EFFECTS OF OIL MIXED WITH CARBONIZED SAND ON AQUATIC ANIMALS

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Explanatory Note

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PREFACE

The study of the effects on aquatic organisms of oil mixed with carbonized sand was undertaken at the request of the Department of the Navy, which has authorized release of this report on findings. The report does not attempt, and should not be construed as an attempt, to relate the observed effects of oil or oils mixed with carbonized sand to the effects of oil pollution generally or to oil pollution as it may affect aquatic organisms in any particular situation.

INTRODUCTION

Oil pollution of inshore waters caused by deliberate or accidental discharges of oil and oily refuses from ships, refineries, and various industrial plants is a problem of major importance for the conservation of our aquatic resources. Aside from being destructive to aquatic animals and plants and a great nuisance to such recreational activities as boating, bathing, and fishing, oil and other organic liquids floating on the surface of the water present a serious fire hazard, especially around piers and other structures built of creosoted wood. It has been recorded numerous times that sparks from welding operations have started serious fires of oil slicks in harbors and that ship repairs have had to be delayed until the oil floating on the surface has been removed.

After a damaging fire at the Norfolk Naval Shipyard, resulting from the accidental ignition of oil floating on the water, the Chemical Laboratory of the shipyard undertook a comprehensive study of the existing methods of removal of oil slicks and began a search for better ones. A description of this review and a proposal of a new and apparently highly satisfactory method developed by this laboratory are given in its report of October 10, 1945 (Chemical Laboratory, Norfolk Naval Shipyard, 1945).

In searching for a substance that would be suitably hydrophobic and organophilic, the experimenters of the United States Navy found that a carbonized sand met their requirements. This material can be prepared simply and cheaply by roasting creosote oil and sand in a revolving kiln-type of furnace at a temperature of approximately 700° F. By using air pressure the carbonized sand is sprayed on the surface of an oil slick on water or on pilings and docks. Coming in contact with oil, the carbon coating of the sand forms a stable bond with the oil. The mixture may then be readily removed. If on the surface of the water, the combined sand and oil may be sunk by a stream of water under pressure from a hose or by some other method of agitation. The bonding of the oil and carbon surface of the sand is permanent and an oil slick thus treated remains anchored on the bottom.

Details of the development of this method, including the descriptions of manufacturing units, means of application, and the results of the two field tests, are given in the report of the Chemical Laboratory of the Norfolk Naval Shipyard (1947). A popular account and graphic story of this new way of removal of oil slicks appeared in "Life" (1947, Vol. 23, No. 19). The caption to one of the photographs accompanying this article stated that the submerged sludge "is lethal to most marine life." Since there was no corroborative evidence of the toxicity of oil bound by carbonized sand, the United States Navy, through its Bureau of Ships, asked the cooperation of the Fish and Wildlife Service in a study of this problem. The present report summarizes the results of the experiments conducted by the authors in compliance with this request.

INJURY TO AQUATIC LIFE CAUSED BY OIL

Oils and oil substances discharged into coastal waters do not remain floating indefinitely, for they are readily absorbed by clay, silt, and other particulate matter suspended in water. Agitation of the water by currents and wave action helps the settling of the oil-saturated material on the bottom, but the oil slick is not securely fixed and may be carried to distant places. This characteristic behavior and its importance to aquatic life has been emphasized by Nelson (1925) and Gowanloch (1935). Injury caused to ducks and other water birds by oil floating on the surface of water is well known, since many instances have been recorded of the finding of oil-smeared birds unable to fly (Lincoln, 1936; Adams, 1936). Likewise, sedentary animals living within the tidal zone and coming in direct contact with oil may be destroyed. A striking mortality of the soft shell clam Mya at low tide on the shores of Staten Island resulting from a heavy film of oil was reported by Nelson (1925). The great damaging action of oil pollution to our aquatic resources is well established and many will agree with Nelson when he says (l. c., p. 178) "Oil is, gallon for gallon as thrown out, the most destructive to aquatic life of all the foreign substances now entering our coastal waters."

The toxicity of oil in sea water has been demonstrated many times experimentally using fishes and marine invertebrates (Seydel, 1913; Nelson, 1925; Roberts, 1926; Gardiner, 1927; Ministry of Transport and the Ministry of Agriculture and Fisheries, Joint Committee on Damages to Fisheries, 1930; Gowanloch, 1934; Galtsoff et al., 1935; Galtsoff, 1936; Veselov, 1948; and others). These investigations have shown that the toxic effect is due to water soluble substances extracted from oil. According to Seydel (1913), the toxic effect of crude oil (from Baku fields) was first demonstrated by Russian investigators who concluded that toxicity is due to naphthenic and volatile acids which are extracted from oil by water. Seydel (l. c.) also described the destruction by oil pollution of crayfishes kept in floats in Bodensee. Observations made in this country and in Europe on various animals kept in oil-polluted waters corroborate these findings and indicate that water soluble toxic material is effective when present in sufficient concentration. In many instances of oil pollution, such concentrations may not be encountered and no ill effect of pollution may be apparent. Under other situations, more sensitive members of aquatic communities may succumb to the presence of poisons. Paucity of fauna and flora in our oil-polluted harbors is probably the best illustration of the destructive effect of oil on marine life.

Because of the importance of oil development for the national welfare and security, and because of the large scale oil drilling operations which are now being conducted in our inshore and coastal waters, it is imperative that comprehensive studies of the toxicology of oil be conducted by a competent and nonpartisan organization. There is no doubt that through sincere and combined efforts of all interested parties a practical method will be found for a safe disposal of oil wastes and for the protection of our inshore waters from the devastating effect of oil pollution. The use of carbonized sand in removing oil slicks may be an important and progressive step in that direction.

Since there is convincing evidence of the leaching out of toxic substances from oils present in sea water, it is desirable to ascertain whether the combination of oil with carbonized sand would alter this action. The combination may either lessen the toxicity of the oil, or it may increase it by bringing the poisonous oil closer to the bottom-dwelling forms, permanently anchoring it there, and allowing a slow and continued extraction.

Before describing in detail our experiments dealing with the toxic effect of oil and sand mixture, we think it is desirable to discuss briefly our observations on the amount of carbonized sand required to submerge a known quantity of oil.

AMOUNT OF SAND REQUIRED TO SUBMERGE VARIOUS OILS ON SEA WATER OF DIFFERENT SALINITIES.

The amount of carbonized sand needed to completely submerge oil floating on the surface of water depends on the quantity and type of oil used, method of mixing, and the salinity and temperature of the water. The sand supplied by the Norfolk Naval Shipyard for the experiments was of very find grade, well coated with carbon. It weighed 1.28 grams per ml. To determine the ratio of sand to oil to be used in our experiments, we poured 10 ml. of oil into a finger bowl 178 mm. in diameter containing 500 ml. of sea water. Sand was sprinkled from a small jar, the lid of which had been punctured with numerous small holes. The amount of sand used was determined by difference in the weight of the jar and its contents before and after the application. To assist in the sinking of oil, the water was agitated by tipping the bowl and stirring with a glass rod. The salinity of the undiluted sea water was 31.65 parts per thousand. The results of our tests, which were made in triplicate, are given in table 1.

For various types of oil on sea water, the amount of sand required to submerge 10 ml. of oil varied from 29.3 to 31.3 grams. As one would expect, an oil slick on fresh water required less sand (25.3 grams). In the case of 50 percent dilution of sea water the amount of sand used occupied an intermediate position (27.0 grams).

Assuming that one ml. of oil weighs 0.96 grams, we infer that the removal of one pound of oil poured on water would require 3.125 pounds of sand. This figure is about 33 percent greater than the amount given in the report of the Chemical Laboratory (1947), which states that the

TABLE 1. --- Comparison of the amount of sand required to sink 10 ml. of different oils in sea water (Salinity 31.65%) and Diesel oil in diluted sea water and tap water

[Each figure average of 3 trials]

Test	Water temperature	Weight of carbonized sand
	°C.	Grams
Crude oil on sea water	22.2	29.7
Fuel oil on sea water	22.2	30.3
Lubricating oil on sea water	22.2	29.3
Diesel oil on sea water	23.0	31.3
Diesel oil on sea water diluted with equal amount of tap water	23.3	27.0
Diesel oil on tap water	22.7	25.3

sinking of one pound of Bunker "C" oil required two pounds of No. 100 sand. The difference may be easily attributed to the differences in the conditions of the tests, as well as to the different grades of oil or sand used.

According to the recommendations by the Chemical Laboratory (1947), the carbonized sand is sprayed in such a way as to float on the oil slick in sufficient amount to absorb and combine with it, but not to force the sand below the surface. After sufficient time is allowed for the sand to absorb the oil, the treated slick is sunk with water from a hose. In the laboratory technique, using small amounts of material in finger bowls, there was a tendency to add more sand than probably was needed and the sinking was accomplished in part by added weight of sand.

The addition of crude oil and carbonized sand has no significant effect on the pH of sea water, which changed from 8.05, before the addition, to 8.08 after the treatment was completed and the oil submerged by spraying it with 27 ml. of sand. After 24 hours, the original value of 8.05 was restored. All pH measurements were made with the Beckman potentiometer.

SELECTION OF EXPERIMENTAL ANIMALS

For our study of the effects of oil slicks and the mixture of oil and carbonized sand deposited on the bottom as a result of the spraying of these slicks with carbonized sand, we selected such forms that would normally live attached to submerged objects, or on the bottom in estuaries and harbors where this type of pollution is most apt to occur. We chose in particular animals of known economic importance, but also included others which aptly lend themselves to the experimental procedures desired. A good test organism should be available in fairly large numbers and be hardy enough to survive in the laboratory tanks, supplied with running sea water, for a reasonable length of time without being weakened or losing its responsiveness. In measuring the reaction of the animal to an altered environment, particularly one caused by the addition of a substance that may prove to be inimical to its existence, the criterion of change in a response that is being studied and the criterion of death must be definite and clear. An added advantage may be had if the reactions of the animal can be automatically recorded or measured, as, for instance, the heart beat, the rate of pumping of water by the gills, the movements of tentacles, etc. After due consideration and several preliminary tests, we decided to use the following organisms: the hydrozoan Tubularia crocea, which grows attached to pilings and docks; the barnacle Balanus balanoides, a very common form growing abundantly on rocks and structures near low tide mark; the embryos of the toad fish Opsanus tau, one of the common bottom-dwelling fishes in harbors and bays which attaches its large eggs to wood, rocks, and other submerged objects; the hard shell clam Venus mercenaria, which inhabits the mud flats; and the oyster Ostrea virginica, found on rocks and on the bottom in all coastal waters. Of these five animals, Tubularia was the most sensitive, while the hard shell clam was apparently the most resistant of the group.

EXPERIMENTS WITH TUBULARIA CROCEA

Colonies of Tubularia crocea were collected in the vicinity of the Cape Cod Canal and, after being brought to the laboratory, were immediately placed in running sea water. Pieces of the colonies having a readily countable number of hydranths were cut with sharp scissors and placed in finger bowls or in experimental dishes. Inasmuch as preliminary experiments have shown that Tubularia cannot be kept in the laboratory for more than a week, all the tests were completed within one or two days. Weakened or dying polyps of Tubularia lose their dark pink color and become slightly opaque. Their tentacles fail to respond to touch and, finally, the entire hydranth, with its whorl of filliform tentacles, separates and drops off, leaving dense tufts of tangled stems. This characteristic change makes it convenient to employ colonies of Tubularia as test animals. The death point of an individual hydranth may be taken as the time when it drops off the stem and the progress of mortality in the group can be easily expressed in the number of lost hydranths.

The tests made with Tubularia consisted in determining the survival of this organism in standing or in running sea water containing known quantities of mixtures of various oils and carbonized sand, and in its survival in water to which an extract of crude oil was added.

1. Effect of oil and carbonized sand mixture in standing water:

In these experiments, the concentration of the substances presumably released from oil and sand mixtures was allowed to build up in standing water. The Tubularia were placed in 200 ml. of laboratory sea water in finger bowls, and the specified amount of oil combined with sand was placed on the bottom of each bowl in such a way that no hydranth was in direct contact with the mixture. The water was not agitated or aerated. Since there was no visible oil slick on the surface of water, the gaseous exchange over the surface of water was not impeded.

The ratios of oil to sea water varied from 1:20 to 1:1,000. The results of four separate tests, summarized in tables 2 to 5, show different degrees of toxicity, depending on the quantity of oil used. In high concentrations of 1:20 and 1:40, nearly all the hydranths perished within twenty-four hours. In the weakest concentration of 1:1,000 (table 3), 17 hydranths out of 50 died within one day, while in the control, only 5 out of 60 succumbed during this time. Some mortality of hydranths in the control dishes occurred in all the tests, but the number of dead was much smaller than that among the Tubularia exposed to oil. From the results of these tests we conclude that under the conditions of the experiments sufficient quantities of toxic material were leached from oil and sand mixtures to have a marked deleterious effect on Tubularia.

TABLE 2. — Toxicity of crude oil mixed with carbonized sand to Tubularia in standing solutions

[Experiments of July 9, 1948. Temperature range 20.6° to 21.3°C.]

Duration of exposure	Number of hydranths remaining on stalks at various concentrations 1/				
	Control in sea water	1:200	1:80	1:40	1:20
Hours					
0	15	15	15	15	15
4.5	15	15	15	14	15
15.5	13	12	10	11	10
24	9	2	2	0	0

1/ Concentration of oil to sea water. Sufficient carbonized sand was mixed to completely absorb the oil.

TABLE 3. — Toxicity of crude oil mixed with carbonized sand to Tubularia in standing solutions

[Experiment of July 12, 1948. Temperature 20.7°C.]

Duration of exposure	Number of hydranths remaining on stalks at the various concentrations 1/				
	Control in sea water	1:1000	1:400	1:200	1:40
Hours					
0	60	50	50	50	58
24	55	33	12	1	3

1/ Concentration of oil to sea water. Sufficient carbonized sand was mixed with the oil to completely absorb it.

TABLE 4. --- Toxicity of crude oil mixed with carbonized sand to Tubularia in standing solutions

[Experiment of August 5, 1948]

Date	Time	Temperature	Duration of exposure	Number of hydranths remaining on stalks at various concentrations 1/			
				Control in sea water	1:200	1:80	1:40
1948		°C.	Hours				
August 5	1500	21.9	0	50	30	42	30
	2100	21.6	6	48	28	40	33
6	0800	21.2	17	26	18	15	8
	1600	21.4	25	23	10	2	0

1/ Average value for 5 countings.
Sufficient carbonized sand was mixed with the oil to completely absorb it.

TABLE 5. — Toxicity of crude oil mixed with carbonized sand to Tubularia in standing solutions

[Experiments of August 7, 1948]

Date	Time	Temperature	Duration of exposure	Number of hydranths remaining on stalks at various concentrations 1/			
				Control in sea water	1 :200	1:80	1:40
1948		°C.	Hours				
August 7	1000	20.8	0	27	20	24	33
	1600	20.6	6	27	19	23	30
	2200	20.6	12	25	17	19	24
8	1000	20.5	24	24	3	1	0

1/ Average value for 5 countings.
Sufficient carbonized sand was mixed with the oil to completely absorb it.

2. Effect of water extract of crude oil:

A toxic effect on oysters of water soluble fractions of crude oil has been demonstrated in the investigations conducted by the Service in Louisiana (Galtsoff et al., 1935). The following experiments, carried out with samples of crude oil supplied by the U. S. Navy, show similar results using *Tubularia*. The extract was prepared by adding 200 ml. of oil to 4 liters of unfiltered sea water and shaking the mixture for 6 1/2 hours on a mechanical shaking machine. The container was then inverted and 48 hours allowed for the separation of the oil and water. The water extract was withdrawn from the bottom and added to sea water in sufficient quantities to give the desired concentrations, which, in the first series of tests, were 1:20, 1:8, 1:4, and 1:2. In a second series more dilute solutions were used, the concentrations being 1:40, 1:200, 1:400, and 1:1,000. These concentrations would correspond to dilutions of the original crude oil from 1:800 to 1:20,000. It was expected, of course, that the vigorous shaking of the crude oil with sea water would result in water soluble extractions in greater amount than might be obtained by simply keeping oil in contact with standing sea water.

All the tests were made in finger bowls using 200 ml. of water which was neither agitated nor aerated. The tests lasted 24 hours. No mortality in the control occurred in the first series, while in the second series only six out of sixty *Tubularia* died in the control dish. As one can see from the examination of tables 6 and 7, the extract was toxic in the concentration up to 1:200 (1:2,000 of the original crude oil sample).

These experiments show that crude oil contains toxic substances that can be extracted by shaking with sea water. Under natural conditions one may expect that strong agitation of water by wind or the back and forth movement of water over submerged oil would tend to increase the concentration of toxic substances.

3. Toxicity of various types of oils and oil and carbonized sand mixtures in running sea water:

In the first set of tests a large container with running sea water served as a common supply from which, by means of a siphon, the water was carried to each of the beakers of 600 ml. capacity containing test materials. From the beakers the water was siphoned to small finger bowls in which the *Tubularia* were placed. The control was arranged in a similar manner but minus the test material. All the siphons were adjusted to allow a flow of 700 ml. per minute (42 liters per hour). The 600 ml. beakers were kept approximately two-thirds full, and the finger bowls with polyps held 250 ml. In each instance 20 ml. of test oil alone, or oil mixed with carbonized sand, were used.

TABLE 6. — Toxicity of crude oil extract to Tubularia

[Experiment of July 9, 1948. Temperature range 20.6 to 21.3 °C.]

Duration of Exposure	Number of hydranths remaining on stalks at various concentrations				
	Control in sea water	1:20	1:8	1:4	1:2
Hours					
0	15	15	15	15	15
4.5	15	14	15	15	15
15.5	15	8	14	10	7
24	15	2	5	4	0

TABLE 7. --- Toxicity of crude oil extract to Tubularia

[Experiment of July 12, 1948. Temperature 20.7° C.]

Duration of exposure	Number of hydranths remaining on stalks at the various concentrations				
	Control in sea water	1:1000	1:400	1:200	1:40
Hours					
0	60	60	50	50	50
24	54	56	44	23	0

The purpose of the experiment was to see if toxic material that may be present at very great dilution has any effect on Tubularia if the organism is removed from the immediate vicinity of the oil.

Only the crude oil and Diesel oil, either alone or mixed with carbonized sand, were employed in these tests. The results summarized in tables 8 and 9 show that under the conditions of the tests no ill effect on Tubularia became apparent during the two and one-half days of exposure to either type of oil. The test was discontinued at this time because of the marked mortality of Tubularia in the control (table 8).

We infer from these experiments that Tubularia suffered no ill effects from oil, presumably because no sufficient concentration of the toxic material was built up in the running water and the organism was removed from the immediate vicinity of the oil.

In the second series of tests lumps of carbonized sand and crude oil were placed in the immediate vicinity of the colony of Tubularia, which were kept in glass battery jars of 2-liter capacity. The sea water was kept running through the jars at a rate of 30 liters per hour. In the first test, the water from the control jar, containing 69 hydranths, was siphoned to the experimental jar, with 78 hydranths. A mixture of 2.5 ml. of crude oil and 7 grams of carbonized sand was placed on bottom close to the colony, but not touching it. During the five days (120 hours) this test was continued the temperature of water fluctuated between 19.8° and 20.4° C. At the end of the test all the hydranths in the control were alive. In the jar containing oil and sand mixture, the first mortality was observed after 48 hours, when 22 hydranths were found dead and were removed. By the end of the fifth day, 35 additional hydranths died, 15 were alive and 6 were not accounted for, apparently having been carried away by the stream.

In another test sea water was supplied directly to each of five battery jars, one of which served as control. On the bottom of four were placed respectively 5 ml., 10 ml., 20 ml., and 30 ml. of crude oil with appropriate amounts of carbonized sand. The rate of flow through each jar was increased over that of the first experiment and kept between 65 and 75 liters per hour. From 50 to 61 Tubularia were placed in each jar. The temperature varied between 20.4° and 20.7° C. At the end of the fifth day there was a noticeable mortality in the control jar (30 hydranths died out of original 50) but a much more marked loss occurred in all the experimental jars (table 10). It is interesting to note that within the first 48 hours there was no death of hydranths in the control and relatively insignificant loss (9 out of 53) in the jar containing 5 ml. of oil.

TABLE 8. ---- Toxicity of crude oil mixed with carbonized sand in close proximity to Tubularia in flowing sea water

[Experiment of July 10 to 15, 1948. Temperature range 20.4° to 20.7° C. Rate of flow, 65 to 75 liters per hour]

Duration of exposure	Number of hydranths remaining on stalks in the various jars containing the amount of oil indicated 1/				
	Control in sea water	5 ml.	10 ml.	20 ml.	30 ml.
Hours					
0	50	53	50	61	59
48	50	44	15	27	8
120	20	10	4	0	0

1/ Sufficient carbonized sand was mixed with the oil to completely absorb it

TABLE 9. — Toxicity of various oils mixed with carbonized sand to Tubularia in standing solutions

[All concentrations 1:40 of the oil in sea water]

Date	Time	Temperature	Duration of exposure	Number of hydranths remaining on stalks in various oil mixtures 1/				
				Control in sea water	Crude oil	Fuel oil	Lubricating oil	Diesel oil
1948		°C.	Hours					
Aug. 5	1500	21.9	0	50	30	32	33	29
	2100	21.6	6	48	33	34	34	23
6	0800	21.2	17	26	8	10	25	13
	1600	21.4	25	23	0	6	19	0

1/ Average value for 5 countings. Sufficient carbonized sand was mixed with the oil to completely absorb it.

