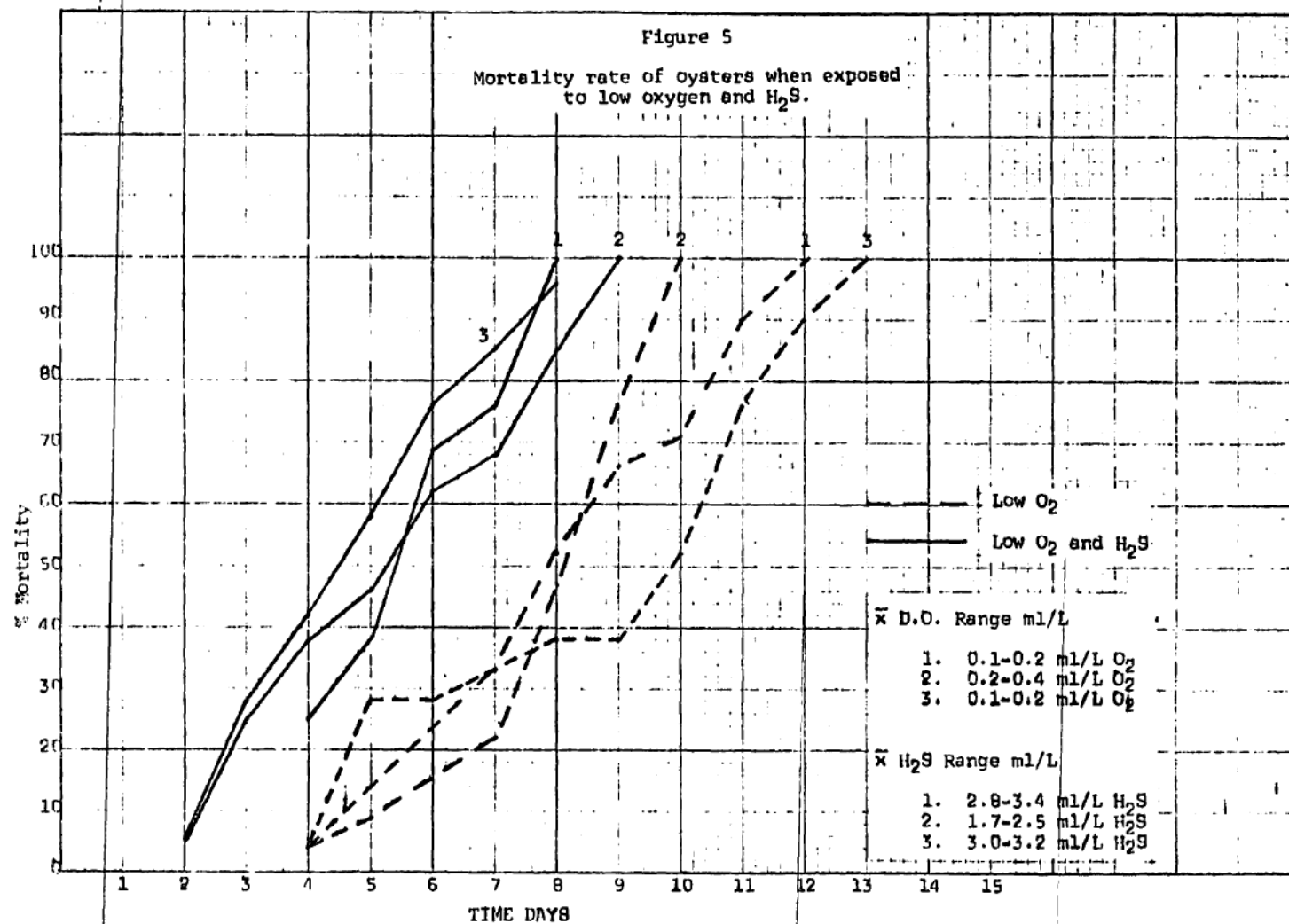


Figure 4

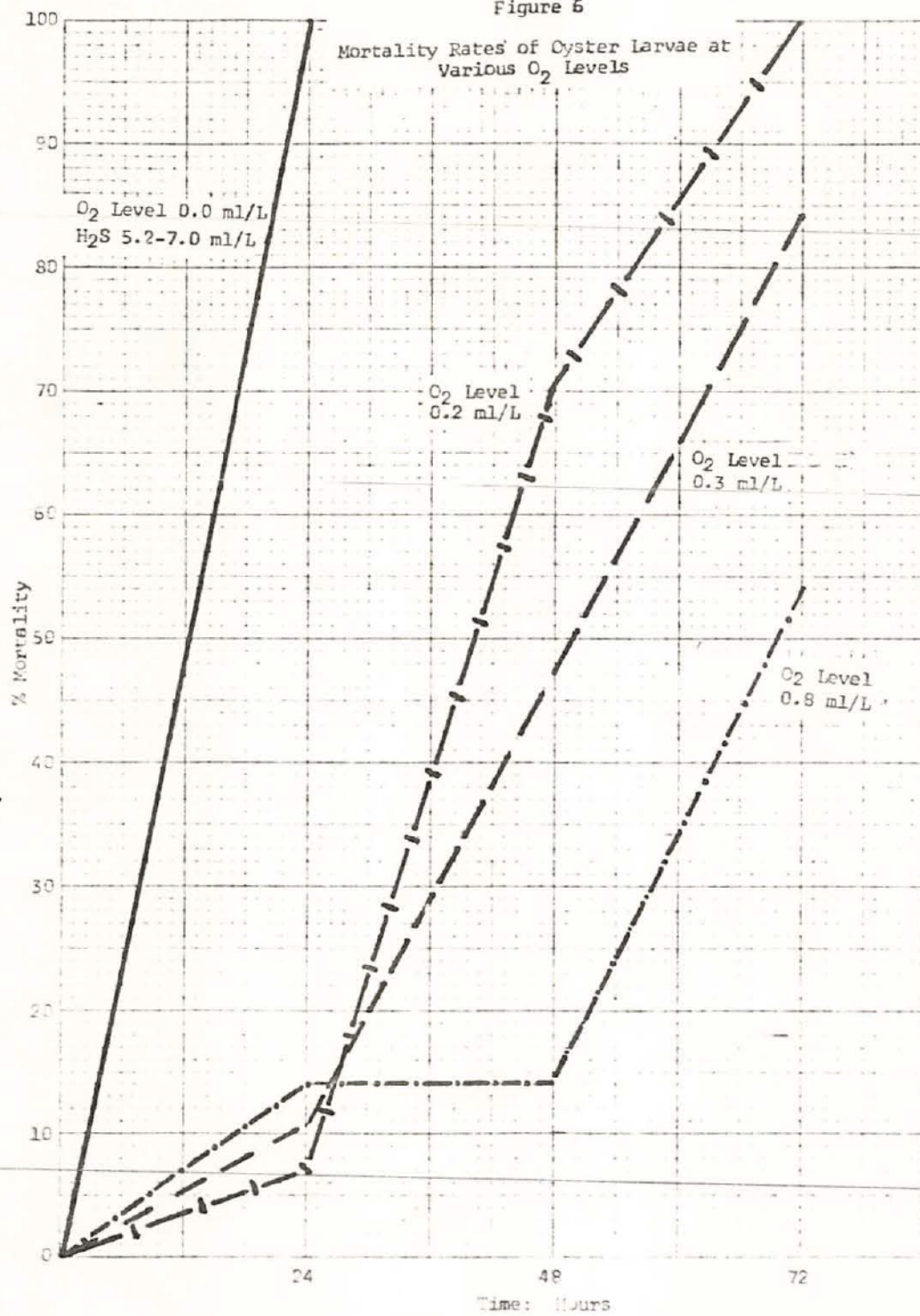
Hydrogen sulfide apparatus in series with peristaltic pump.





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Figure 6



NO. 343 111 DIETZEN SQUARE BANGOR

EUGENE DIETZEN CO. MADE IN U.S.A.