TABLE 10. ---- Toxicity of Diesel oil and Diesel oil mixed with carbonized sand to Tubularia in flowing sea water

[Flow rate 700 ml. per minute, 20 ml. of oil]

Date	Time	Temperature	Duration of exposure	Number of hydranths remaining on stalks in the experimental flow indicated. 1/		
				Sea water control	Sea water and Diesel oil	Sea water and Diesel oil mixed with sand
1948		°C.	Hours			
July 26	1600	22.0	0	23	22	30
	2130	21.5	5.5	23	21	29
July 27	0830	21.2	16.5	22	21	30
	1700	22.2	25.0	22	21	30
July 28	0800	21.4	40.0	21	21	29
	1330	22.0	45.5	21	20	29
	1930	22.2	51.5	12	20	26
July 29	0830	21.3	64.5	12	19	24

1/ Average value for 5 countings

We conclude from these experiments that in running sea water a toxic effect becomes apparent after 48 hours if 5 ml. or more of oil mixed with sand are placed in the immediate vicinity of Tubularia.

4. Relative toxicity of different types of oil in standing water:

Samples of oil received from the Navy for testing comprised the following types: crude oil, Navy Grade Special fuel oil, lubricating oil (SAE 20), and Diesel oil. In conducting the test, 5 ml. of each of these oils, with a sufficient amount of carbonized sand to anchor them, were placed on the bottom of each of four finger bowls containing 200 ml. of sea water. One finger bowl of the same size and capacity served as a control. The test was continued for 25 hours, during which time the number of remaining live hydranths was counted at the 6th, 17th, and 25th hour. The results, summarized in table 11, show that lubricating oil was least toxic, while crude oil appeared to be the most toxic. There was considerable mortality in the control (27 out of 50) but the loss of hydranths in the water contaminated with oil was much greater, with the exception of that with the lubricating oil.

EXPERIMENTS WITH BARNACLES, BALANUS BALANOIDES (Ag.)

Among the common invertebrates of the inshore region, barnacles occupy a prominent position as one of the principal fouling organisms, attaching themselves to ship bottoms and covering the shells used by oystermen as cultch for the catching of oyster spat. Many toxicological studies have been conducted with the view of combating fouling by preventing, or at least minimizing, the attachment and growth of barnacle larvae. The adult organism also can be conveniently used in toxicological tests. Being sessile, the barnacles can be easily arranged in a desired position in the experimental set-up. The effect of a toxic substance can be studied by observing and timing the sweeping of their cirri.

Keeping the barnacles in the laboratory presents no difficulty. In running sea water or in containers in which the water is renewed daily, they remain active and apparently in good condition for many days and weeks.

In the present experiments, specimens of Balanus balanoides growing on rocks were obtained from Woods Hole Harbor close to the low tide mark. Stones with 25 to 50 individuals were placed in finger bowls containing 250 ml. of sea water. One finger bowl served as control, while to each of three others were added 5 ml., 10 ml., and 20 ml. of crude oil mixed with carbonized sand in quantities sufficient to anchor the oil on the bottom. It was found that the water in the control bowl was rapidly fouled by abundant discharge of fecal material. This was not the case, however, in the experimental containers in which

TABLE 11. ---- Toxicity of crude oil and crude oil mixed with carbonized sand to Tubularia in flowing sea water

[Flow rate 700 ml. per minute, 20 ml. of oil]

Date	Time	Temperature	Duration of exposure	Number of hydranths remaining on stalks in the experimental flow indicated 1/		
				Sea water control	Sea water and crude oil	Sea water and crude oil mixed with sand
1948		°C.	Hours			
July 27	1700	22.2	0	30	22	31
July 28	0800	21.4	15	30	26	32
	1400	22.0	21	30	26	29
	1730	22.2	24.5	26	27	30
July 29	2330	22.1	54.5	28	24	31

1/ Average value for 5 countings

the water remained clear, presumably because the presence of oil depressed the functioning of the intestinal tract of barnacles. To avoid undesirable effects, the water in the control was renewed daily.

The oil and carbonized sand mixture in the water had pronounced effect on the behavior of the barnacles. At first their cirri continued to beat at the normal rate. As the exposure in oil-contaminated water continued, there was a slowing of the beat and a reduction in the extent of each sweep. With the progress of poisoning, both the rate and the extent of sweep were further reduced until the test animals came to rest and stopped their movement, the cirri half extended. In contrast, the control barnacles, when coming to rest, were tightly closed with the cirri completely withdrawn. At the earlier stage of poisoning, the barnacles would respond to touch by withdrawing their cirri and closing their shells. The reaction ceased as the animals succumbed to the poison. This effect of oil on the behavior of barnacles is similar to that of other poisons which were studied by Clarke (1947). During the 70 hours of observations there was no change in the behavior and no death among the control barnacles. In the oil-contaminated water the first symptons of poisoning, shown by slowing down of the sweeping of the cirri, became apparent after six hours of exposure. The poisoning progressed in proportion to the concentration of oil, all the barnacles passing through the same stages characterized by incomplete sweep of cirri, opening of shells, failure to react to touch, and death (table 12).

The test very clearly demonstrates the toxic effect of crude oil and sand mixtures placed in the immediate vicinity of barnacles.

EXPERIMENTS WITH TOADFISH EMBRYOS, OPSANUS TAU LINN

Toadfish embryos present excellent material for bioassay; they are large, fairly transparent, and are attached by egg membranes to pieces of wood, stone, shells, and similar objects. They can be easily handled in the laboratory without being injured. The fish itself, a very common bottom dweller along the coast of North America south of Cape Cod, is hardy and resistant to unfavorable environment. Around Woods Hole the eggs are laid early in May and may be found until the middle of July. They can be kept in good condition in the laboratory tanks in running sea water if care is exercised in removing dead or dying eggs. After hatching, the larvae still remain attached to the substratum by their yolk sacs. At this stage they somewhat resemble tadpoles and exhibit active movements of their tails and gills. The beating of their hearts and the circulation of blood can be easily observed with adequate illumination and suitable optical equipment.

For the test we used larvae from 8 mm. to 10 mm. long which were detached from the substratum and placed on the bottom of finger bowls together with known quantities of oil and sand mixtures. The embryos,

TABLE 12. — Toxicity of different amounts of crude oil mixed with carbonized sand to barnacles

[Sufficient sand was added to keep the oil on the bottom]

Date	Time	Temperature	Duration of exposure	Reaction of barnacles to oil added in the amounts indicated 1/			
				Control in sea water	1:50	1:25	1:12.5
1948		°C.	Hours				
August 7	1100	21.0	0	+ + + +	+ + + +	+ + + +	+ + + +
	1700	21.2	6	+ + + +	+ + +	+ + +	+ + +
8	1000	20.5	23	+ + + +	+ +	+	+
	1700	21.2	30	+ + + +	+	+	+
9	0800	21.0	45	+ + + +	+	+	0
	1700	21.8	54	+ + + +	+	0	
10	0900	21.4	70	+ + + +	0		

1/ Reaction of 80 to 90% of the barnacles.
Symbols same as Clarke (1947) as follows:

+ + + + Normal rapid sweep of cirri
+ + + Full sweep but slow
+ + Incomplete sweep
+ Open, inactive, but reacts to touch
0 Dead

fi.e in each finger bowl, were placed in such a manner as to avoid direct contact with oil. The first experiments consisted in testing the relative toxicities of crude oil, Diesel oil, and lubricating oil in the concentrations of 1:10, 1:20, and 1:40. The results are summarized in tables 13, 14, and 15. Crude oil added in the ratio of 1:40 killed three out of five test embryos within 47 1/2 hours. Toxicity of Diesel oil was noticeable within 52 hours in the concentration of 1:20, while lubricating oil was ineffective even in the concentration of 1:10 (50 hours).

In view of high toxicity of crude oil, a series of tests were made in which the quantities of oil added to the water varied from 1:20 to 1:200. As the examination of table 16 shows, even the lowest concentration of 1:200 was sufficiently toxic to kill all five embryos in 11 days. The mortality of embryos in greater quantities of oil was more rapid. There was no death among the controls.

The relationship between the time of death and the quantity of oil used is shown in figure 1. The open circle curve, forming the lower limit of the band, corresponds to the time of the first death; the dark circles to the time required to kill all five embryos. Thus, the lower and upper boundaries of the band represent respectively the relationship between the quantity of oil used and the time of survival of the weakest and the hardiest specimens.

If the log of the survival time is plotted against the log of concentration, the toxicity curve approximates a straight line (fig. 2). The linear relationship obtained by such plotting can be approximately represented by a general equation of the type $y = a x^{c}$ and the constants a and c may be computed from the empirical data. We see no particular advantage at present in making these computations, since the nature of toxic substance extracted from oil is not known. It is, however, significant that the relationship between the time of survival of embryos and the quantities of oil used follows the type of a time-concentration curve typical for a number of coal tar disinfectants, phenol and other toxic substances (Clark, 1933).

Tests with different types of oil showed that, as in the experiments with Tubularia and barnacles, the crude oil was the most toxic. All the tests were made using the oil in ratio of 1 part to 40 parts of sea water (table 17) and keeping the embryos exposed for five days. No toxic effect was noticeable in the water containing lubricating oil.

EXPERIMENTS WITH HARD SHELL CLAMS, VENUS MERCENARIA LINN

The hard shell clam, Venus mercenaria, chosen for experiments because of its economic value, is frequently found in polluted bottoms of harbors and bays. Because of their ability to close themselves within their shells,

TABLE 13. —— Toxicity of Diesel oil mixed with carbonized sand to toadfish embryos

Date 1948	Time	Temperature °C.	Duration of exposure Hours	Number alive in the designated concentration 1/			
				Control in sea water	1:40	1:20	1:10
July 29	1200	22.1	0	5	5	5	5
	1630	22.8	4.5	5	5	5	5
	1900	22.7	7	5	5	5	5
	2230	22.4	10.5	5	5	4	4
July 30	0830	22.2	20.5	4	5	4	2
	1300	22.2	25	4	5	4	1
	1900	23.3	31	4	5	4	1
July 31	0800	22.3	44	4	5	3	0
	1600	23.1	52	4	5	2	0

1/ Concentration of oil to sea water. Sufficient carbonized sand was mixed with the oil to completely absorb it.

TABLE 14. ---- Toxicity of lubricating oil of SAE 20 mixed with carbonized sand to toadfish embryos

Date	Time	Temperature	Duration of exposure	Number alive in the designated concentration 1/			
				Control in sea water	1:40	1:20	1:10
1948		°C.	Hours				
July 29	1330	22.6	0	5	5	5	5
	1630	22.8	3	5	5	5	5
	1900	22.7	5.5	5	5	5	5
	2230	22.4	9	5	5	5	5
July 30	0830	22.2	19	5	4	5	4
	1300	22.2	23.5	5	4	5	4
	1900	23.2	29.5	5	4	5	4
July 31	0800	22.2	42.5	5	4	5	4
	1600	23.1	50	5	4	5	4

1/ Concentration of oil to sea water. Sufficient carbonized sand was mixed with the oil to completely absorb it.

TABLE 15. ---- Toxicity of crude oil mixed with carbonized sand to toadfish embryos

[Experiment of July 29, 1948]

Date 1948	Time	Temperature °C.	Duration of exposure Hours	Number alive in the designated concentrations 1/			
				Control in sea water	1:40	1:20	1:10
July 29	1630	21.8	0	5	5	5	5
	1900	22.7	2.5	5	5	5	5
	2230	22.4	6	5	5	2	3
July 30	0830	22.2	16	4	5	2	3
	1300	22.2	20.5	4	5	2	2
	1900	23.2	26.5	4	3	2	0
July 31	0800	22.3	39.5	4	3	1	0
	1600	23.1	47.5	4	2	0	0

1/ Concentration of oil to sea water. Sufficient carbonized sand was added to the oil to completely absorb it.

TABLE 16. --- Toxicity of crude oil mixed with carbonized sand to toadfish embryos

[Experiment of August 2, 1948]

Date	Time	Temperature	Duration of exposure	Number alive in the designated concentrations 1/				
				Control in sea water	1:200	1:80	1:40	1:20
1948		°C.	Hours					
August 2	2100	22.7	0	5	5	5	5	5
3	1000	22.4	13	5	5	5	5	5
	2000	22.4	23	5	5	5	5	4
4	0800	22.0	35	5	5	5	4	1
	2100	22.6	48	5	5	5	2	0
5	0900	21.1	60	5	5	5	1	
	1900	21.7	70	5	5	4	1	
6	0800	21.1	83	5	5	3	0	
	2000	21.2	95	5	5	3		
7	0800	20.6	107	5	5	2		
	1900	20.7	118	5	5	1		
8	0900	20.5	132	5	5	1		
	1900	21.3	142	5	5	0		
9	0900	21.0	156	5	5			
	1900	21.8	166	5	4			
10	0900	21.3	180	5	2			
	2000	21.6	191	5	2			
11	0900	21.3	204	5	1			
12	0900	21.5	248	5	1			
13	0900	21.4	272	5	0			

1/ Concentration of oil to sea water. Sufficient carbonized sand was mixed with the oil to completely absorb it.

TABLE 17. --- Relative toxicity of different oils mixed with carbonized sand to toadfish embryos

[Concentrations of 1:40]

Date	Time	Temperature	Duration of exposure	Number surviving in the test oil and sand indicated				
				Control in sea water	Lubricating oil	Diesel oil	Fuel oil	Crude oil
1948		°C.	Hours					
August 7	1100	21.0	0	5	5	5	5	5
	1700	20.6	6	5	5	5	5	5
8	1000	20.5	23	5	5	5	5	5
	1700	21.2	30	5	5	4	5	3
9	1100	21.0	48	5	5	3	3	1
	1700	21.8	54	5	5	2	2	0
10	1000	21.3	71	5	5	0	0	
11	0900	21.3	94	5	5			
	1900	22.1	104	5	5			
12	0900	21.5	118	5	5			

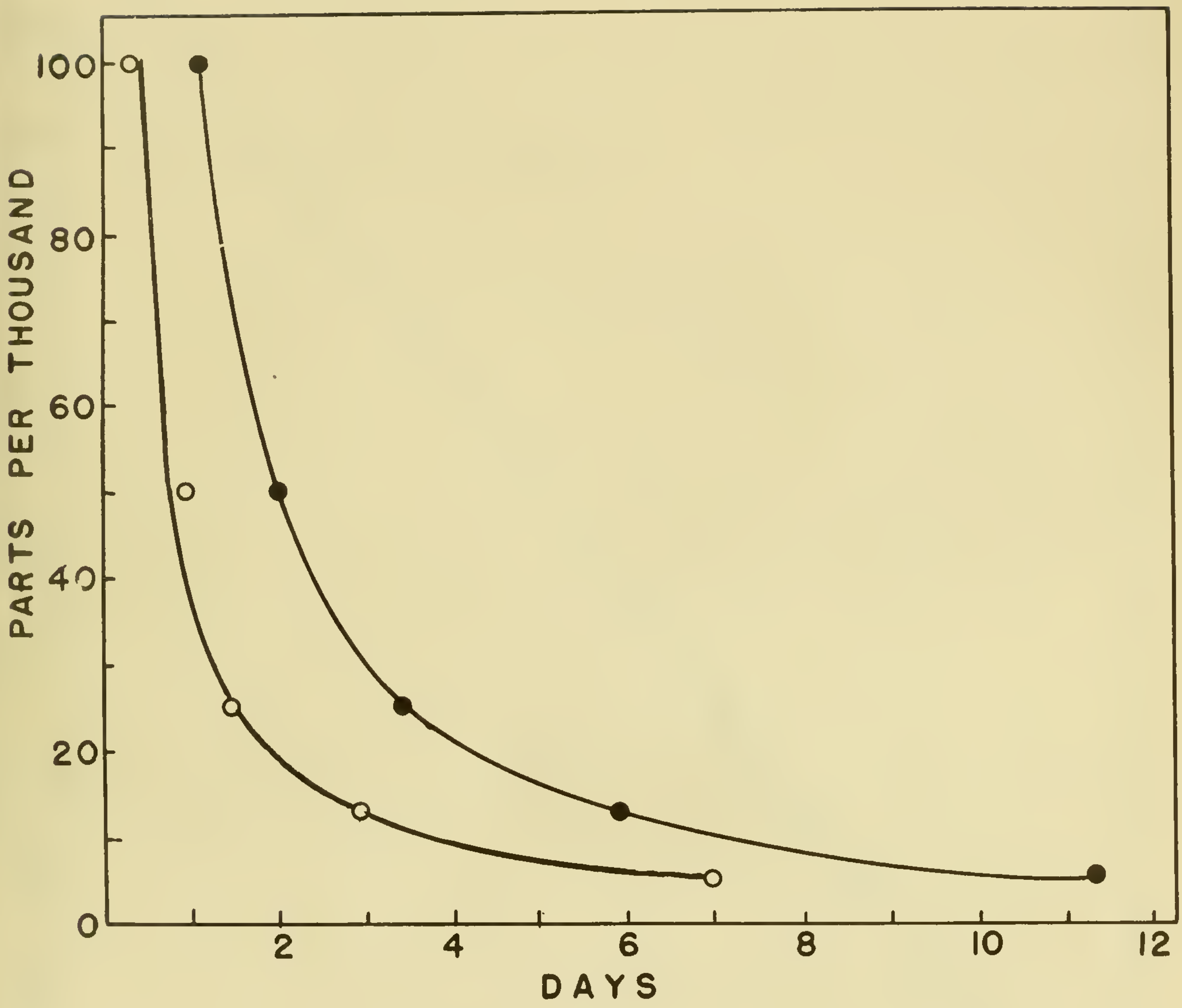

Figure 1. ----Effects of increasing concentration of crude oil mixed with carbonized sand on the survival of toadfish embryos. The survival time in days is plotted against the concentration in parts per thousand. The open circle curve corresponds to the time of the first death, the dark circle to the time required to kill all embryos.

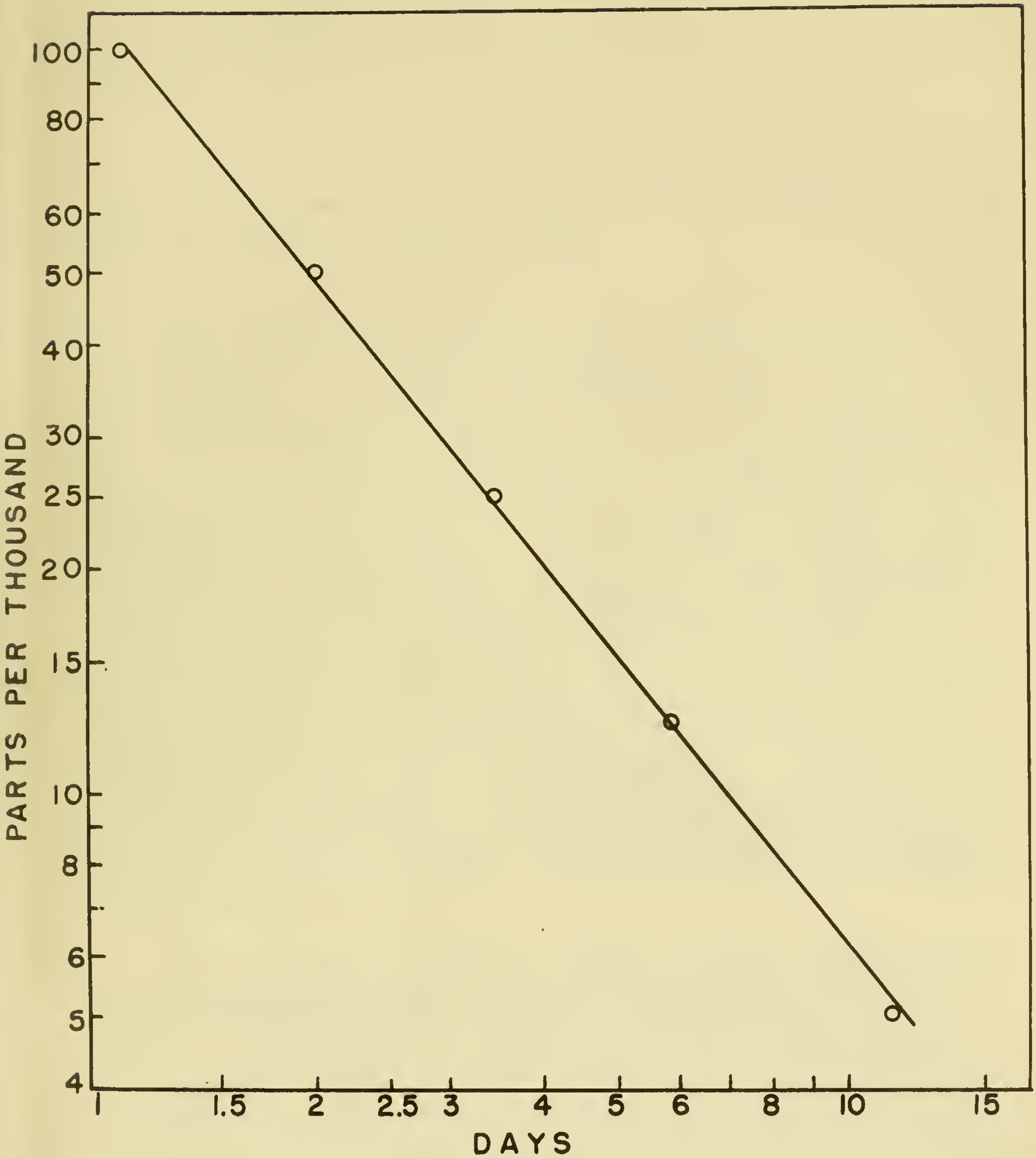

Figure 2. ---- Relationship of survival time to concentration of crude oil mixed with carbonized sand in tests using toadfish embryos. Survival time in days is plotted against concentration in parts per thousand using log-log paper.

clams, like oysters, are capable of slowing down their activities to a low minimum for rather protracted periods of time. In this way they may reduce the immediate effect of unfavorable conditions. If the latter prevail for a long time, clams eventually may be injured since their movements are rather limited.

Specimens for testing were collected from Eel pond at Woods Hole. They ranged in size from one to one and three-quarter inches in length. A group of ten clams were placed in each of nine large finger bowls, 178 mm. in diameter. One bowl was used as a control, the remaining eight receiving water contaminated with oil.

The experiment was designed to test the effect of various types of oil, either floating on the surface of water or mixed with carbonized sand and sunk to the bottom. To avoid the placing of clams in close proximinty to oil, the following arrangement was used: A large container, through which the sea water was freely running, served as a common supply from which the water was siphoned to eight beakers, each containing 20 ml. of oil, or of a mixture of the same amount of oil with carbonized sand. From each beaker the water was siphoned to the finger bowl with clams. Overflow siphons kept the level of water in each bowl below the rim. The flow was regulated to provide an exchange of water at the rate of 250 ml. per minute (21 liters per hour). Four types of oil were tested: crude oil, Diesel oil, lubricating oil, and fuel oil. During 12 1/2 days the clams were examined each morning and evening and the number of those with extended siphons was recorded. Protrusion of the siphons is a reliable sign that the clam is active. This was corroborated by observing the accumulation of waste material, which was so great that it was necessary to clean each bowl every other day. The record of the observations is summarized in table 18. None of the clams died during the test and there was no evidence of their weakening. It was noticed that the majority of clams had their siphons extended in the evening, but were tightly closed each morning. This difference may have been associated with the change in illumination, since the laboratory was becoming darkened at the time of evening observations. There was no significant difference between the behavior of clams in the control and in the water contaminated with various types of oil.

If the experiments had been so arranged, close and intimate contact with oil might have produced toxicity in the experiments with the hard shell clam, Venus mercenaria. This direct contact with the oil was avoided. Thurlow Nelson, as quoted by Lane, Bauer, Fisher, and Harding (1925, page 179), describes a mortality of soft shell clams (Mya) from oil pollution as follows: "A most striking example of the effects of oil may be seen at low tide on the shores of Staten Island. On a warm day, with consequent increased oxidation, soft clams (Mya) may be seen coming up to the surface of the flats by the thousands, dying soon after reaching the top. The surface of the flats is covered by a heavy film of oil, while the bodies of the animals reek with it. This region,

Table 18. - - - Number of clams with siphons extended while in flowing sea water passing in contact with various oils or oils mixed with carbonized sand

Date	Time	Temperature	Number with siphons extended in the test solution indicated								
			Control in sea water	Diesel oil		Crude oil		Lubricating oil		Fuel oil	
				without sand	with sand	without sand	with sand	without sand	with sand	with out sand	with san
1948		°C.									
July 30	2130	22.5									
31	0800	22.3	2	2	3	1	2	2	6	7	6
	2000	22.7	7	7	6	6	4	6	7	8	5
Aug. 1	0900	22.2	2	3	1	2	1	1	3	4	1
	1900	22.8	8	6	7	6	5	8	7	7	6
2	0830	21.9	2	2	1	1	0	0	1	0	2
	1900	22.4	9	5	8	6	7	9	7	6	8
3	1000	22.3	3	2	1	1	0	1	2	0	1
	1930	22.2	9	6	8	8	8	10	8	7	8
4	0800	22.0	1	3	1	1	1	2	2	1	1
	2100	22.6	5	4	5	6	6	6	6	5	4
5	0900	21.3	1	1	1	1	0	0	0	0	0
	1900	21.6	6	6	5	7	6	4	6	6	4
6	0800	21.0	1	1	1	0	0	1	2	0	0
	2000	20.9	9	8	10	9	6	8	7	9	10
7	0800	20.7	1	1	3	4	1	3	5	2	4
	1900	20.7	8	8	9	9	9	9	9	9	9
8	0900	20.7	1	1	1	1	0	1	3	1	1
	2000	21.4	10	8	9	8	7	8	8	7	10
9	0800	21.0	3	1	1	2	0	0	2	1	1
	2000	21.8	4	7	5	3	3	4	7	4	2
10	0900	21.3	1	0	0	0	0	0	1	0	0
	1900	22.0	4	6	2	1	2	0	3	3	4
11	0800	21.3	1	0	0	0	0	0	0	0	0
	1900	22.1	4	6	3	2	1	3	5	5	3
12	0800	21.5	0	1	0	0	0	0	0	0	0

however, receives industrial wastes, and hence is open to more than one interpretation, although there is no doubt in my own mind that oil is the chief cause of the destruction evident."

EXPERIMENTS WITH OYSTERS, OSTREA VIRGINICA GM

Because of its great economic importance, the oyster has been studied both in this country and abroad more than any other marine invertebrate. Consequently, its physiology, habits, and life histroy are better known than those of clams and other lamellibranchs. The oyster is one of the most common organisms of inshore waters where it lives attached to rocks or lying on the bottom from levels of about halfway between high and low water marks to depths of about 40 feet or more. In this situation it is frequently affected by oil wastes discharged into waters. Having no means of moving from unfavorable environments, the oyster protects itself by tightly closing its valves. If the inimical condition persists, the oyster is eventually damaged or killed. Methods for studying the response of the oyster to altered water conditions have been worked out by previous investigations conducted by the Service and have been applied several times in pollution studies (Galtsoff, 1928; Hopkins et al., 1931; Galtsoff et al., 1947; Chipman, 1948). They consist in recording the time of opening and closing of the valves of the oyster, observing the character of the muscular movement, and the rate of pumping of waters by the gills. The methods of observing and testing the reactions of oysters to the presence of oil in water employed by us in the present work are given separately with the accounts of the different experiments.

1. Toxicity of oil and of oil and carbonized sand mixtures in standing solutions:

The purpose of these tests was to find out whether oysters can survive high concentrations of oil in standing water, and whether the toxic effect is reduced by treatment of the oil with carbonized sand. The conditions of the experiment must be regarded as comparable to the most adverse case of oil pollution that the oyster may suffer.

The tests were conducted in the College Park laboratory using adult Chesapeake Bay oysters which were carefully washed and scrubbed with a fiber brush to remove the attached fouling organisms. Three groups, each comprising twelve oysters, were placed in glass aquaria containing 10 gallons of sea water collected at the same place and time that the oysters were taken. The salinity of water in the Bay was 13 parts per thousand. It was maintained at this level in the aquaria by additions of distilled water to compensate for evaporation. The water was aerated. A continuous record of temperature was obtained by means of a recording thermometer, the bulb of which was immersed in a glass cylinder filled with distilled water and set in the control aquarium. Two sets of tests were made using 200 ml.

of Diesel oil and 76 ml. of crude oil, the resultant ratios of water to oil being 1:500 and 1:189 respectively. In one aquarium of each set the oil was left floating on the surface, while in a second one the oil slick was sprinkled with carbonized sand until the oil was completely combined with the sand and settled on the bottom. The third had no oil and served as a control.

Samples of water, taken at frequent intervals close to the bottom, were analyzed for dissolved oxygen and determination made of the pH. No abnormal conditions with respect to these two factors were observed during the tests.

In the aquaria in which oil was floating on the surface, it was observed that after some time the bottom and sides of the aquaria and the shells of the oysters became coated with an oil film. No such film appeared when oil and sand mixtures were used, the oil being permanently bound to the carbonized sand.

In the experiment with Diesel oil (ratio 1:189) the first death occurred on the third day in the aquarium with an oil layer on the surface, and on the fourth day in the aquarium with oil and sand mixture. By the end of the test on the 13th day, the mortality was 67 percent in the test with oil on the surface against 25 percent in that with the oil treated with carbonized sand (table 19). There was no mortality among the control oysters. The results show that the toxicity of Diesel oil anchored on the bottom by carbonized sand is somewhat reduced. At the end of the experiment, all the oysters that survived were removed, washed with tap water, and opened for examination. Their meats had a marked oily odor.

Experiments with crude oil added in the ratio of 1:500 gave similar results. In these the first death was observed on the ninth day and the mortality was less pronounced, due probably to the smaller quantity of oil used (table 20).

2. Effect of prolonged exposure to crude oil mixed with carbonized sand in running sea water:

To observe the effect on oysters, small quantities of crude oil mixed with carbonized sand were placed on the bottom of tanks of running sea water or introduced into a flowing sea water system. The following tests were performed:

Fifty adult oysters were placed on the bottom of each of three wooden tanks 28 1/4 x 58 1/2 inches. A depth of eight inches of water was maintained in each tank by a standpipe. One tank was maintained as control. To the other tank 500 ml. of crude oil were added and oil slick immediately sprinkled with carbonized sand until all the oil had been removed from the surface and settled on the bottom. Some of the oil and sand mixture was directly on oysters. The third tank contained no oil but received the water that was running over a layer of oil and sand. This was accomplished

TABLE 19. —— Toxicity of Diesel oil and Diesel oil mixed with carbonized sand to oysters in standing solutions

[Concentration of oil to sea water 1:189]

Date	Duration of exposure	Number of oysters alive in the various test materials indicated		
		Control in sea water	Sea water with Diesel oil	Sea water with Diesel oil mixed with carbonized sand
1948	Days			
March 17	0	12	12	12
18	1	12	12	12
19	2	12	12	12
20	3	12	11	12
21	4	12	10	11
22	5	12	10	11
23	6	12	10	10
24	7	12	9	10
25	8	12	8	10
26	9	12	8	10
27	10	12	8	10
28	11	12	7	9
29	12	12	5	9
30	13	12	4	9

TABLE 20. --- Toxicity of crude oil and crude oil mixed with carbonized sand to oysters in standing solutions

[Concentration of oil to sea water 1:500]

Date	Duration of exposure	Number of oysters alive in the various test materials indicated		
		Control in sea water	Sea water with crude oil	Sea water with crude oil mixed with carbonized sand
1948	Days			
April 15	0	12	12	12
16	1	12	12	12
17	2	12	12	12
18	3	12	12	12
19	4	12	12	12
20	5	12	12	12
21	6	12	12	12
22	7	12	12	12
23	8	12	12	12
24	9	12	11	11
25	10	12	10	11
26	11	12	9	10

by placing a small aquarium 25 x 13 inches over the top of the tank, pouring 500 ml. of crude oil on the water of the aquarium, and treating the oil with carbonized sand. The depth of water was maintained at two and three-fourths inches. Through an overflow the water from this aquarium was delivered to the tank with oysters. The rate of flow of water through each tank was maintained at the rate of three liters per minute. The oysters used in the tests were four and five year old marketable stock from private grounds operated by a commercial company in Narragansett Bay, Rhode Island.

The results of the test were negative. Within the first three days from two to six oysters died in each tank, undoubtedly due to weakness from handling, but no further mortality occurred during the 35 days of exposure in any of the tanks. The experiment was conducted in August and September when the temperature fluctuated between 22° and 18° C. There was considerable accumulation of feces and pseudofeces in all of the tanks, indicating that oysters were feeding. At the end of the test, a fairly good growth of shell, varying from one-eighth to one-fourth of an inch, was noticed in many oysters.

From these observations we conclude that the 500 ml. of oil introduced into a water system running at the rate of 180 liters per hour and anchored by carbonized sand was insufficient to cause mortality or to inhibit the growth of the shells of adult oysters.

3. Effect of oil, oil extracts, and oils mixed with carbonized sand on the rate of filtration of water:

The maintenance of a steady flow of water through the gills of an oyster is essential for its feeding and respiration. The current is produced by the beating of the lateral cilia of the gills, while the access of water to the branchial chambers is regulated by the position of the opposing edges of the mantle and by the extent to which the shells are open. Numerous small openings (ostia) on the surface of the gills regulate the flow of water to the branchial tubes. Thus, the oyster has several mechanisms by which the rate of flow of water may be controlled. The ciliary epithelium may be compared to a pump, the capacity of which depends on the rate of beating of the cilia, while the mantle and the adductor muscle are comparable to a check valve which regulates the flow of water. There is a certain degree of independence in the functioning of the mantle and of the adductor muscle. The latter may be relaxed and the valves of the oyster wide open while the two edges of the mantle come together and completely seal the entrance to the branchial cavity, thus intercepting the access of water.

The measurement of the rate of filtration of water is a very sensitive means of studying the effect of changes in the environment of the oyster, for the organism rapidly reacts to the presence of toxic substances which may be introduced into natural waters. Methods are available at present for measuring the efficiency of the ciliated mechanism alone or for obtaining the over-all picture of the function of the entire pumping system. Both methods have their own advantages and limitations, which are briefly discussed below.

The materials used in the tests were: Diesel oil, crude oil, water extracts of these oils, and oil treated with carbonized sand.

a. Effects on the efficiency of ciliated epithelium of the gills (carmine cone method):

The slowing down of the work of the ciliated epithelium of the gills by water soluble fractions of crude oil was first demonstrated in 1931-1932 by Galtsoff and Smith (Galtsoff et al., 1935, pp. 167-193) using the carmine cone and the drop counting methods. In these methods the functions of the adductor muscle and the mantle of the oyster are eliminated and measurement is made of the efficiency of the pumping mechanisms alone. The carmine cone technique is simple in operation and allows the accumulation of considerable data in a minimum of experimental time. This advantage warrants its use for many purposes. The carmine cone method as used in these studies has been fully described by Galtsoff et al. (1947) and by Chipman (1948).

Oysters for these experiments were obtained from the Chesapeake Bay close to the mouth of the Severn River. They were kept in sea water tanks for a period of several days to allow adjustment to laboratory conditions. The salinity of the sea water varied from 14.09 to 14.70 parts per thousand. All of the experiments were run at room temperature, which ranged from 22.2° to 26.0° C., but the greater number of tests were carried on at 24° or 25° C. Fluctuations during any one experiment usually did not exceed ± 0.2° C.

Comparison of the rate of pumping before and after the addition of the test material was used as a basis for determining the effect. Control oysters in non-polluted sea water were observed at the same time to check any effects that may be due to other causes.

The following procedure was followed in conducting these tests: The oyster, with the shell kept open by a small glass rod and with rubber tubing inserted into the cloaca, was placed in a large enamelware tray containing five liters of water. The rubber tubing was then connected to a glass tube for measuring the velocity of current, and series of measurements made during a period of two or more hours. Then the material to be tested was introduced, the water well stirred, and the measurements continued for several hours. In testing oil or oil extracts, the desired volume of water was withdrawn from the tray and replaced by an equal volume of test liquid. The results, representing the mean of 10 consecutive readings, expressed as the rate of current of water in mm. per second, were plotted against time.

In the first test 20 ml. of Diesel oil were used, which gave the ratio of one part of oil to 250 parts of sea water. The results, plotted in figure 3, clearly indicate a marked depression in the rate of current, which reaches its maximum about one hour after the introduction of oil. It is significant that the rate of current of water in the control oysters gradually increases, while in both experimental oysters it is materially depressed. We conclude from these observations that oil contains substances which, after being leached by water, exert a depressive effect on the ciliated epithelium of the oyster.

To corroborate this inference we prepared an extract by shaking a sample of Diesel oil with an equal volume of sea water and tested its effect. The mixture of oil and sea water was poured in a large Erlenmeyer flask with only sufficient air space to allow good agitation and shaken for three hours on each of three succeeding days. Then the sample was transferred to a large separatory funnel and, after separation of the oil and water, the transparent and colorless water fraction was withdrawn and used in the desired concentration. As can be seen from figure 4, 10 percent had no effect, slight reduction was caused by 20 percent, while very marked depression occurred in the concentrations of 30 and 50 percent.

Because of the known greater toxicity of crude oil, only 200 ml. in four liters of sea water were used in preparing an extract. The sample was shaken for five hours and then allowed to separate overnight. Marked depression in the rate of cloacal current was produced in the concentrations of 50 and 20 percent of this extract (figures 5 and 6).

In a second test, the oil was used in a ratio of one part to 100 parts of sea water. The mixture was poured into a carboy and allowed to stand for six days; during this time it was aerated by streams of fine air bubbles which kept the oil agitated. No reduction in the rate of current was noticed in the samples containing 30 and 50 percent of this extract (figures 7 and 8).

A mixture was prepared of 100 ml. of crude oil and a quantity of carbonized sand sufficient to remove all the oil from the surface and submerge it. The mixture was then placed on the bottom of a five liter tray containing experimental oysters. The resultant ratio was equivalent to 1 part of oil to 50 parts of sea water. A marked depression in the rate of cloacal current (figure 9) indicates the effect caused by a gradual release of the physiologically active materials. By the end of the third hour after the introduction of the mixture, the rate of current produced by the oyster was still declining, while in the control oyster it was gradually increasing.

In another experiment we tested a water extract prepared from an oil and sand mixture. In this case 200 ml. of oil combined with sufficient amount of sand were shaken for six hours in four liters of sea water. A solution containing 50 percent of this extract in sea water greatly depressed the rate of current (figure 10).

From these experiments we conclude that anchoring of oil by carbonized sand does not prevent the release of physiologically active substances which depress the activity of the ciliated epithelium of the gills of the oyster.

b. Effects on the rate of pumping of water by the intact oyster (apron method):

The apron method for measuring the rate of pumping of water by oysters presents several advantages that are lacking in the carmine cone technique. With the latter, the functioning of the adductor muscle and the mantle of the oyster is prevented by the inserting of a glass rod between the valves and the introducing of a rubber tube into the cloaca. In the apron method the rate of pumping of the intact oyster is measured. Partial wrapping of the oyster in thin rubber dam to channel the cloacal current is the only deviation from a normal condition. The functioning of the internal organs is not in any way affected by this arrangement and, since the adductor muscle and the mantle continue to react normally, there is no interference with the spontaneous movements of the shell, important for the well being of the oyster. Thousands of observations made in our and other laboratories since the apron technique was suggested by Thurlow Nelson (1936) show that this experimental procedure does not interfere with the normal behavior of the oyster. In the work conducted by us since 1935, first at the laboratory at Yorktown, Virginia (Galtsoff et al., 1947), and at Woods Hole, Massachusetts, in connection with our studies on respiration, we have accumulated several thousand records of oyster activities under normal and altered conditions and found that oysters wrapped in the rubber apron according to Nelson's method and kept in sea water running freely through Galtsoff's constant-level tanks (Galtsoff, 1926) suffer no ill effects. Using this method we have been able to obtain uninterrupted records of shell movement and of rate of pumping of water by the oyster kept under observation for fortnight periods and longer. The complexity

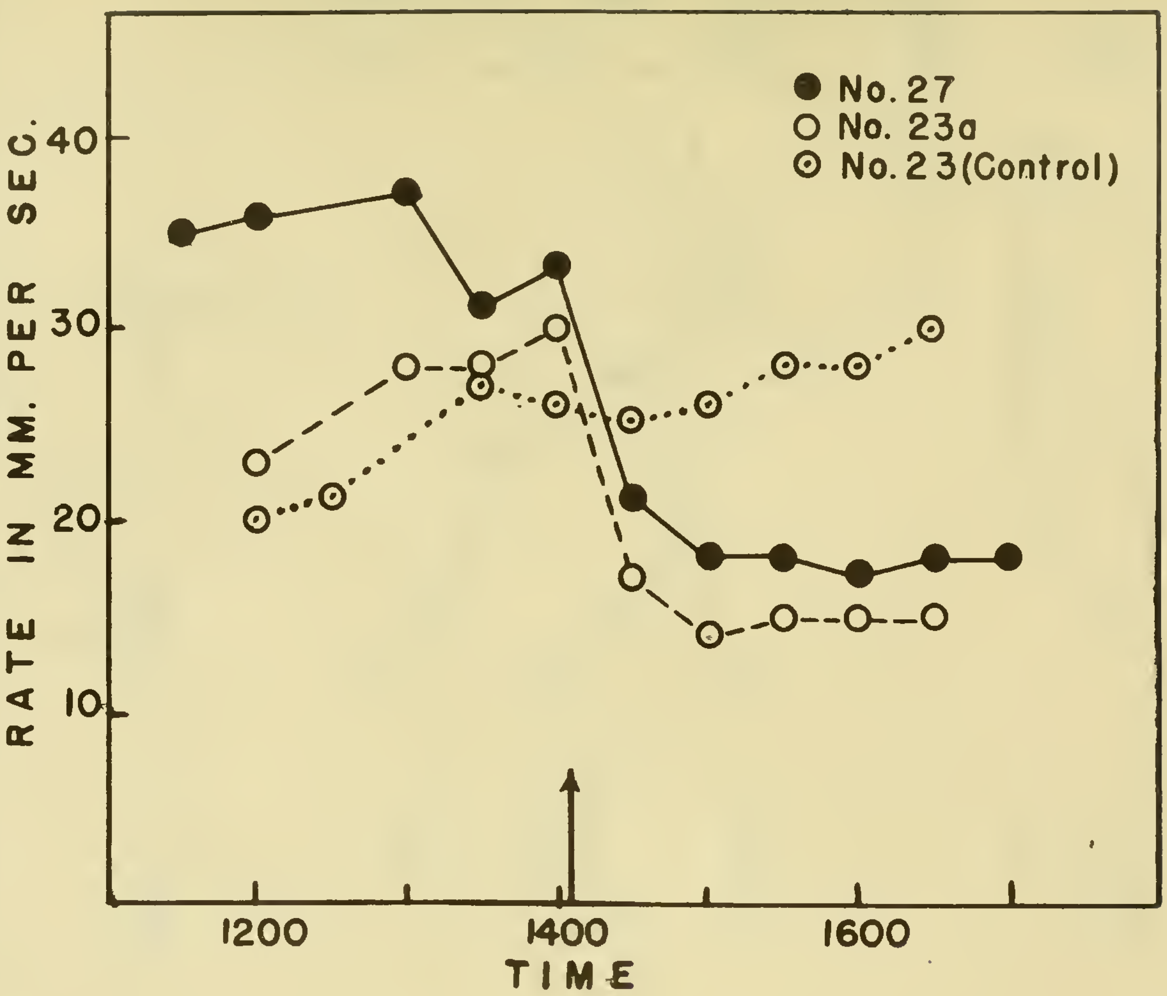

Figure 3. - - - Effects of Diesel oil on the rate of water filtration of oysters measured as a velocity of cloacal current in mm. per second. Addition of 20 ml. of oil to the 500 ml. of sea water in which oyster was being observed indicated by arrow. Carmine cone method. Time shown in hours of the day.