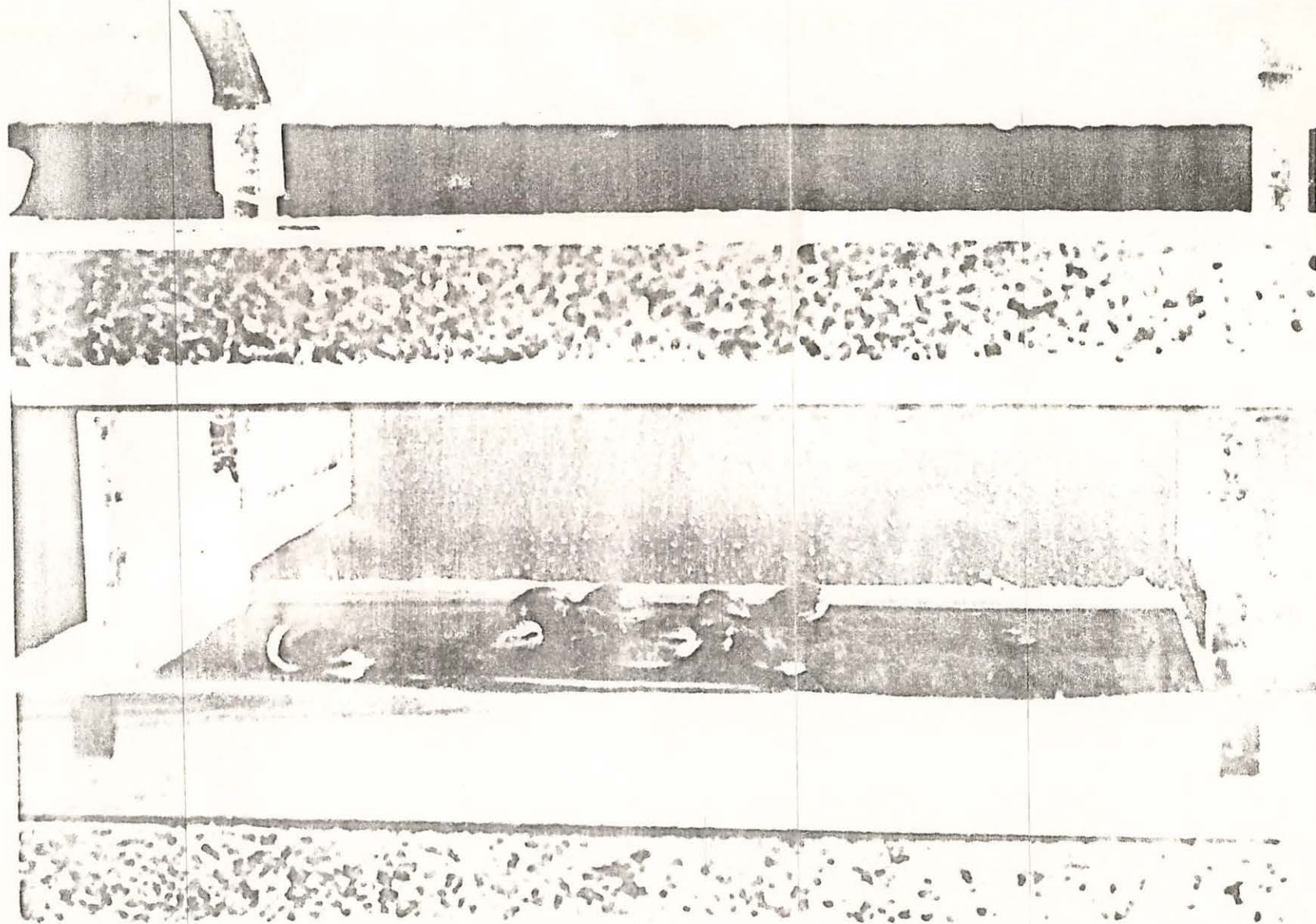


Figure 7

(clams being subjected to low oxygen and hydrogen sulfide.)