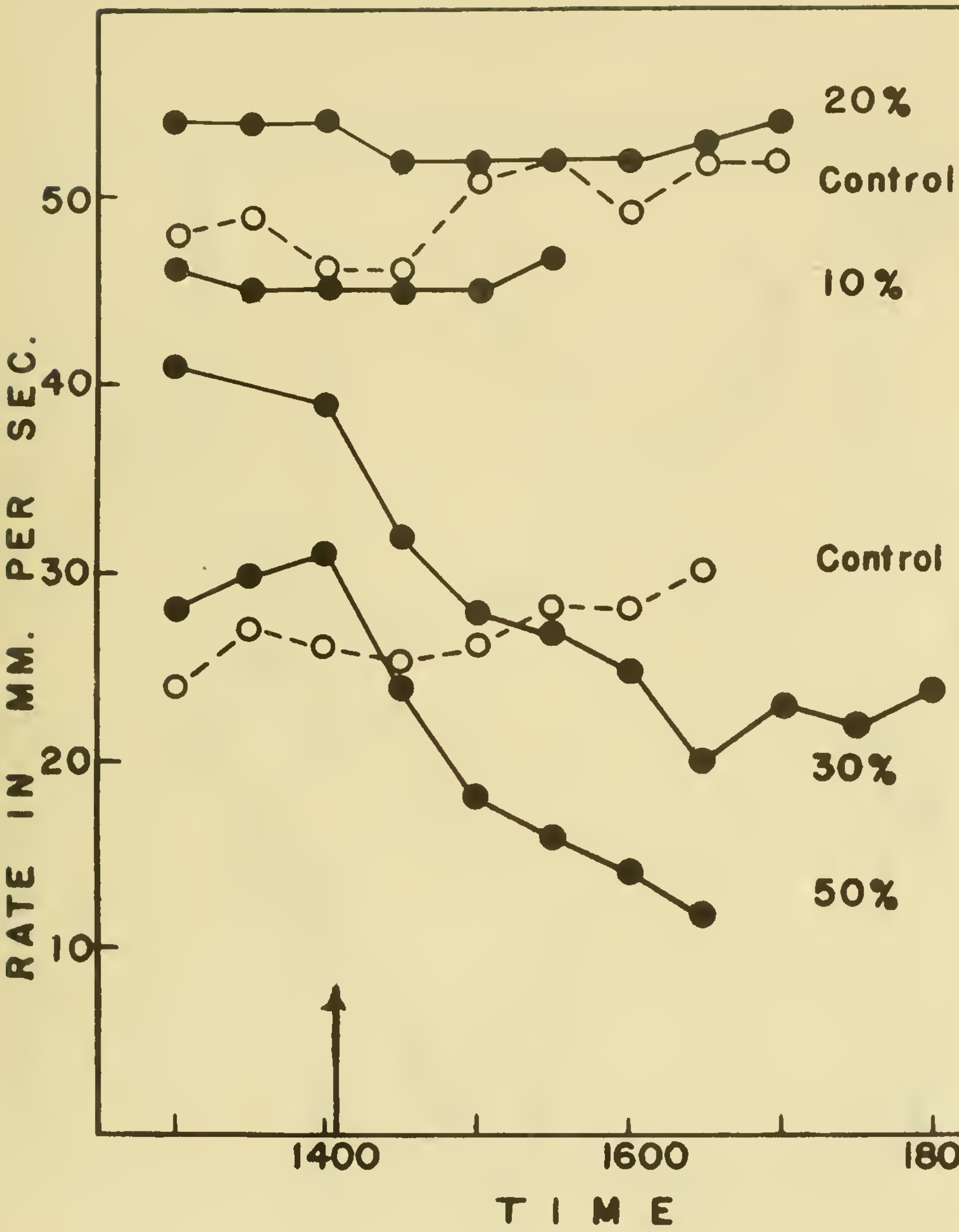

Figure 4. --- Effects of various concentrations of extract of Diesel oil in sea water on the rate of water filtration of oysters. Extract was made by using 1,500 ml. of Diesel oil in 1,500 ml. of sea water and shaking three hours on each of three successive days. Arrow indicates time of addition of extract. Carmine cone method. Ordinate - velocity of cloacal current in mm. per second; abscissa - time of day.

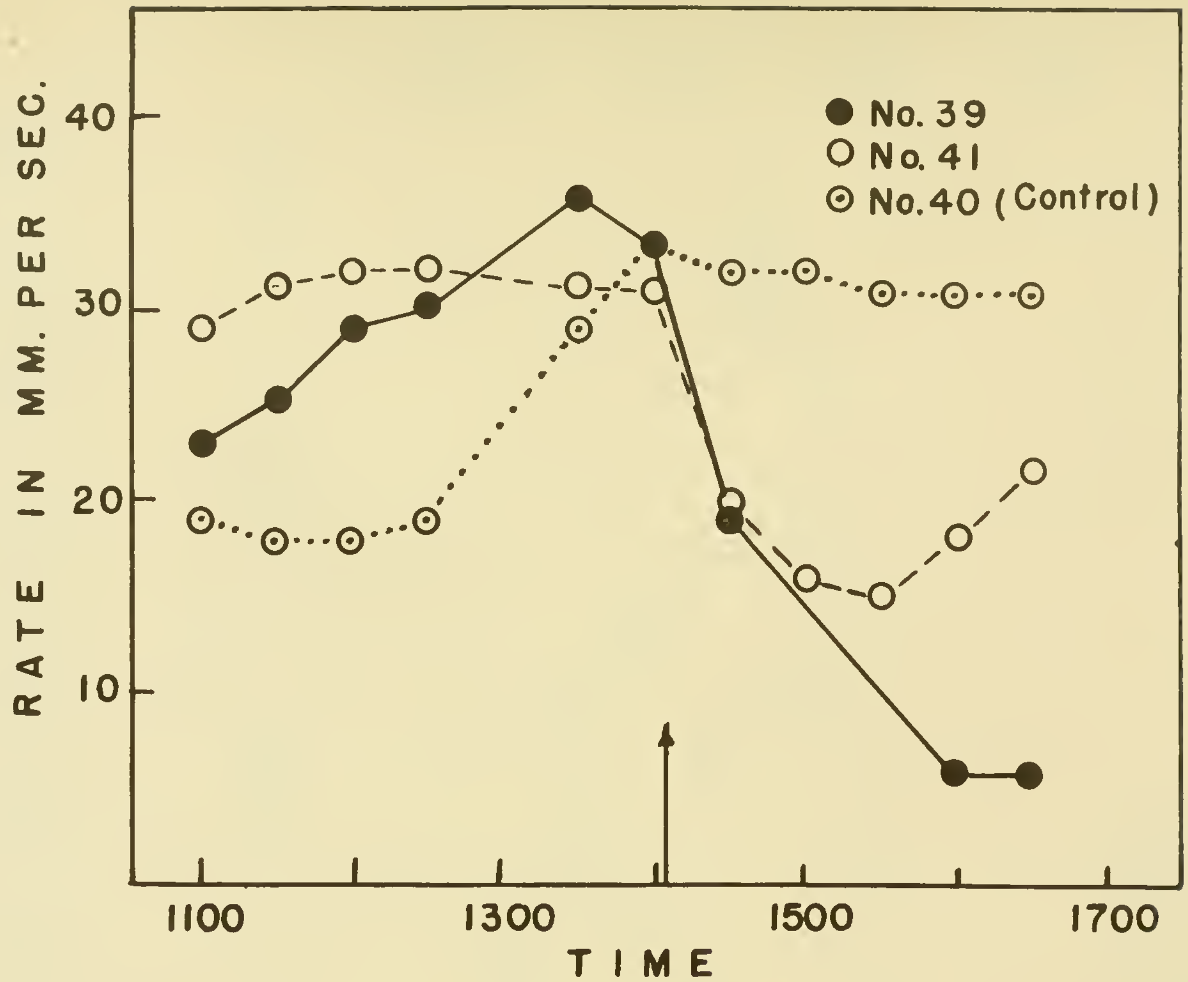

Figure 5. --- Effects of 50 percent crude oil extract on the rate of water filtration of oysters. Extract of 200 ml. of crude oil shaken for five hours in 4,000 ml. of sea water. Carmine cone method. Arrow indicates time of addition. Plotting as in figures 3 and 4.

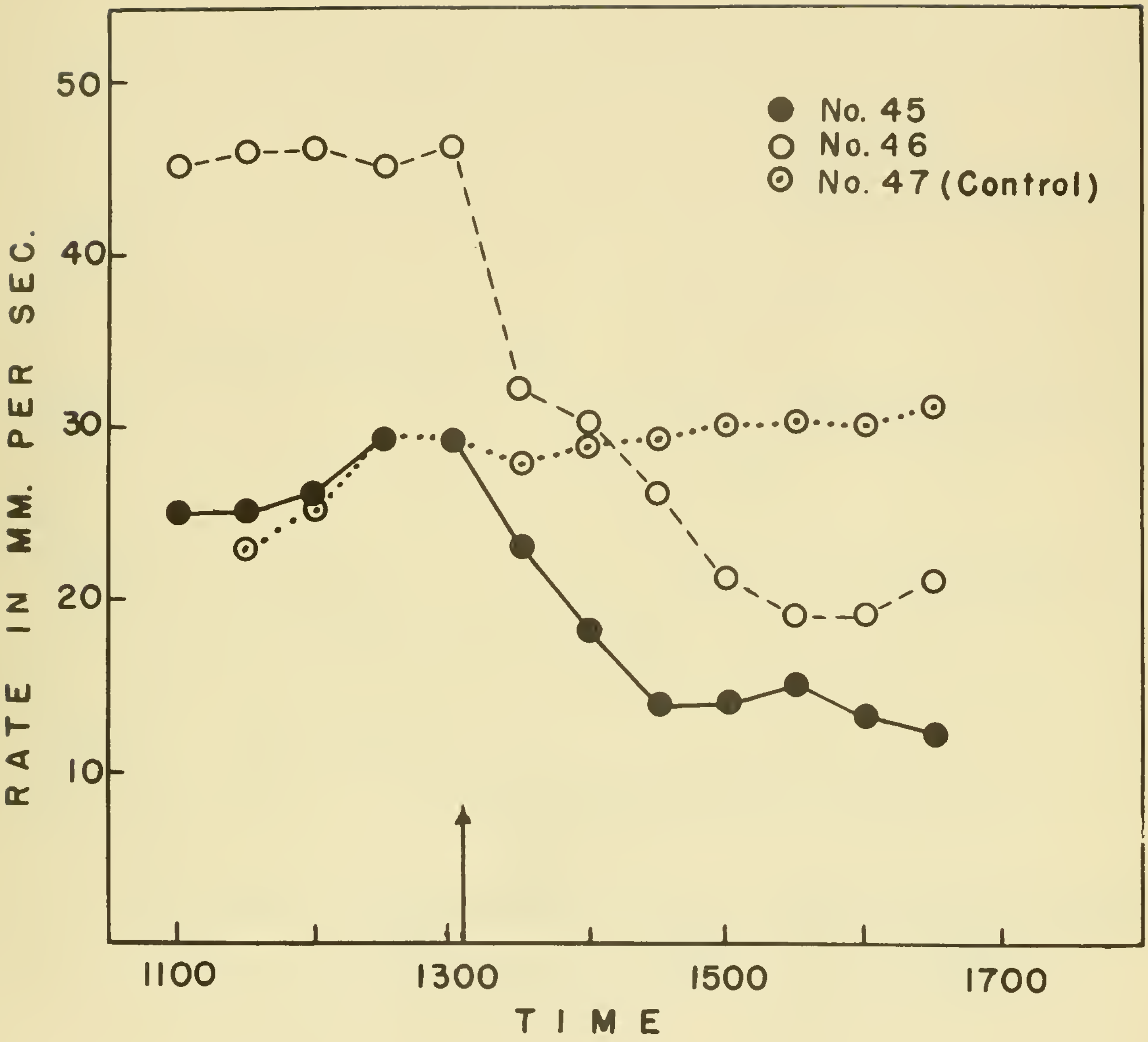

Figure 6. --- Effects of 20 percent of crude oil extract on the rate of water filtration of oysters. Extract of 200 ml. of crude oil shaken for five hours in 4,000 ml. of sea water. Carmine cone method. Arrow indicates time of addition. Plotting as in figures 3 and 4.

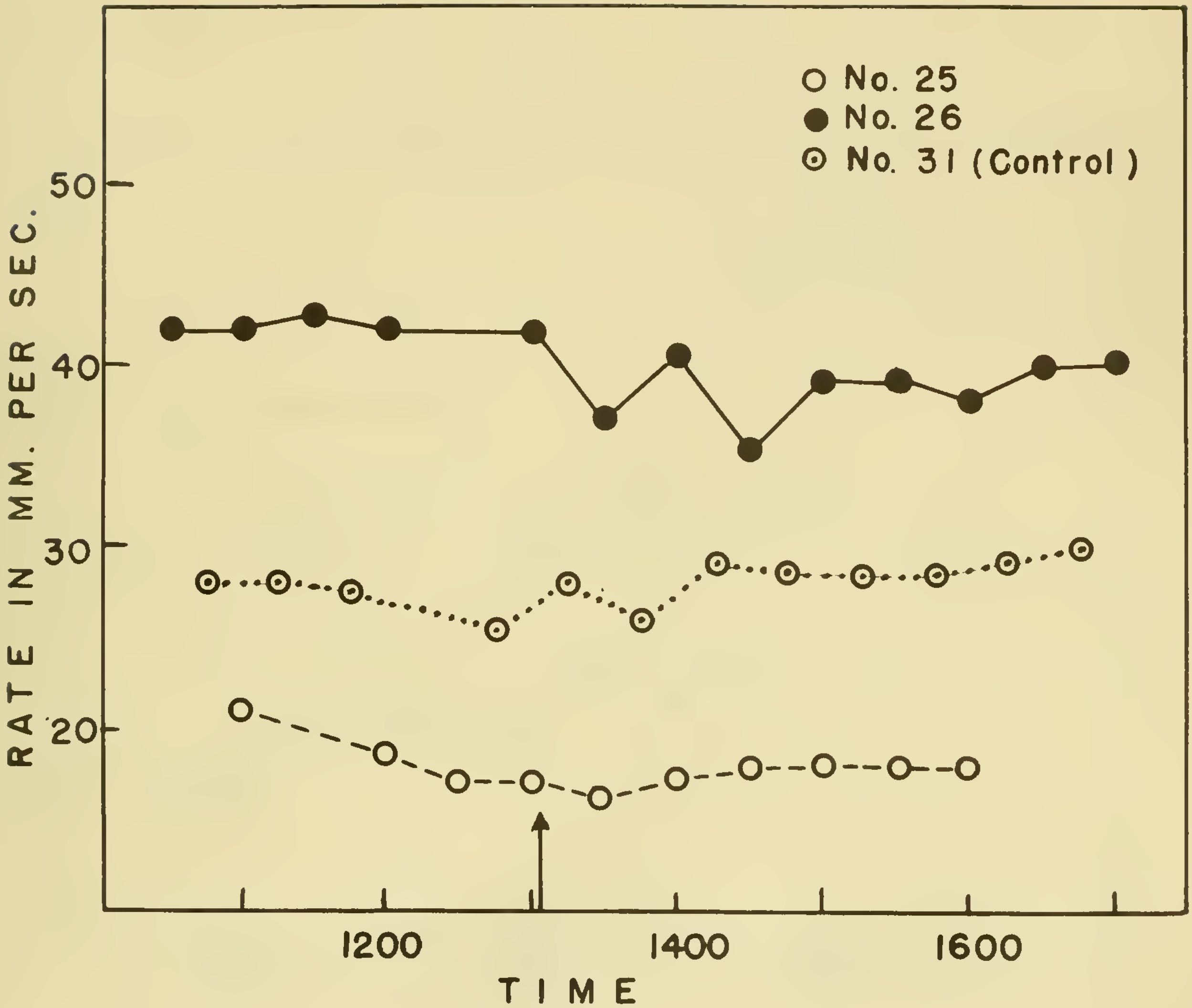

Figure 7. --- Effects of aerated extracts of crude oil in a concentration of 30 percent on the rate of water filtration of oysters. A mixture of 160 ml. crude oil and 16 liters of sea water was left standing and aerated for six days. Arrow indicates time of addition. Carmine cone method. Plotting as in figures 3 and 4.

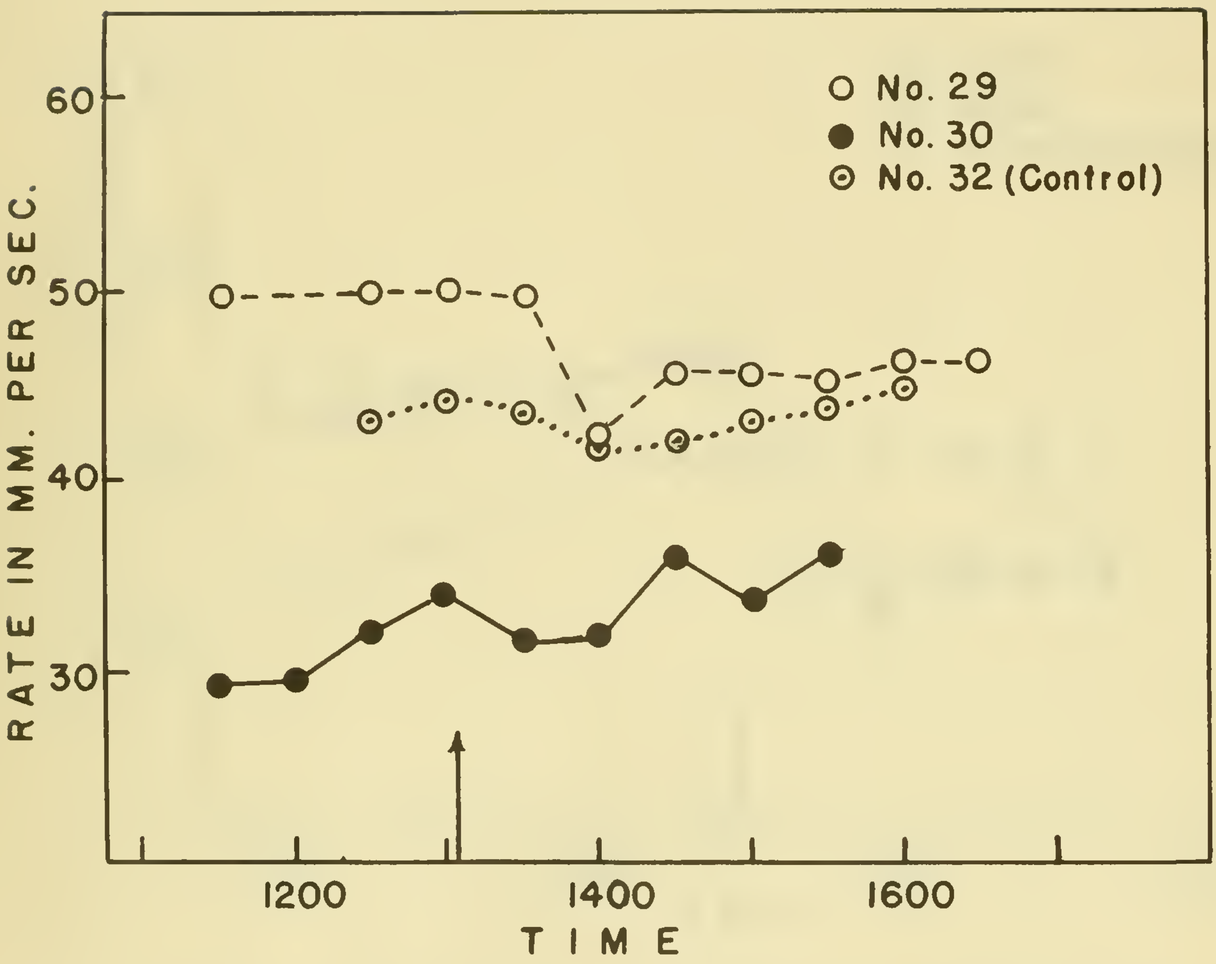

Figure 8. --- Effects of aerated extract of crude oil in a concentration of 50 percent on the rate of water filtration of oysters. Preparation of extract as in figure 7. Arrow indicates time of addition. Carmine cone method. Plotting as in figures 3 and 4.

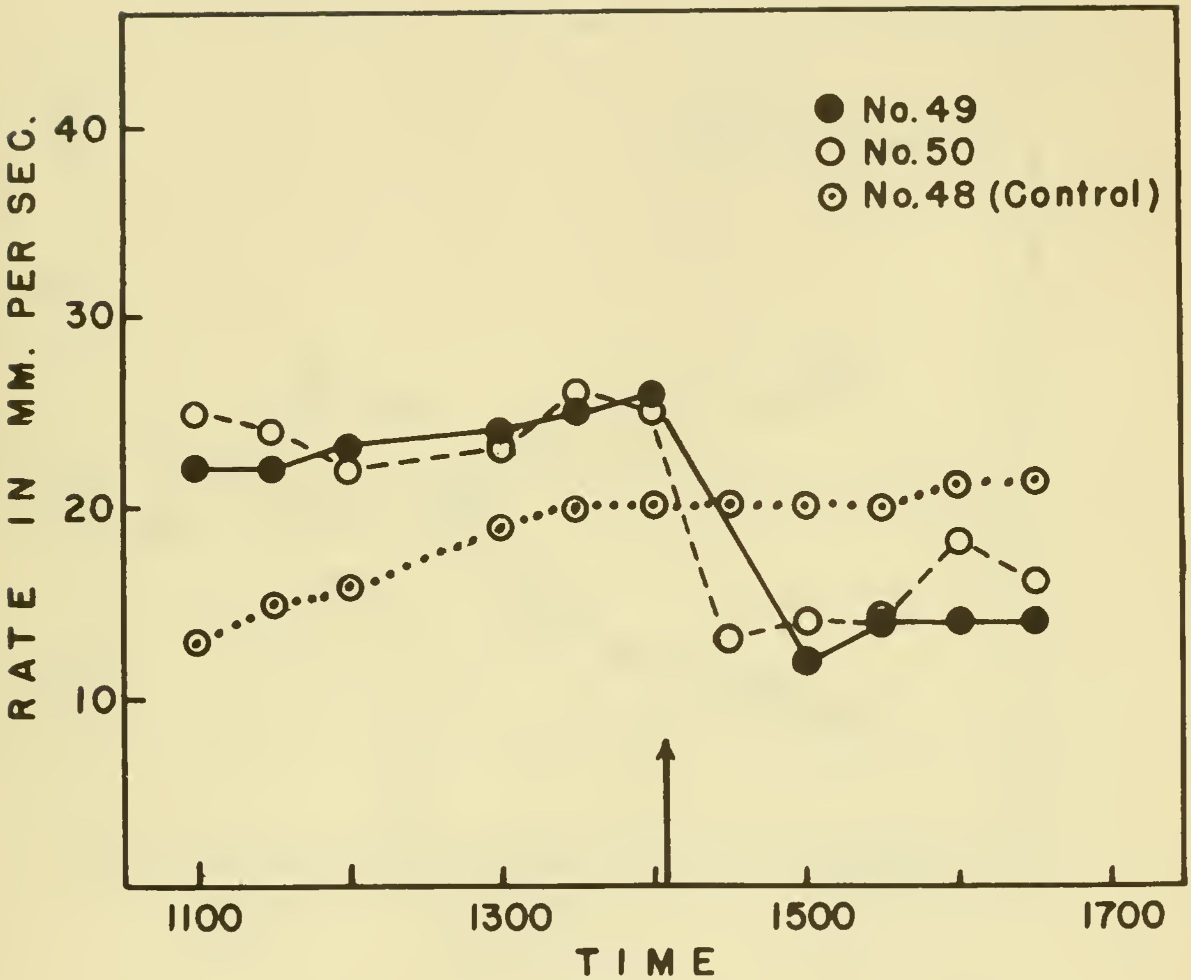

Figure 9. --- Effect of crude oil mixed with carbonized sand on the rate of water filtration of oysters. Addition of 100 ml. crude oil treated with carbonized sand to 5,000 ml. of sea water in which the oyster was being observed indicated by arrow. Carmine cone method. Plotting as in figures 3 and 4.

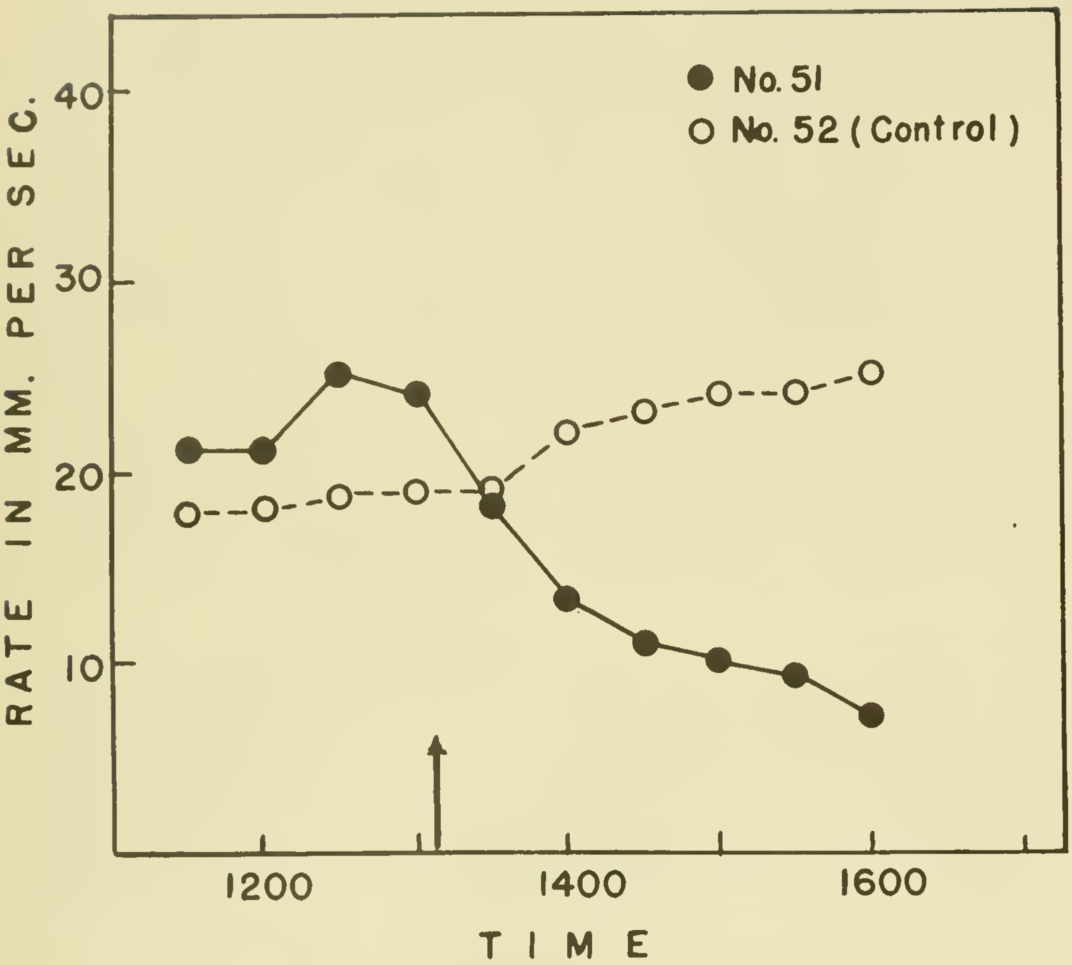

Figure 10. --- Effect of the addition of extract of a mixture of crude oil and carbonized sand on the rate of water filtration of oysters. A mixture of carbonized sand and 200 ml. of crude oil in four liters of sea water were shaken for six hours and a portion of the extract diluted with an equal volume of sea water for the test. Carmine cone method. Arrow indicates time of addition. Plotting as in figures 3 and 4.

of the experimental set-up, which requires continual attention, and the length of time needed to complete the test limit the use of the method and restrict the number of animals that can be used in bio-assaying. From this point of view the carmine cone method presents greater advantages, for the latter test may be completed on a larger number of specimens in a relatively short time.

The experimental set-up for recording the rate of pumping of oysters by the apron method, as it has been developed in the laboratories of the Section of Shellfishery Investigations at College Park and Woods Hole, has been fully described and illustrated in a previous publication (Galtsoff, 1946, pages 7-11) and requires no additional discussion. (See Appendix 1.) Only minor changes in the construction of the set-up have been made for the present work. One of these involved the use of plexiglass in place of the cellulose nitrate employed previously for the construction of the apparatus. A change was made also in the construction of the dumping vessel. It consisted in mounting above the dumping chamber a small rectangular container to receive the water filtered by the oyster. A glass rod inserted into a drain on the bottom of the container keeps the drain open when the dumping vessel is in an upright position, as shown in figure 11. When the float (inside the dumping vessel) releases the catch and the vessel turns over, the glass rod in the drain tube drops down and a small metal ball, above the drain, closes the outlet. In this way no water is lost when the dumping vessel turns over. When the vessel returns to its upright position, the glass rod is pushed upward, the ball is lifted, and the water from the container flows into the vessel.

In the experiments described below, crude oil was mixed with carbonized sand in the ratio of 50 ml. of oil to 127 grams of sand. After rinsing the mixture in sea water and wiping off excess oil, the paste-like mass was placed in the two middle compartments of the mixing chamber (B in figure 2, Appendix 1) through which the sea water was running at a rate slightly in excess of the rate of pumping of water by the oyster. The latter precaution is important since accurate results cannot be obtained if the level in the tank in which the oyster is kept does not remain constant (Galtsoff, 1928).

Oysters used in the experiments were obtained either from Hadley Harbor, a short distance from Woods Hole, or from Narragansett Bay, Rhode Island. Prior to experimentation they were kept in the laboratory tanks for at least a week to allow for a complete adjustment to the new environment. Two oysters were used for each test. One oyster received sea water flowing over a submerged layer of oil and carbonized sand and the other, as a control, was supplied uncontaminated sea water.

At the beginning of each experiment, a fresh sample of oil and sand was prepared and used without being renewed until the end of the test. No oil slick was noticeable on the surface, but the water in the oil-contaminated tank was slightly fluorescent in ultra-violet light. No fluorescence was observed in the control tank. In a few days after the beginning of each test, the oil and sand mixture became covered with a layer of brown organic sediment which was not disturbed.

The kymograph paper was changed once every day, always at 0800. This moment was considered the beginning of each 24-hour period. Water temperature was recorded two or three times a day. After being set and adjusted, the constant level tanks and dumping vessels worked automatically without serious mishaps. Only few records were lost because of the failure of the dumping vessel to turn over or because of the stoppage of the spring kymographs.

Time was recorded at 30-minute intervals using a Telechrone electric motor. To the axis of this was mounted a plexiglass disc with two copper springs set opposite each other at the periphery of the disc. In the lowermost position, the spring made contact with two terminals mounted on a suitable base completing the electric circuit which activated small signal magnets connected to writing levers.

In analyzing the records, the time of opening and closing of the oyster and the number of dumps were summarized for each hour. The dumping vessels were carefully calibrated before the experiments - their capacity was 265 and 276 ml. The number of dumps per hour, recorded by kymograph, was multiplied by the corresponding figure and the maximum, minimum, and the mean rate of pumping were computed. The mean rate of pumping given in the tables refers to the periods the oyster was open and therefore considered as able to feed. By this method the efficiency of the ciliary mechanisms can be more accurately expressed than by the mean rate of pumping for every 24 hours, irrespective of whether the oyster was open or closed. Thus, the figures of the mean rate of pumping given in this report refer to the situations when the oysters had an opportunity to filter water through their gills. The percentage of time the oyster remained open each day is given in a separate column.

No attempt was made to control the temperature since it fluctuated only within narrow margins not exceeding $\pm$ 1.5° C. during a day. The salinity of the water, as usual for Woods Hole, remained fairly constant, varying between 31 and 32 parts per thousand.

In observing the condition of the sea water as it was running through the mixing chambers, it was noted that a fairly large number of copepods accumulated in the last compartment of the chamber. On several occasions the number of copepods was counted by using a magnifying glass and exploring the entire depth and width of the compartment. In the control, the number usually varied from 50 to 75, while in the experimental set-up, after the addition of oil, it fluctuated between 15 and 25. No more detailed study of this was made because no sample of water from the mixing chamber could be removed without interfering with the operation of the constant level tank. This observation suggests, however, that the presence of substances leached from crude oil may exert some repelling effect on copepods.

Three tests, lasting 16, 33, and 15 days respectively, were performed during July and August of 1948. The conditions of the test, so far as temperature, salinity, rate of renewal of water, and amount of oil used, were almost identical. The results are summarized in tables 21, 22, and 23 and are presented graphically in figures 12, 13, and 14.

The reactions of the oysters to the water contaminated with oil can be easily observed in the changes in the muscular movement and in the rate of pumping. A very distinct case of almost immediate response is shown in figure 15, which represents photographic reproduction of the original record. The following changes become apparent shortly after the introduction of oil into the sea water system: (1) Partial contraction of the adductor muscle (1st line) and the ensuing reduction in the degree of opening of the valves and restriction of the access of water to the mantle cavity; (2) increase in the number of the contractions of the adductor and decrease in the extent of each contraction, shown by the vertical excursion of the up and down strokes of the lever, (3) marked decrease in the rate of pumping of water (2d line). No such changes take place in the control oyster (figure 16) which continues to pump water at a very high rate. Sometimes these changes are less pronounced and become apparent only in about one hour after the addition of the oil-contaminated water (figure 17). In all three experiments, however, a change from pure to oil-contaminated water sooner or later resulted in a significant decrease in the percentage of time the oysters remained open and in a marked reduction in the rate of pumping.

The depressing effect of the substance or substances leached from oil lasts for a long time. This can be seen by examining the data summarized in tables 21, 22, and 23, and shown graphically in figures 12, 13, and 14. In the first two experiments (figures 12 and 13) there was, apparently, a common factor which affected both the experimental and the control oysters and was responsible for the rise and fall in the rate of pumping after the eighth day of the test. The nature of this factor has not been definitely established but it is reasonable to postulate that it was correlated with the prespawning condition of the oysters. In the summer of 1948, oysters kept in laboratory tanks spawned several times between July 8 and 31. Mass spawning was observed on July 24, the day which corresponds to the eighth day of the second experiment (figure 13). Our previous experience in studying spawning of the oyster shows that the maximum rate of pumping by the oysters may be expected at the time they are ready to shed their sex products. It is therefore reasonable to infer that gradual increase in the rate of pumping observed in our tests was due to this cause. This tentative explanation

TABLE 21. --- Experiment No. 1: Effect of crude oil mixed with carbonized sand on the percentage of time the oyster remains open and on the rate of pumping of water July 1 to 16, 1948, Woods Hole, Massachusetts

[Rate of flow of water through each experimental tank was 15.0 $\pm$ 0.5 liters per hour]

Day	Temperature range		Oyster No. 1					Oyster No. 2				
			Time open	Liters per hour pumped			Total liters pumped	Time open	Liters per hour pumped			Total liters pumped
				Maximum	Minimum	Mean			Maximum	Minimum	Mean	
	°C.	°C.	Percent					Percent				
1	19.8	19.0	97	3.2	1.3	2.7	65.5	18	3.31	0.28	1.4	8.6
2	19.1	19.1	92	3.7	1.06	2.8	54.8	61	0.83	4.14	2.6	38.9
3	19.1	20.0	69	3.4	1.86	2.9	44.5	100	3.86	6.07	5.3	127.6
4	19.8	20.0	43	0.5	3.7	2.5	37.3	87	8.6	0.5	6.1	137.3
5	20.0	20.2	93	9.0	0.8	6.0	133.8 1/	94	8.5	1.9	7.3	161.5
6	20.2	19.3	85	7.4	1.0	5.0	106.4	89	9.7	0.2	6.4	157.4
7	19.3	19.3	59	3.7	0.2	2.5	37.1	94	9.4	3.3	8.5	191.8 2/
8	18.8	20.5	48	2.6	0.8	2.3	25.7	81	13.0	0.28	8.1	177.0 2/
9	19.7	20.0	33	5.0	0.3	1.7	15.7	93	11.0	1.7	7.5	167.0
10	20.0	19.9	48	4.8	0.3	3.9	51.4	95	12.1	0.6	10.5	239.4
11	19.9	20.0	100	7.2	9.0	7.9	190.0	92	13.0	5.0	12.9	249.2
12	20.0	20.2	80	9.8	1.06	6.2	130.9 3/	87	13.0	0.5	9.3	223.8 3/
13	20.2	20.2	42	3.4	0.3	1.8	22.0	---	---	---	---	---- 4/
14	20.4	20.4	29	4.0	0.8	2.5	17.5	79	10.8	0.5	5.3	111.8
15	20.4	19.6	45	1.6	0.3	1.1	9.0	88	11.0	0.5	4.2	110.1
16	19.6	20.2	39	2.9	0.8	2.2	15.4	95	10.0	0.5	3.3	75.3

1/ 50 cc of crude oil in 127 grams of carbonized sand placed in mixing chamber at 1830
2/ Total daily volume of water estimated. Record out of order for 8 hours.
3/ Supply of the water changed at 2100. Oyster No. 1 receives uncontaminated water; oyster No. 2 is supplied with water which is in contact with carbonized sand saturated with oil.
4/ Apparatus out of order.

TABLE 22. --- Experiment No. 2: Effect of crude oil mixed with carbonized sand on the percentage of time the oyster remains open and on the rate of pumping of water July 17 to August 18, 1948, Woods Hole, Mass.

[Rate of flow of water through each experimental tank was 15.0 ± 0.5 liters per hour]

Day	Temperature range		Oyster No. 3					Oyster No. 4				
			Time open	Liters per hour pumped			Total liters pumped	Time open	Liters per hour pumped			Total liters pumped
				Maximum	Minimum	Mean			Maximum	Minimum	Mean	
	°C.	°C.	Percent					Percent				
1	20.8	20.8	43.7	4.5	0.3	2.4	21.8	85.1	10.5	3.0	7.3	138.8
2	20.8	21.6	63.0	6.9	0.8	3.6	51.1	96.0	12.4	0.5	9.50	208.9
3	21.2	21.2	95.0	10.3	0.8	7.2 1/	144.6 1/	96.0	12.4	1.4	11.1	266.3
4	21.2	21.8	66.0	11.0	2.6	8.6	138.0	88.0	12.4	8.0	11.3	249.2
5	21.3	21.8	65.6	11.9	0.3	9.04	153.6	100.0	24.3	9.9	16.0	384.6
6	21.1	20.8	79.5	11.9	0.3	9.36	187.8	95.5	23.2	1.1	19.1	457.8
7	20.8	21.1	52.1	11.1	0.8	5.9	59.1	100.0	24.3	1.1	18.9	453.7
8	20.6	21.2	66.0	12.8	4.2	11.1	177.8	83.0	18.5	1.9	13.5	311.0
9	20.6	21.9	86.5	11.1	2.9	8.9	178.6	90.3	16.8	0.3	13.9	320.8
10	21.1	21.5	76.4	12.2	1.3	8.3	157.1	83.3	19.6	5.5	15.0	330.1
11	21.5	21.8	57.3	12.2	2.7	9.2	128.8	81.2	19.3	0.6	14.6	307.0
12	21.5	21.5	72.9	11.9	0.8	9.2	173.8	80.9	20.1	6.6	16.3	359.1
13	22.4	22.0	95.8	10.6	0.5	4.9	113.7	91.1	18.5	1.7	13.5	308.6
14	22.0	22.6	62.5	4.8	0.0	1.9	26.2	92.7	19.9	0.0	13.1	302.8
15	22.6	21.8	33.3	4.8	0.3	2.8	22.2	66.7	16.0	6.3	13.3	213.2
16	21.8	22.2	25.0	2.4	0.3	1.5	6.1	58.3	13.2	2.8	9.2	119.2
17	22.1	22.5	25.0	3.2	1.9	2.6	10.3	70.0	17.4	0.0	10.6	191.3
18	22.0	23.4	58.3	0.0	0.0	0.0	0.0	64.6	15.2	0.6	8.6	146.3
19	22.3	21.7	34.4	----	---	---	----- 2/	50.0	11.9	0.5	8.6	77.5
20	21.5	21.0	45.8	1.6	0.3	1.1	12.2	50.0	19.3	1.1	15.6	187.4
21	20.7	21.4	57.0	3.4	1.3	2.3	25.7	84.0	17.9	6.9	14.9	298.1
22	20.7	20.7	84.7	3.4	0.3	1.9	32.0	83.4	17.7	9.4	14.6	321.8
23	20.7	21.0	0.0	0.0	0.0	0.0	0.0	93.8	17.7	0.0	12.1	289.7
24	21.0	21.8	32.0	2.4	0.0	1.0	10.9	90.6	19.3	0.3	13.8	330.4
25	21.2	21.8	49.6	1.5	0.5	0.9	7.3	86.1	18.4	0.3	15.6	342.7
26	21.3	22.0	53.1	0.8	0.3	0.6	1.9	87.2	19.9	0.3	16.4	287.5
27	21.6	21.8	42.4	0.0	0.0	0.0	0.0	88.2	13.8	0.0	7.8	149.9
28	21.3	21.6	79.2	4.7	0.0	2.1	37.6 3/	64.6	19.8	0.2	14.6	219.3 3/
29	21.6	21.3	81.1	5.3	0.0	1.6	29.1	48.0	18.2	0.5	14.1	155.2
30	21.3	21.6	55.2	5.3	0.0	2.8	36.0	58.3	15.7	0.8	11.5	160.9
31	21.6	21.2	64.6	5.3	0.3	2.3	38.6	45.8	14.9	0.2	8.3	108.2
32	21.5	21.8	63.6	5.3	0.5	3.1	43.2	36.5	8.8	0.8	3.7	33.4
33	21.5	20.8	60.5	1.3	0.2	0.8	7.9	33.3	8.3	0.3	0.5	3.6

1/ 50 cc of crude oil in 127 grams of carbonized sand placed in mixing chamber at 1400.
2/ Apparatus out of order.
3/ Oil-contaminated water shifted from oyster No. 3 to oyster No. 4 at 2100.

TABLE 23. --- Experiment No. 3: Effect of crude oil mixed with carbonized sand on the percentage of time the oyster remains open and on the rate of pumping of water August 4 to 17, 1948, Woods Hole, Mass.

[Rate of flow of water through each experimental tank was 15.0 ±0.5 liters per hour]

Day	Temperature range		Oyster No. 5					Oyster No. 6				
			Time open	Liters per hour pumped			Total liters pumped	Time open	Liters per hour pumped			Total liters pumped
				Maxi-mum	Mini-mum	Mean			Maxi-mum	Mini-mum	Mean	
	°C.	°C.	Percent					Percent				
1	22.4	21.5	100.0	3.4	2.1	2.72 1/	32.6	100.0	3.0	0.5	1.7	38.1 2/
2	21.5	21.0	91.7	7.7	1.3	4.4	89.0	85.4	4.1	0.3	2.2	42.2
3	21.4	20.7	95.1	12.4	1.8	8.6	207.2	91.0	9.1	0.8	5.8	127.2
4	20.7	21.4	95.1	15.6	1.5	12.2	292.3	95.8	15.4	0.6	11.9	286.0
5	20.7	21.6	92.4	18.0	0.3	13.5	310.6	100.0 3/	13.8	9.7	12.4	287.0
6	21.0	21.2	88.9	16.1	0.5	11.8	280.4	----- 4/	17.6	14.6	15.7	-----
7	21.2	21.8	88.6	10.6	0.3	1.6	34.2 5/	85.4	15.7	1.4	13.9	227.8
8	21.3	22.0	50.0	0.8	0.3	0.5	5.2	85.4	16.5	1.1	10.1	202.6
9	21.6	21.3	27.1	0.8	0.3	0.5	3.1	57.3	11.6	0.3	9.4	150.7
10	21.3	22.2	28.1	1.3	0.5	0.8	5.5	57.0	14.4	4.1	11.2	157.3
11	21.6	21.3	36.9	0.3	0.0	0.1	0.8	76.7	17.9	0.3	12.3	234.4
12	22.0	21.6	20.8	0.0	0.0	0.0	0.0	81.6	19.0	9.9	15.1	287.8
13	21.6	22.0	18.8	0.5	0.0	0.2	1.0	85.8	15.4	0.3	11.9	261.7
14	21.5	20.2	19.8	0.8	0.3	0.5	2.9	76.2	13.8	3.0	10.9	142.1
15	----	----	----	---	---	---	--- 6/	75.6	13.0	2.1	9.8	176.3

1/ Estimated on basis of 12-hour record.
2/ Estimated on basis of 22-hour record.
3/ Estimated on basis of 8-hour record.
4/ Shell lever out of order.
5/ 50 cc of crude oil with 127 grams of carbonized sand placed in mixing chamber at 1100.
6/ Apparatus out of order.

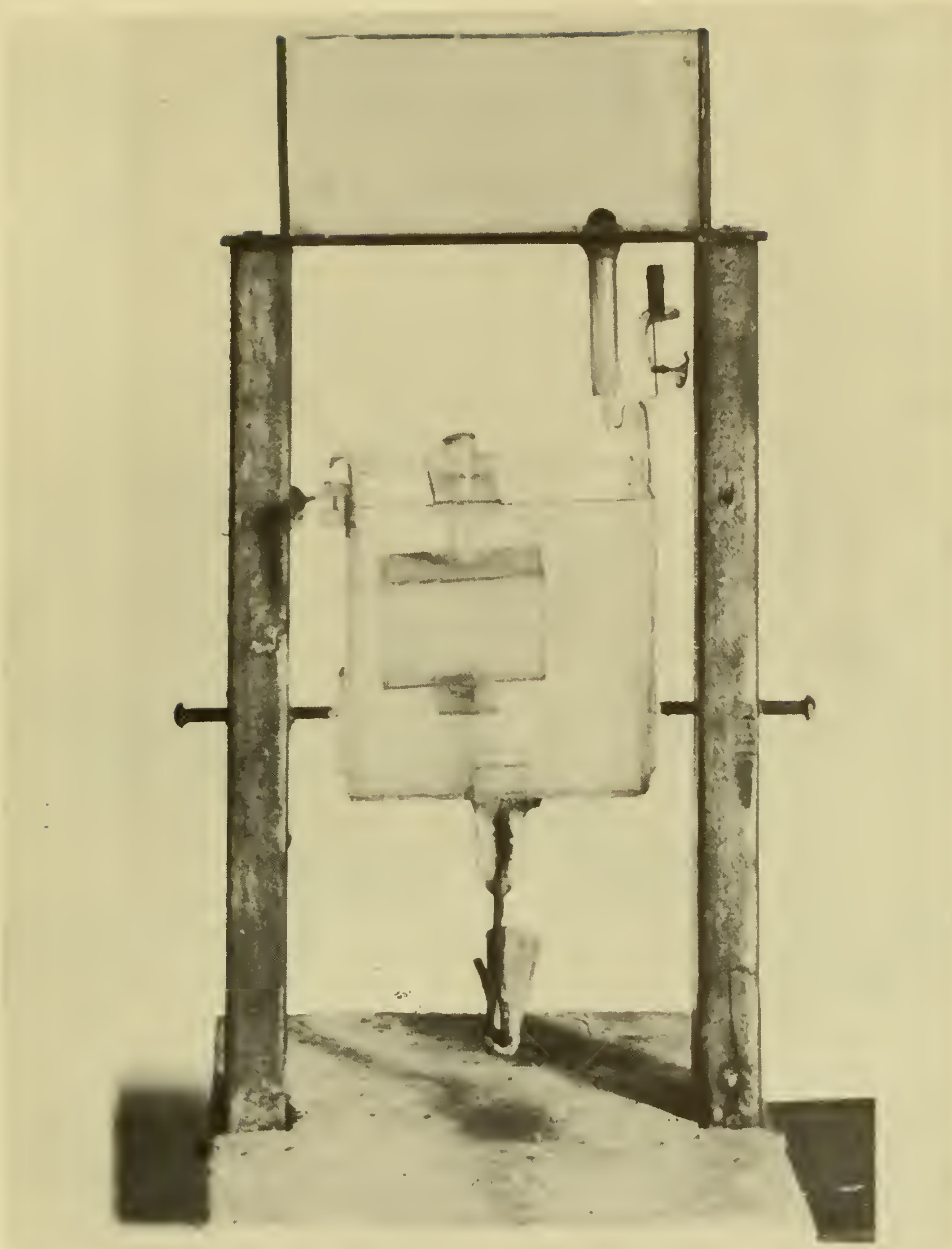

Figure 11. -- Dumping vessel used in connection with the set-up for recording the amount of water filtered by the oyster. Explanation in text. All parts, except the frame and screws, are made of plexiglass. The capacity of the vessel is 265 ml.

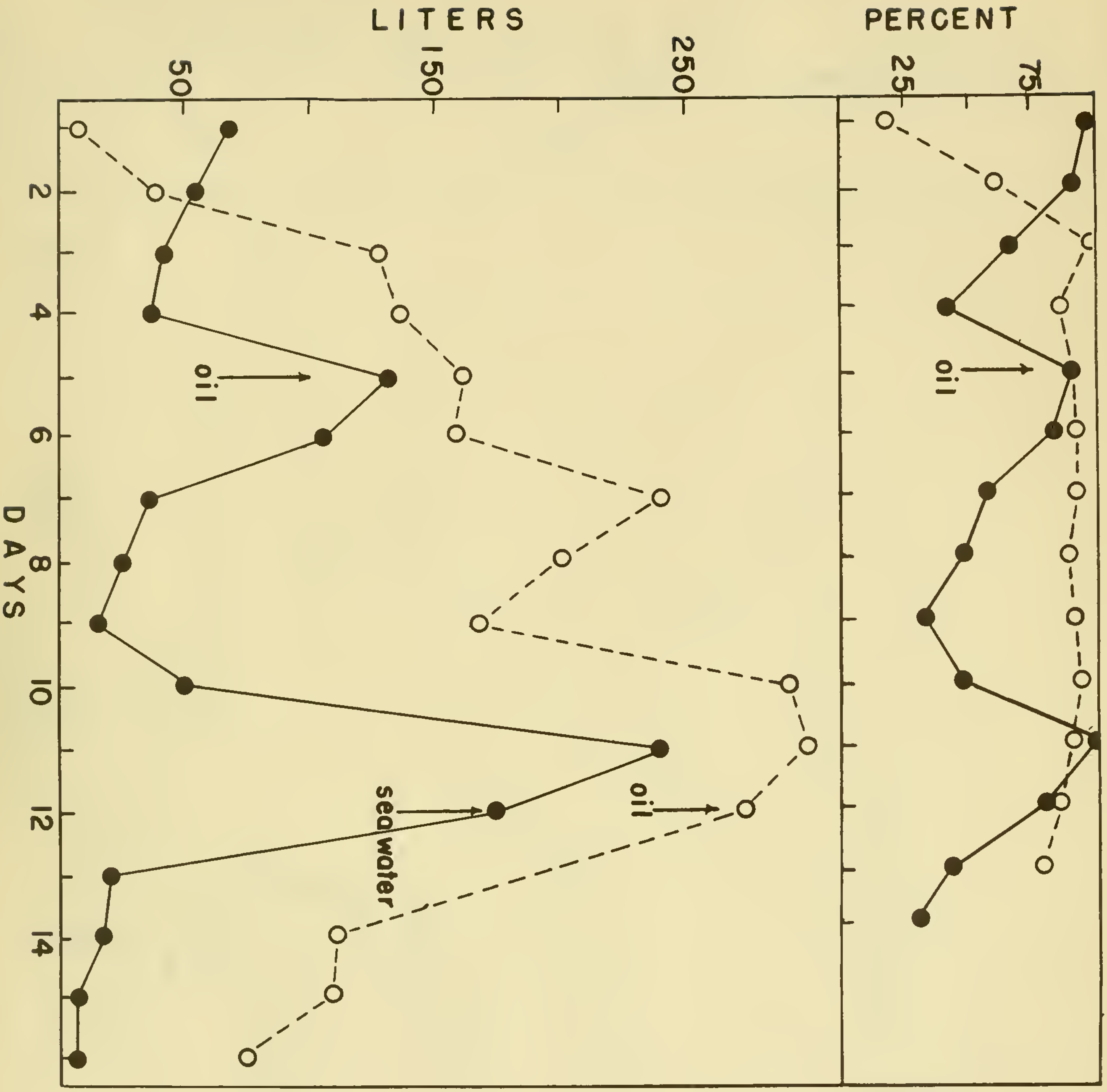

Figure 12. --- Effect of crude oil mixed with carbonized sand on the percentage of time oysters remain open and on the total volume of water pumped each day. Oyster No. 1, experimental; and oyster No. 2, control. The control is indicated by open circles. Rubber apron method. July 1 to 16, 1948.

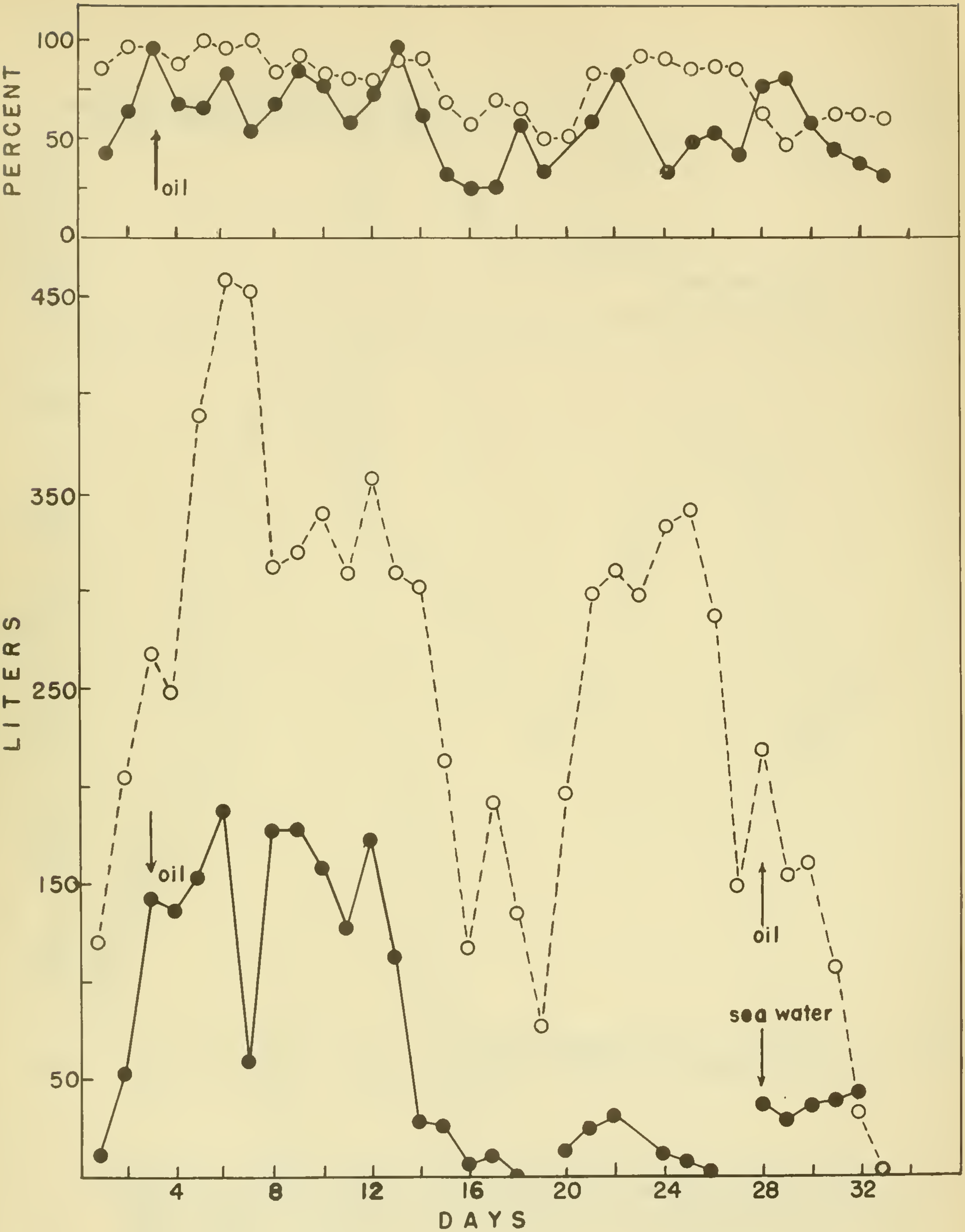

Figure 13. --- Effects of crude oil mixed with carbonized sand on the percentage of time oysters remain open and on the total volume of water pumped. Oyster No. 3, experimental; and oyster No. 4, control. The control is indicated by open circles. Rubber apron method. July 17 to August 18, 1948.

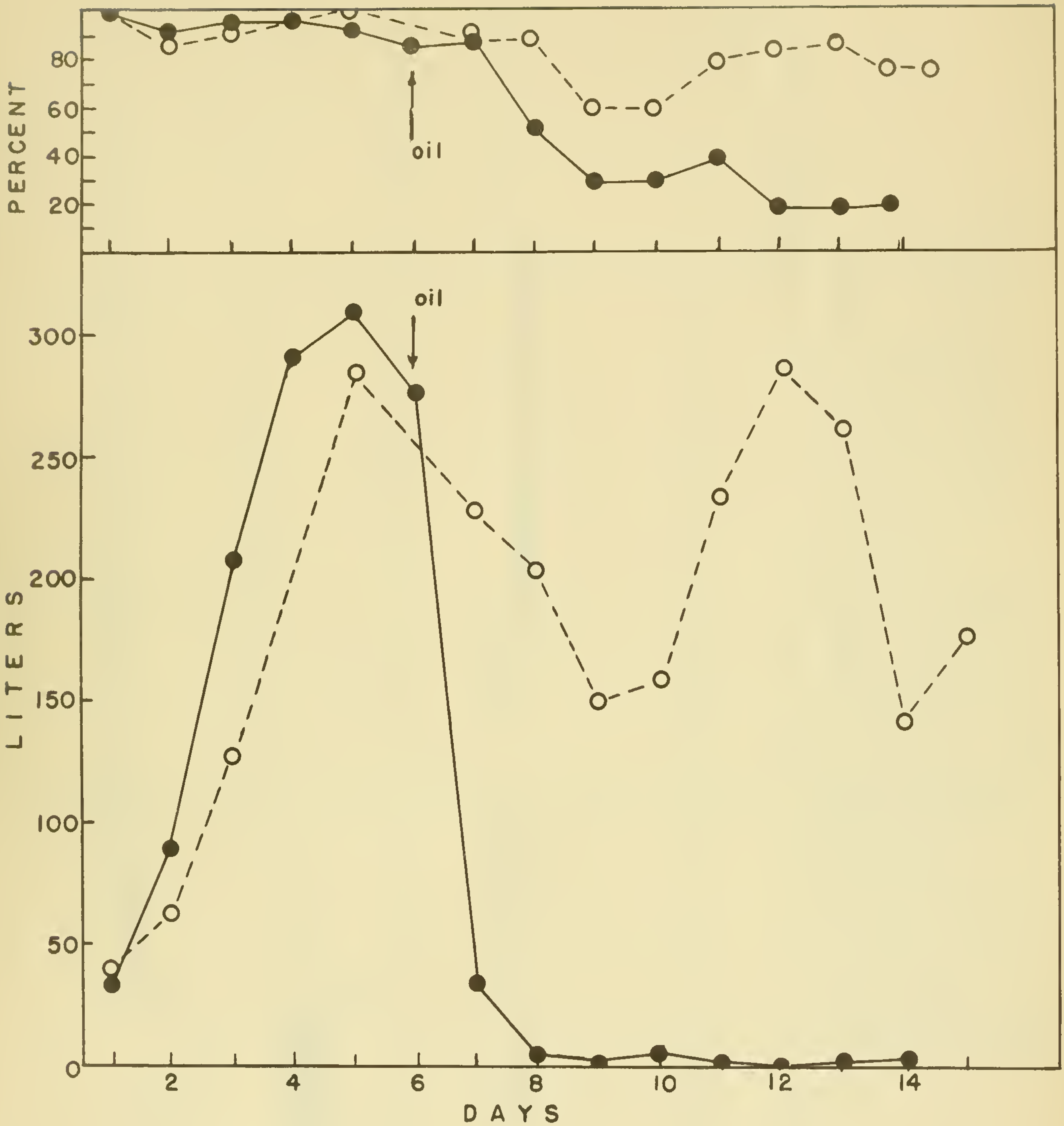

Figure 14. --- Effect of crude oil mixed with carbonized sand on the percent of time oysters remain open and on the total volume of water pumped. Oyster No. 5, experimental and oyster No. 6, control. The control is indicated by open circles. Rubber apron method. August 4 to 17, 1948.

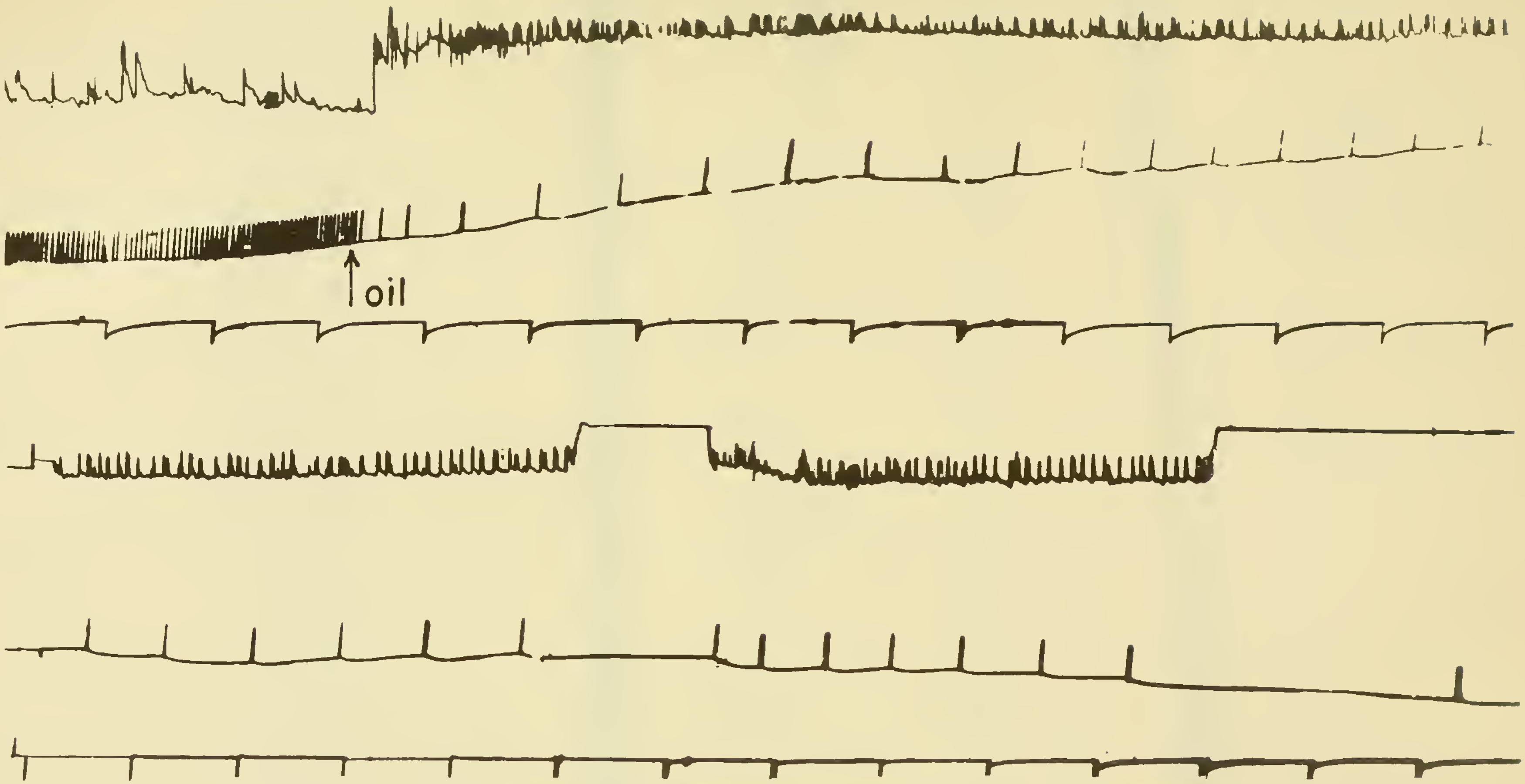

Figure 15. --- Photograph of a kymograph record of shell movements (upper line) and rate of pumping (2d line) of an oyster (No. 6) after the introduction of 50 ml. of crude oil mixed with carbonized sand into running sea water. Note the change in the tissues of the adductor and marked decrease in the rate of pumping of water. Each vertical stroke on the second line represents the discharge of 272 ml. of sea water. Time interval (3d line), 30 minutes. The lower group of lines represents the record obtained nine hours later. t^{o} - 21.2^{o} to 21.5^{o} C.

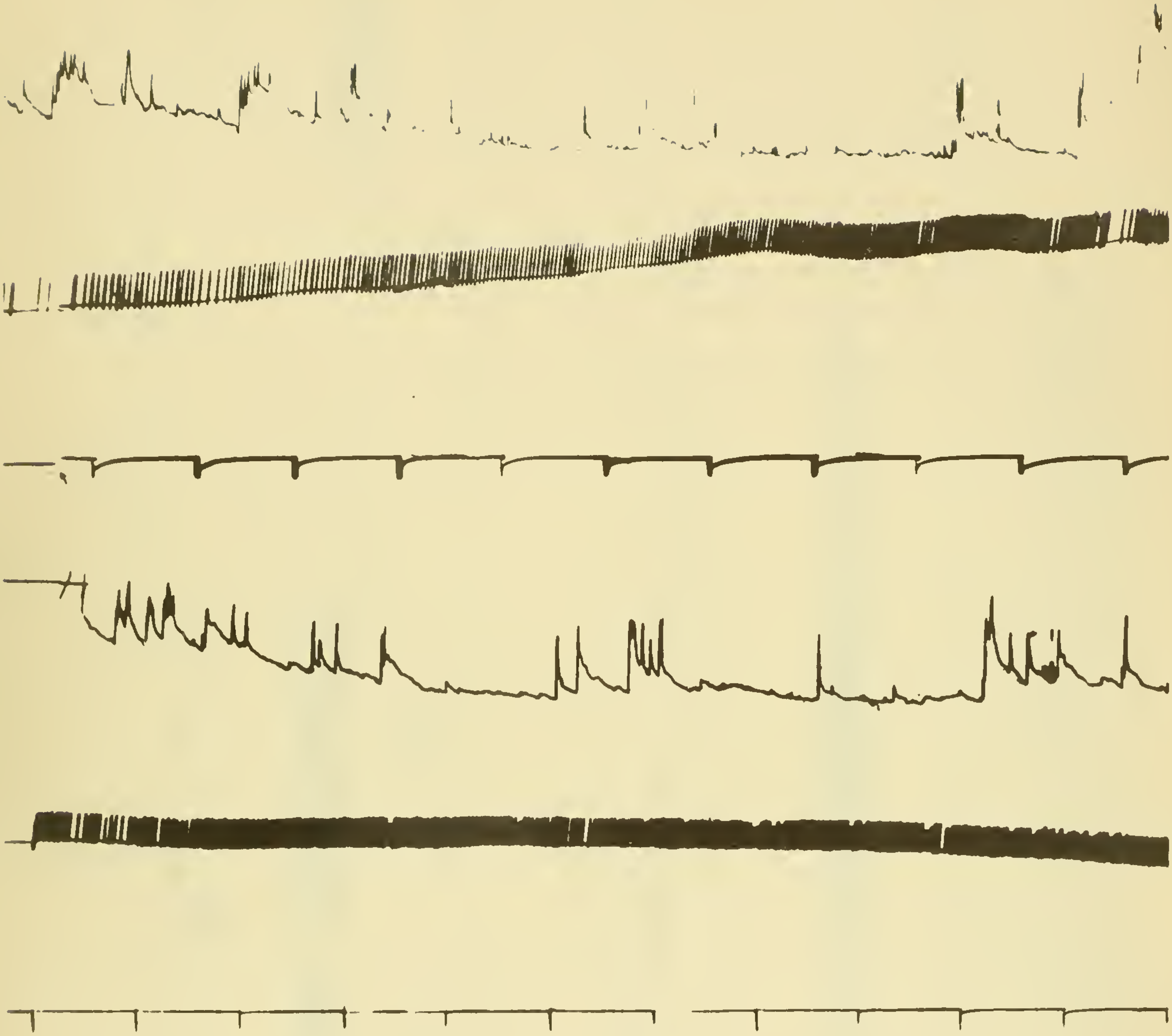

Figure 16. --- Photograph of a kymograph record of shell movement and rate of pumping of water by the control oyster (No. 5), of the experiment shown in figure 15. Each stroke of the second line of each group represents the discharge of 265 ml. of sea water. Other explanations the same as in figure 15 with the exception that no oil was introduced into the sea water.

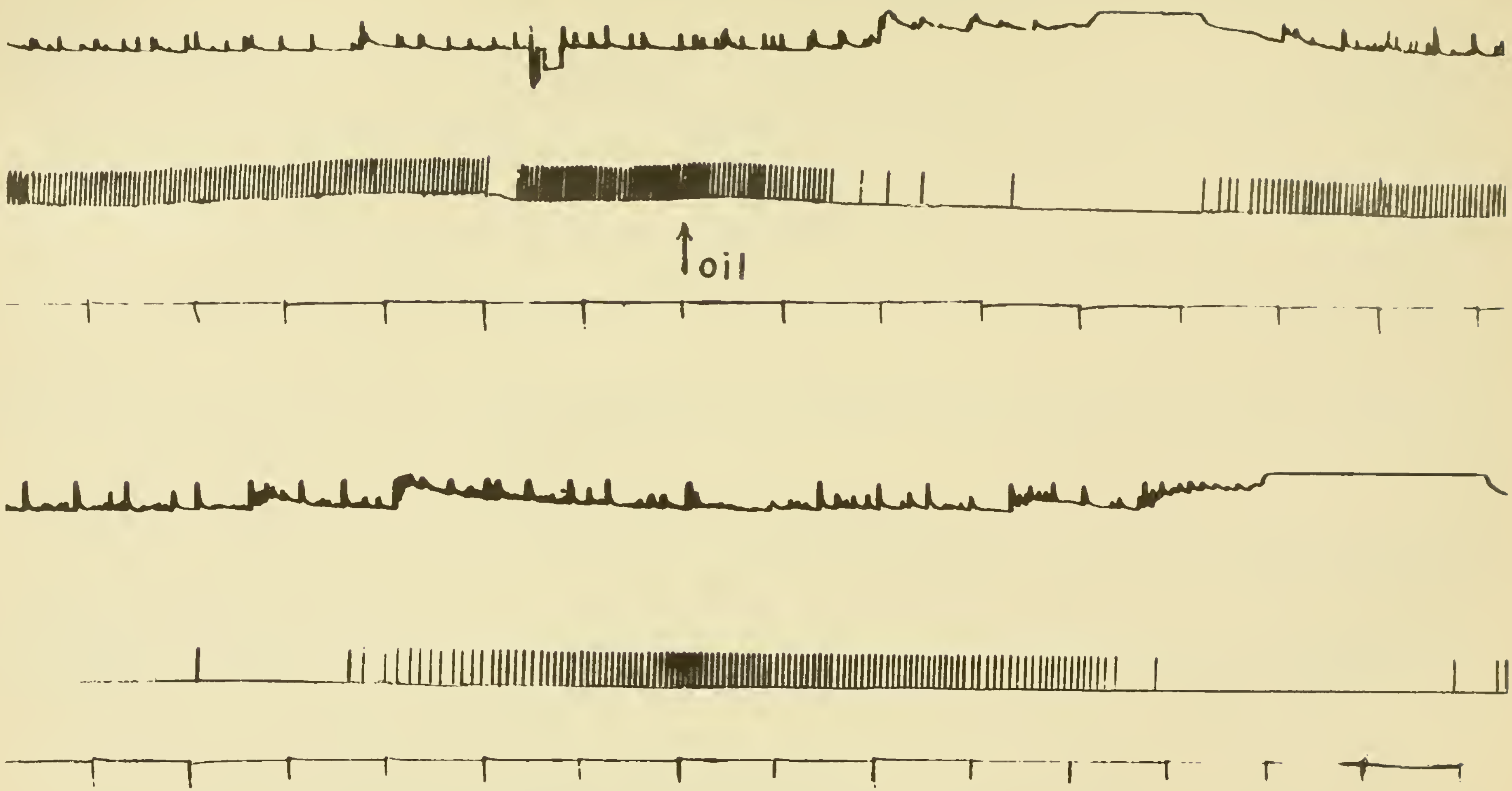

Figure 17. --- Photograph of a kymograph record of shell movements and rate of pumping of water by oyster No. 3 after the introduction of 50 ml. of oil and carbonized sand into the running sea water. Other explanation as in figure 15. Temperature 21.0° to 21.2° C. No such changes as shown here occurred in the control.

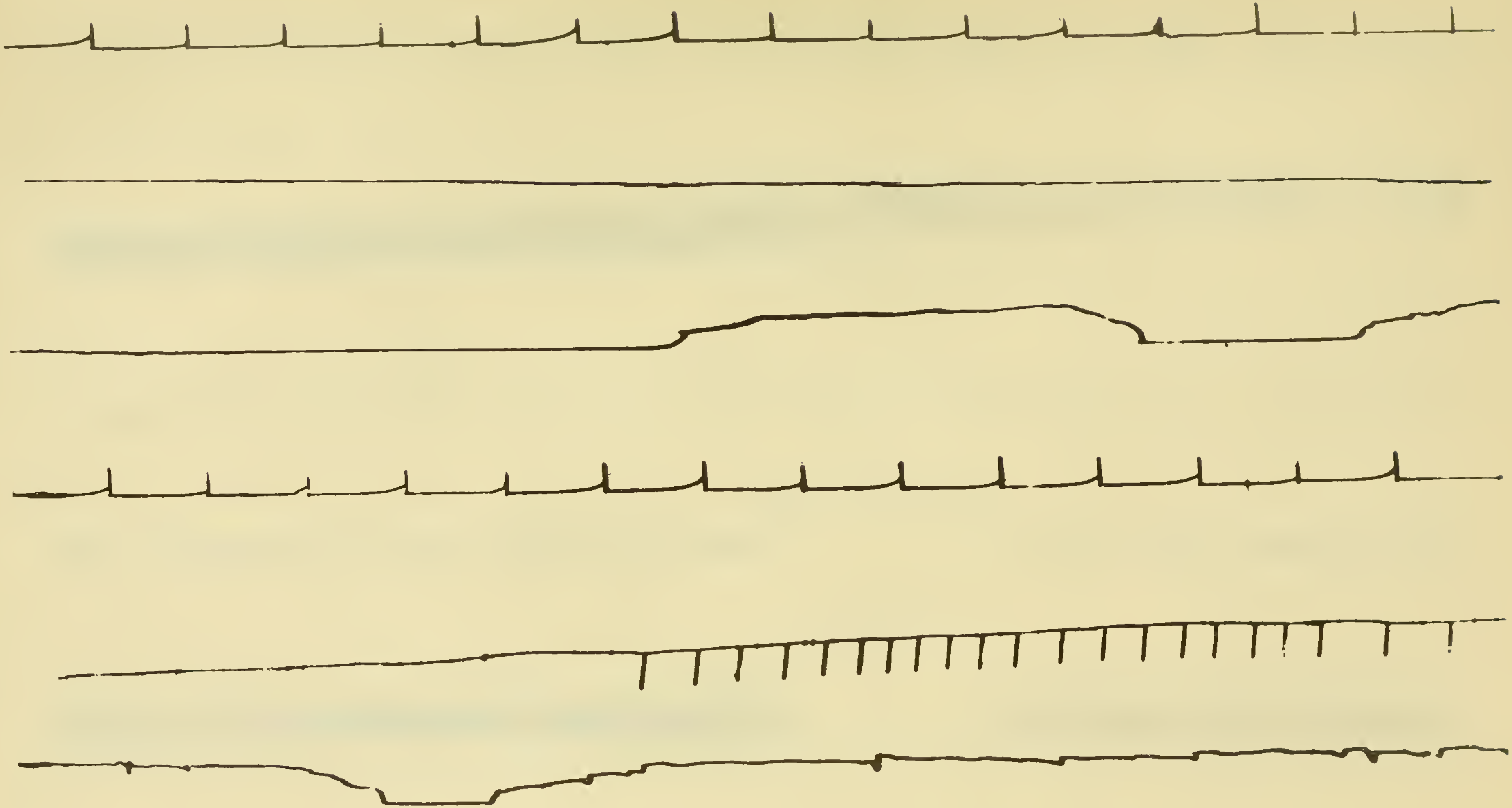

Figure 18. --- Photograph of a kymograph record of shell movements and amount of water filtered by oyster No. 3 (Experiment 2) on the 25th day of exposure to water running over crude oil held in carbonized sand. For other explanations see figure 15.

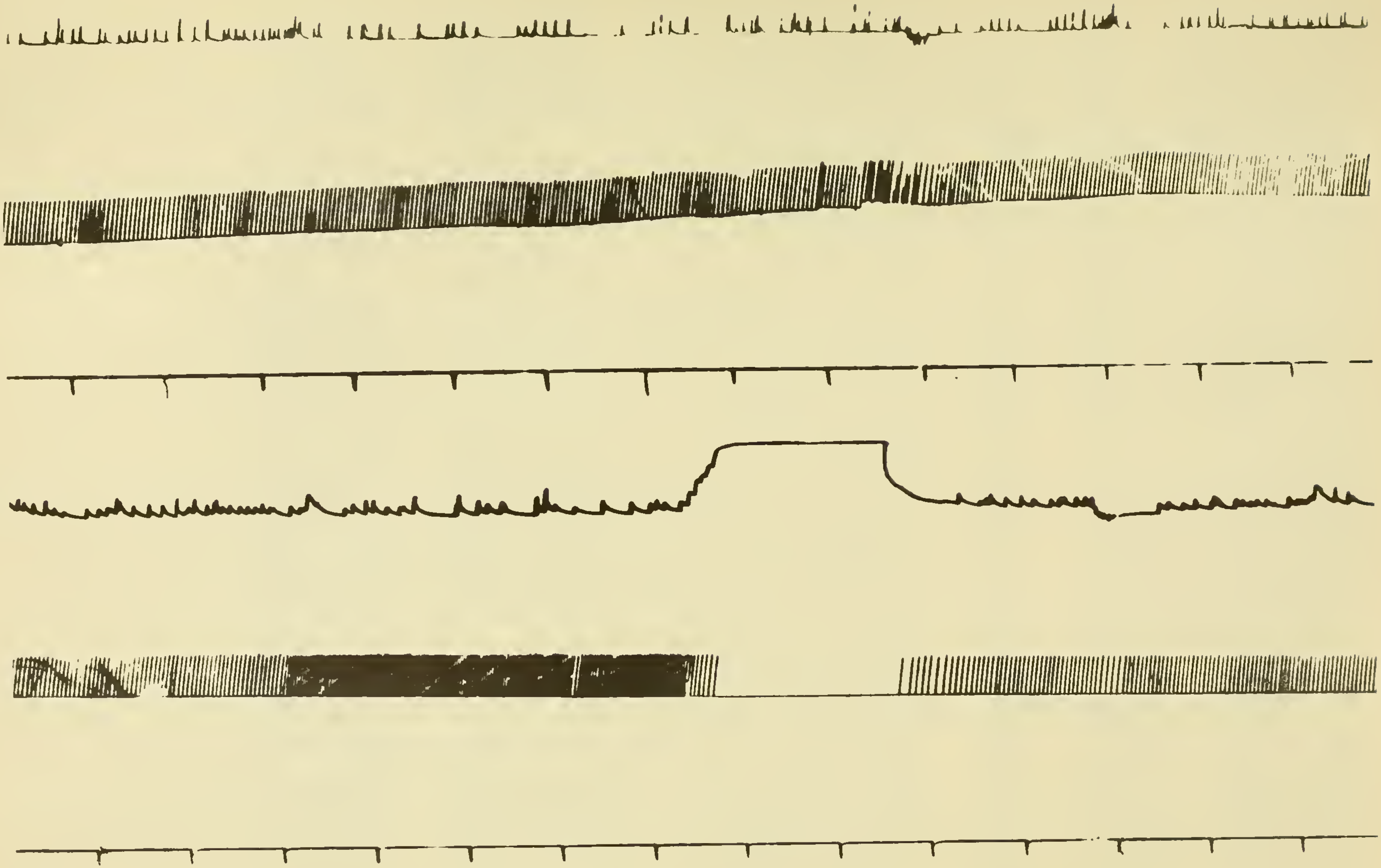

Figure 19. —- Photograph of a kymograph record of shell movements and amount of water filtered by the control oyster (No. 4, Experiment 2) on the 25th day of test. For other explanations see figure 15.

is advanced only as one of the possibilities, for other factors, such, for instance, as the changes in the concentration of microplankton or the presence of the products of plankton metabolism may either suppress or stimulate the rate of pumping of water by the oyster. The sex of oysters used in the tests is not known for, when they were examined at the completion of the experiments in September, the gonads were in an indifferent phase and the sex was unidentifiable.

In a third experiment (table 23) conducted from August 4 to 17, 1948 after the spawning of oysters had been completed, the interfering factor was absent and the depressing effect of oil was more pronounced, as can be seen by examining figure 17.

Regardless of the temporary increases, in all instances the rate of pumping of water by the oysters kept in oil-contaminated water was reduced in comparison with that of the controls.

Other significant changes affecting the physiology of the adductor muscle became apparent as the exposure in oil-contaminated water continued. The shell movements were greatly decreased, and the oysters remained open for a long time without changing the position of the valves. This abnormal behavior of the adductor is clearly seen in figure 18. In many instances no water was pumped by the gills, although the shells remained wide open. These symptons are obvious signs of the weakening of the neuro-muscular and ciliary systems of the oyster. In contrast to the experimental oysters, the controls at the same day of the experiment showed normal behavior at the very end of the experiment and the rate of pumping was sometimes even greater than at the start (figure 19).

Toward the end of the first and second experiments, the supply of water was reversed and oil-contaminated water was diverted to the controls, while the experimental oysters were given pure water. This change is indicated in the graphs (figures 12 and 13) by vertical arrows. No recovery in the rate of pumping was observed in the first experiment but there was a marked decrease in the rate of pumping in the control. In the second experiment (figure 13) there was some recovery in the oil-treated oyster and a marked decrease in the rate of pumping in the control.

These observations confirm our findings that sea water which was allowed to flow over crude oil bound by carbonized sand contained a certain toxic substance or substances which impede the ciliary activity of the gill epithelium and interferes with the normal behavior of the adductor muscle. We also infer from experiment No. 2 that after 28 days in running sea water the crude oil and sand mixture still retained its toxicity.

DISCUSSION

The purpose of the experiments described in this report was to find out whether crude oil and other oils, combined with carbonized sand and submerged to the bottom, are toxic to marine life. The tests, made with various organisms commonly found in the environment where oil pollution is apt to occur, were designed to answer this particular question. We made no attempt to study the chemical nature of the toxic substance or substances leached from oil, the rate of their leaching, and the stability of these substances in sea water. The study of these important problems is impossible without special funds, personnel, and equipment which were not available. We hope, however, that the results of our bioassays, which show the toxic effect of various types of oil on marine organisms, will stimulate such an investigation. Rapid development of the oil drilling operations in the inshore waters and the extension of explorations for new oil fields to the offshore areas of the continental shelf indicate the necessity and urgency of toxicological studies of crude oil and oil wastes. Sound national policy of conservation demands that the development of mineral resources should not spell the doom of destruction to the inshore and coastal fisheries, the products of which are needed for the welfare of millions of people. Control of pollution is, therefore, a very urgent problem which demands immediate action.

Our experiments confirm previous findings made by us and others that oil added to sea water results in a toxic environment to marine animals. The hydrozoan Tubularia crocea, one of the more sensitive organisms of shallow waters, was quickly killed if placed in water containing relatively small amounts of water extract of oil. Likewise, Tubularia perished if a mixture of oil and carbonized sand was placed near the colony. The lethal effect of such a mixture was noticeable even when sea water was steadily flowing through the container.

Different results were obtained, however, in the tests in which the oil and sand mixture was not in the immediate vicinity of Tubularia but was introduced into the containers from which the water was fed to the finger bowls with this hydroid. Under these conditions no toxic effect was observed, presumably because of greater dilution and the failure of substances leached from the oil to reach threshold concentrations.

The toxicity of oil and carbonized sand mixtures was also demonstrated in experiments with adult barnacles, Balanus balanoides; embryos of the toadfish Opsanus tau; and the eastern oyster, Ostrea virginica. No toxic effect was apparent in the experiment with the hard shell clam, Venus mercenaria. It seems probable that in the latter test the poisonous extractives of oil were in too dilute concentration to produce an effect under the conditions of the experiment.

Of the different oils investigated, fresh lubricating oil mixed with carbonized sand was not toxic to Tubularia or toadfish embryos. It seems apparent that this oil liberates little toxic material to the sea water as compared with crude oil, Diesel oil, or Navy Grade Special fuel oil. It is possible that waste crankcase oil, which may enter our waters as a pollutant, may contain added materials that may be highly toxic but this material was not tested in the present experiments.

In the experiments with toadfish embryos it was found that the toxicity of crude oil and carbonized sand mixture varies with the amount of oil used. As can be seen from figure 2, the plot of log y (concentration) against log x (time of survival) approximates a straight line, indicating that the relationship may be expressed by a simple equation of the general type $y = ax^c$.

Although weaker concentrations of oils, oil extracts, and small quantities of mixtures of carbonized sand and oil may not result in an immediate lethal effect, they exert a deleterious action which may eventually bring about death of the organisms. An altered physiology of oysters was observed when these substances were added to the water. There was a disruption in the action of the water-pumping mechanisms of the gills, which in all lamellibranch mollusks serve to bring fresh water for respiration and feeding. In addition, the presence of oils caused the oysters to remain closed within their shell for longer periods, greatly lessening the time available for feeding.

Nelson's experiments with the effect of oil on adult oysters show a damaging action, agreeing in many respects to the findings of the present experiments. He summarizes his tests as follows (Nelson, 1925, page 179): "Regarding the effects of oil upon adult oysters let me cite experiments which I performed in 1920 in preparation for testimony in the case against an oil company at Providence, R. I., in May, 1921. The oysters were kept in two tanks of bay water of known purity; one tank served as control, the other was kept covered by a film of oil. The waste in both tanks was frequently agitated to simulate wave action, and at no time did the oxygen saturation fall below 80 per cent. (It has been shown that oysters do not suffer until the saturation falls below 30 per cent.) After 15 days the oysters from the two tanks were opened and compared. Those from the oil-covered tank showed marked contraction of the mantle, the blood vessels revealed many bluish nodules characteristic of oysters living under unfavorable conditions, and the bodies of the oysters were distinctly thinner and poorer than those of the controls. Preserved samples of each lot show the difference clearly even now." The experiments indicate a disturbance in the rate of water filtration and feeding, such as was found in the tests performed in the present paper.

The toxic action of oils on molluscan larvae has been pointed out by Nelson (1921). He states (page 3) "A film of oil on the surface of the water will kill molluscan larvae within a few hours, owing largely to their habit of swimming close to the surface." This statement was based on laboratory experiments, which are discussed by him in a later paper (Nelson, 1925, page 178). He continues with "Such larvae of shellfish as escape the oil at the surface will, when the time comes to attach to the bottom, find this unsuitable on account of the oil which is accumulated there and will therefore perish." It seems apparent that harmful effects on the larvae and young of oysters could be expected. Tests were not included in the present report on any but adult animals.

Our observations of the toxic effects of oil are in agreement with the data reported by earlier investigations. European students of this problem attributed the toxic effect of the Baku petroleum to the presence of naphthenic acids, which they found to be fatal to dace and ruff (small European perch) in concentrations varying from 4 to 16 parts per million (quoted from Seydel, 1913). Crayfish weighing 35 grams was killed in 18 to 60 hours in the solutions of 5 to 60 mg. per liter. Among various materials of refinery wastes listed by the U. S. Public Health Service (1939) as destructive to aquatic life, mention is made of phenol, injurious to trout and goldfish, and naphthenic acids, which killed minnows in 72 hours in a concentration of five parts per million. Naphthenic acids may be extracted from crude oil or distillates by aqueous NaOH solution in which the acids dissolve, yielding sodium naphthenates (Ellis, 1934). Mention should be made here also of the results of many experiments conducted in England on the damages to fisheries by various tars used for road surfacing (Ministry of Transport and Ministry of Agriculture and Fisheries, 1930). Laboratory tests conducted jointly by these agencies demonstrated the toxicities to fish of the extracts of tar and various heavy oils (l. c., p. 131). In a recent paper Veselov (1948) has described experiments showing an extremely marked toxicity of Ischimbaev crude oil in concentrations as weak as 0.4 ml. of oil per liter of water to small fresh-water fish (Carassius carassius L). Water soluble fractions, obtained by shaking 15 ml. of crude oil in a liter of water for 15 minutes, were found to be highly toxic also.

Many of the oils when shaken with water form emulsions, both water and oil, and oil in water. In personal communication to P. S. Galtsoff, Dr. C. E. Coates (1936) states that he had an emulsion of oil and water which stood for ten to fifteen years without separating completely. He points out that in passing through the gills of the oyster these emulsoids, caught by the cilia, may produce anything from sickness to death. This point is of great interest and requires further study. From the point of view of conservation, the question of the physical status of the oil mixed with water is, however, of little importance. The fishery biologist is primarily concerned with the toxicity of oil, and the question whether the poisonous effect is caused by water soluble fractions of oil or by emulsion is of secondary importance in solving the conservation problem. Some of the samples of oil extracts in water used in this work were examined under a microscope at the magnification x625. The samples of extract were not opalescent and contained no oil globules. After centrifuging a small amount of sediment was collected. It consisted of irregularly shaped, angular black particles apparently derived from crude oil. Furthermore, in the tests using oil held by carbonized sand, shaking and agitation was completely eliminated, yet the water slowly running over the layer of the mixture had a definite toxic effect on marine animals.

Oil placed on clean water will spread to a thickness of one molecule. This ultimate result may not be reached if the surface tension of the water is lowered by the presence of other contaminating substances. There is a definite relation between the thickness of the oil films, their appearance, and the quantity of oil required to produce them. It is known that a trace of irridescent color may be observed in an oil film of an approximate thickness of 0.000006 inch. A film of that type is produced by 100 gallons of oil spread over one square mile of sea surface. Smaller quantities of oil produce barely visible films, the thinnest one, visible only under most favorable light conditions, corresponds to 25 gallons of oil per one square mile of area. Films of dark dull color are about 0.00008 inches thick. They require about 1,332 gallons or more per square mile. The American Petroleum Institute (1933) puts much emphasis on the fact that thin oil films are very unstable and even those of the average thickness, of the order of 0.00004 inches, disappear from the surface of the sea in less than 24 hours. On the basis of their data, the American Petroleum Institute infers (l. c., p. 9) "that oil discharged into surface waters at the rate of 666 gallons per day, or 28 gallons per hour, per square mile, may not be cumulative under average conditions. This rate would probably result in a continuous irridescent film." This conclusion is based primarily on observations of the disappearance of oil slick from the surface of the water but does not take into consideration the fact that, in silt-laden waters, oil is rapidly combined with the suspended material and is loosely deposited on the bottom. Thus, from a biological point of view, the absence of oil from the surface of the water does not indicate that the pollution has ceased.

Oil discharged on the surface of coastal waters, with their complex system of currents, may be carried some distance from the source of original pollution. In loose combination with the material suspended in water, it may settle on the bottom, but strong currents, wave action, and storms may stir it up and oil slicks may reappear on the surface and be carried farther with the currents.

The harmful effect of a submerged oil slick on the bottom-dwelling forms may continue because of the leaching of toxic substances. It is true that under usual conditions in the open bays and estuaries the dilution of the materials leached by sea water would be great, and for this reason the injuriousness of these substances to marine life may not always be observed. A different situation exists, however, in the bays and harbors with slow currents and limited circulation. In such situations, the cumulative toxic effect of submerged oil may become very pronounced.

An oil slick treated with carbonized sand is much more firmly bound and is permanently anchored at the place of deposition. It has no tendency to shift with the currents. In this way, the pollution is localized and its spread is prevented.

In sinking oil slicks by carbonized sand, the toxic nature of the oil is not appreciably lessened. As a matter of fact, it is brought in close proximity to bottom-dwelling forms where the concentration of leached poisons will be made greater than in the case where the slick is allowed to float freely on the surface. In our harbors and areas close to docks, shipyards, piers, etc., most marine life that is at all sensitive has been either killed or has moved because of pollution by a variety of industrial and domestic wastes. In reality these waters are almost biological deserts. If additional toxic environment is created in such locations by the deposition of oil, very little, if any, increase in damage can be expected over what has already taken place. Indeed, the anchoring of the oil and limiting it to such areas has the distinct advantage of keeping the pollution away from adjacent places that may be productive of economically important estuarine forms, such as oysters, clams, shrimps, etc.

It seems logical to assume that carbonized sand will not be used over valuable shellfish-producing beds except in emergencies. In the latter case, some damage may be expected, but if the quantities of discharged oils are small, the effect may be insignificant and of temporary nature.

From our studies we conclude that dusting with carbonized sand is a highly efficient method of removal of oil from the surface of the water. It is useful around docks and piers to combat fire hazards and has also distinct advantage in preventing the movement of oil slicks to productive areas where great injury to sea food resources may occur. We hope that the method will be adopted by the oil industry, especially in active oil fields, and that eventually it will be generally used in combatting oil pollution in coastal waters.

SUMMARY

The results of the tests performed with a selected group of marine animals may be summarized as follows:

1. Crude oil, Diesel oil, and Navy Grade Special fuel oil added to sea water are toxic to the hydrozoan Tubularia crocea; barnacles, Balanus balanoides; embryos of the toadfish, Opsanus tau; and the eastern oyster, Ostrea virginica. The one experiment

performed with the hard shell clam, Venus mercenaria, failed to show toxicity, probably from insufficient concentration of oil used.

2. The toxicity of these oils is apparent whether they are present as oil slicks on the surface of the water or are held on the bottom bound to carbonized sand.

3. Strongly toxic fractions obtained by shaking crude oil and Diesel oil with sea water and separating the two phases show that the toxic effect is due to a substance or substances leached by water.

4. The toxicity of crude oil to toadfish embryos was found to increase with concentration; the relationship may be approximately expressed by an equation $y = ax^c$, where y is survival time, x is concentration, and a and c are constants.

5. The presence in water of crude oil and Diesel oil mixed with carbonized sand and of extracts of these oils interferes with the pumping mechanisms of the gills of oysters and reduces the rate of filtration of water. Continued exposure of oysters to sea water flowing at a slow rate over a mixture of crude oil and carbonized sand results in a marked reduction in the amount of water pumped by oysters and in a decrease in the number of hours that the oysters remain open.

6. There is definite advantage in the use of carbonized sand in treating oil slicks for it localizes the oil pollution, prevents the spread of oil over the surface of water, and submerges and permanently anchors the oil near the source of pollution. In view of the fact that bottoms of harbors and bays near industrial ports are grossly polluted and nonproductive, the sinking of oil in these localities will not increase the damages to the fisheries. It is considered that the presence of oil, bound to carbonized sand, in such localities will not affect productive shellfish grounds located in the nearby unpolluted areas because the dilution of toxic substances would be too great and would not reach a threshold concentration for harmful effects.

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APPENDIX 1. --- Set-up for simultaneously recording shell movements and rate of filtration of water by the oyster (from Galtsoff, 1946 "Reaction of Oysters to Chlorination," Research Report 11, U. S. Fish and Wildlife Service). Constant-level tank (C) with oyster (M); constant-level tank (L) regulating the flow of sea water; float (F); mixing chamber (B); kymograph (K); floating vessel (T) from which the solution is delivered to the mixing chamber; delivery tube (G) leading from vessel (T) to the mixing chamber; tube (H) for the delivery of sea water; overflow (O). The lower part of the diagram shows dumping vessel (D) (side view at the left and front view at right). The float (F) pushes the lever and releases the catch (A); (detail of the construction of catch is shown at the right lower corner of the diagram). Platform (P) with hook for a thread leading to the writing lever. All parts are made of plastocoele.

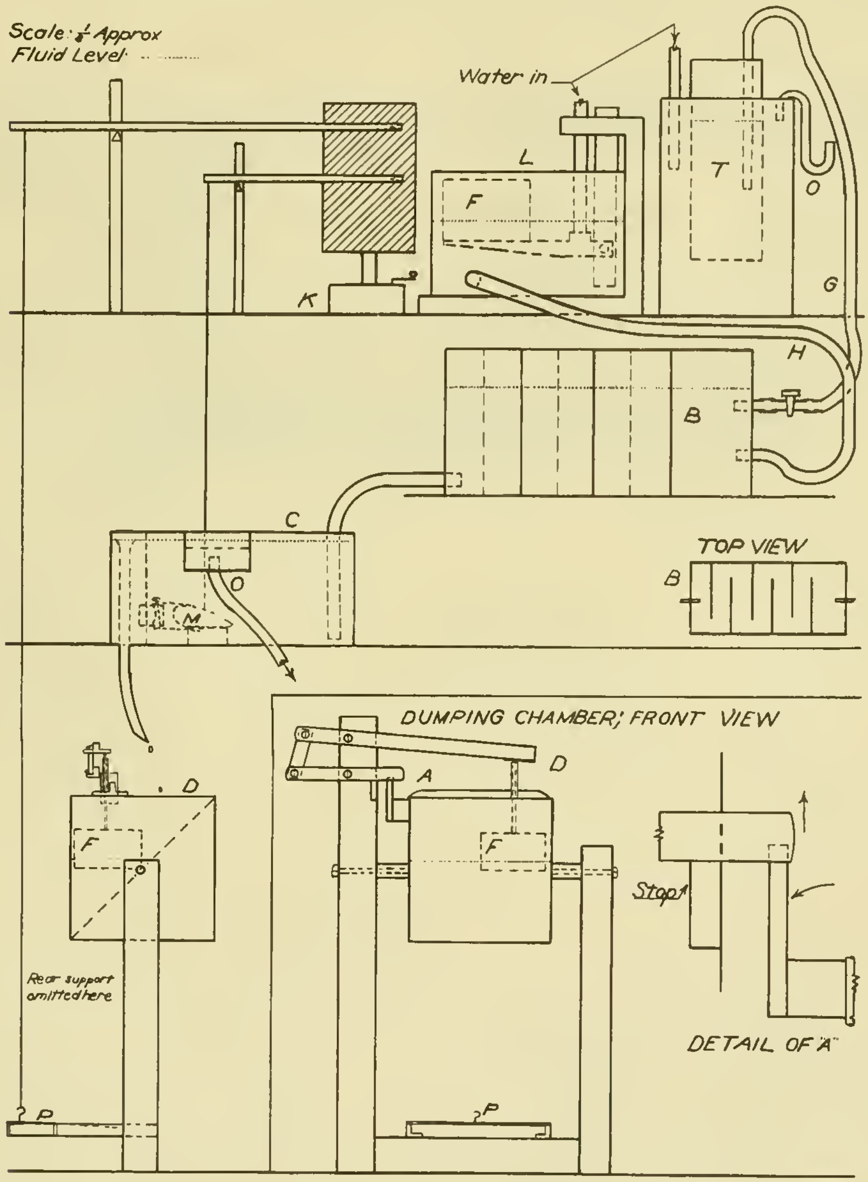

Figure 20. - - - Three stages of the removal of oil slick from the surface of the water.

1. Crude oil floats and spreads over the surface of sea water.

2. Oil slick is treated with carbonized sand.

3. After agitating the surface, large lumps of carbonized sand with absorbed crude oil sink to the bottom. The surface of the water is clear.

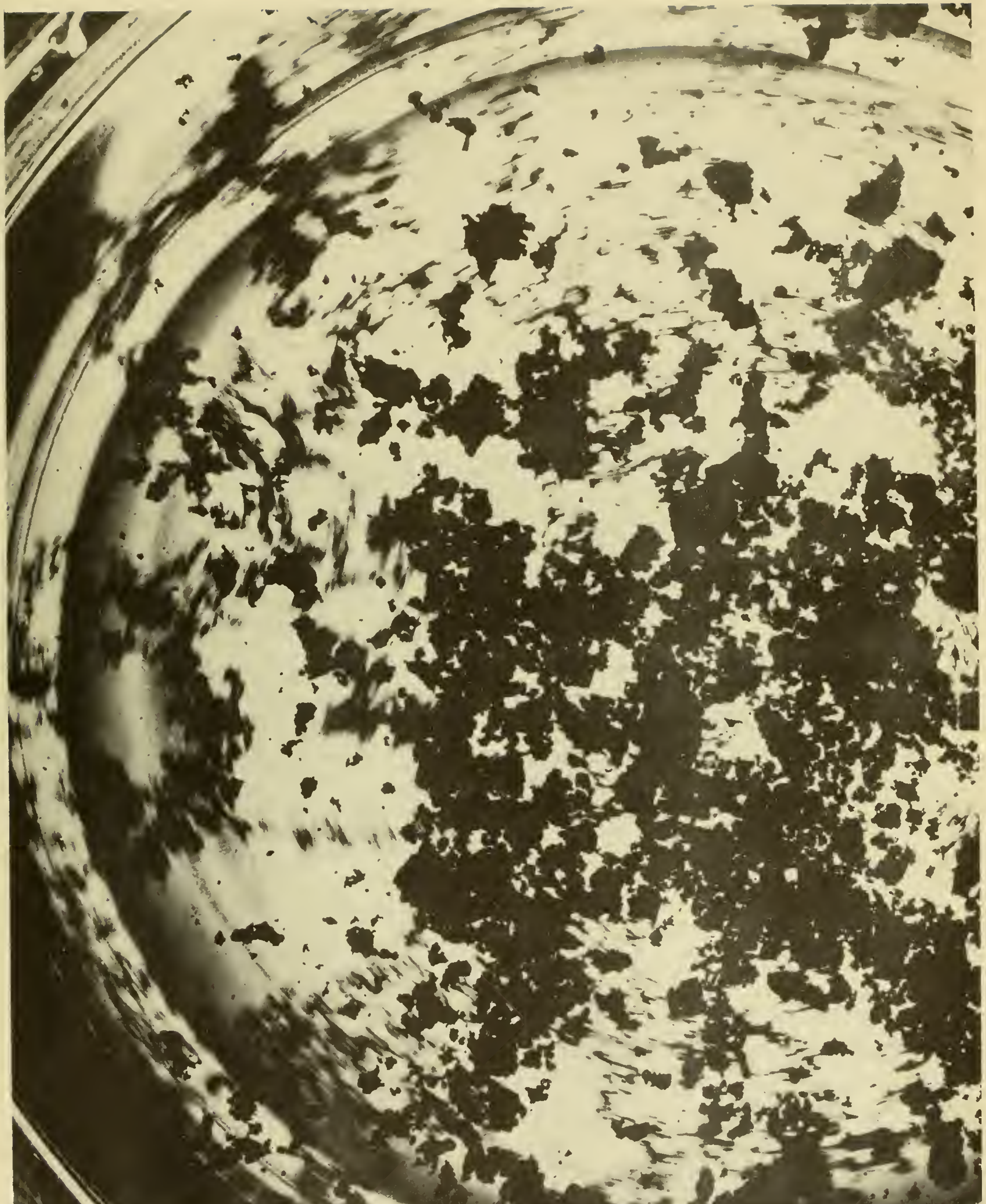

Figure 21. --- <u>Apparatus used in testing the effect of carbonized sand and oil mixture on oysters.</u>

Upper level: On the left, timing device consisting of telecrome motor and disc making an electric contact every 30 minutes. On the right, constant level tanks (2) from which sea water is supplied to the mixing chambers.

Center: Two kymographs which record on smoked drum the muscular activity (upper line); rate of pumping (second line); and time interval (third line).

Lower level: Two dumping vessels (standing in large white tank) for recording the amount of water pumped by the oyster. Mixing chamber (6) is seen on the extreme right. It supplies water to the constant level tank (1) in which the oyster is kept.

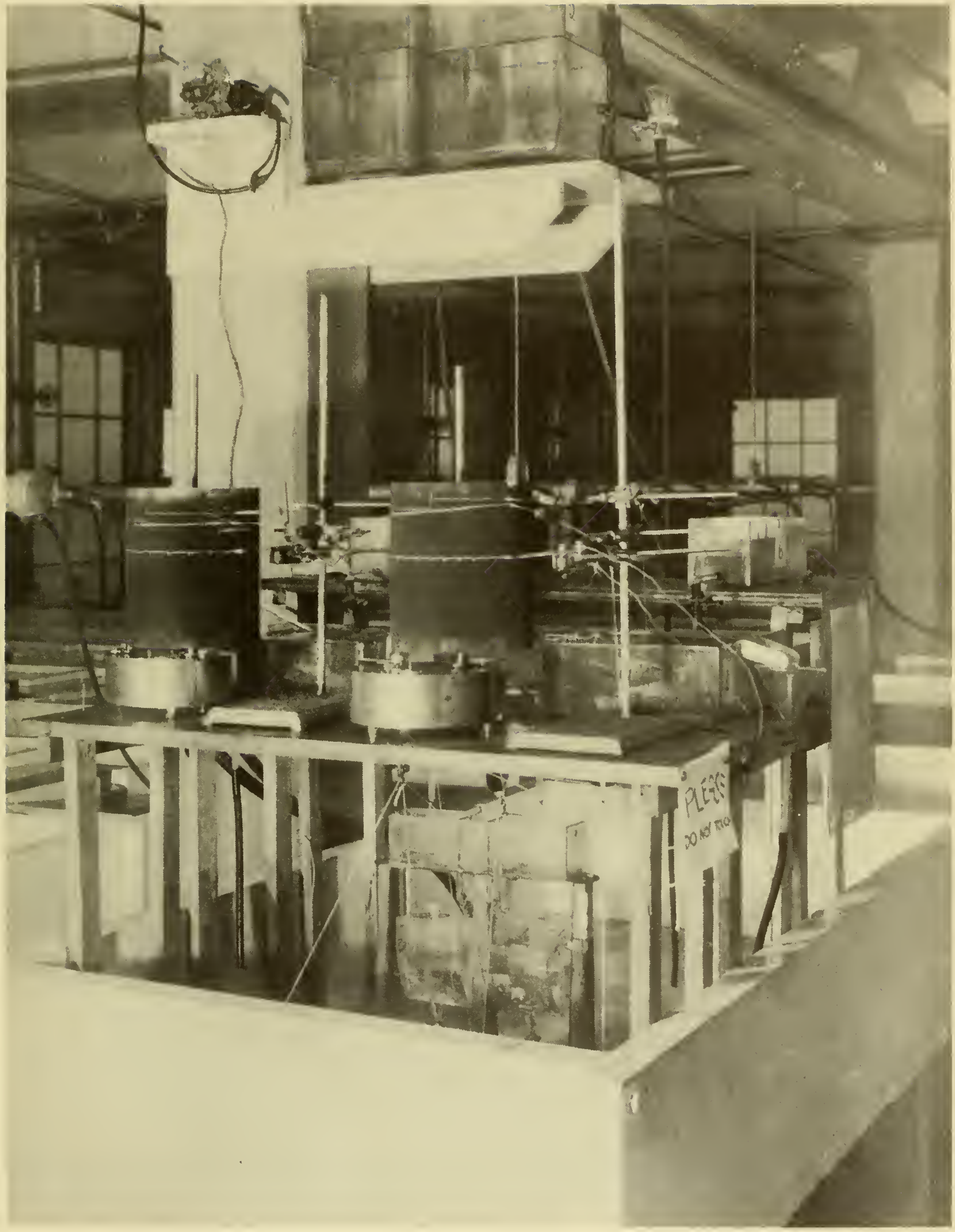

Figure 22. --- Tubularia crocea. Control experiment in standing sea water, 24 hours after the beginning of the experiment. All hydranths are alive and attached to stems.

Figure 23. --- Tubularia crocea in standing sea water with 2 ml. of crude oil mixed with 5 grams of carbonized sand, 24 hours after the beginning of the experiment. Notice that all the hydranths have separated from stems and are on the bottom of the dish, partially decomposed.

Figure 24. --- Tubularia crocea attached to mussel shell. In running sea water with 12.5 grams of carbonized sand on the bottom; 50 hours after the beginning of the experiment. Notice that the majority of hydranths are alive and attached to the stems.

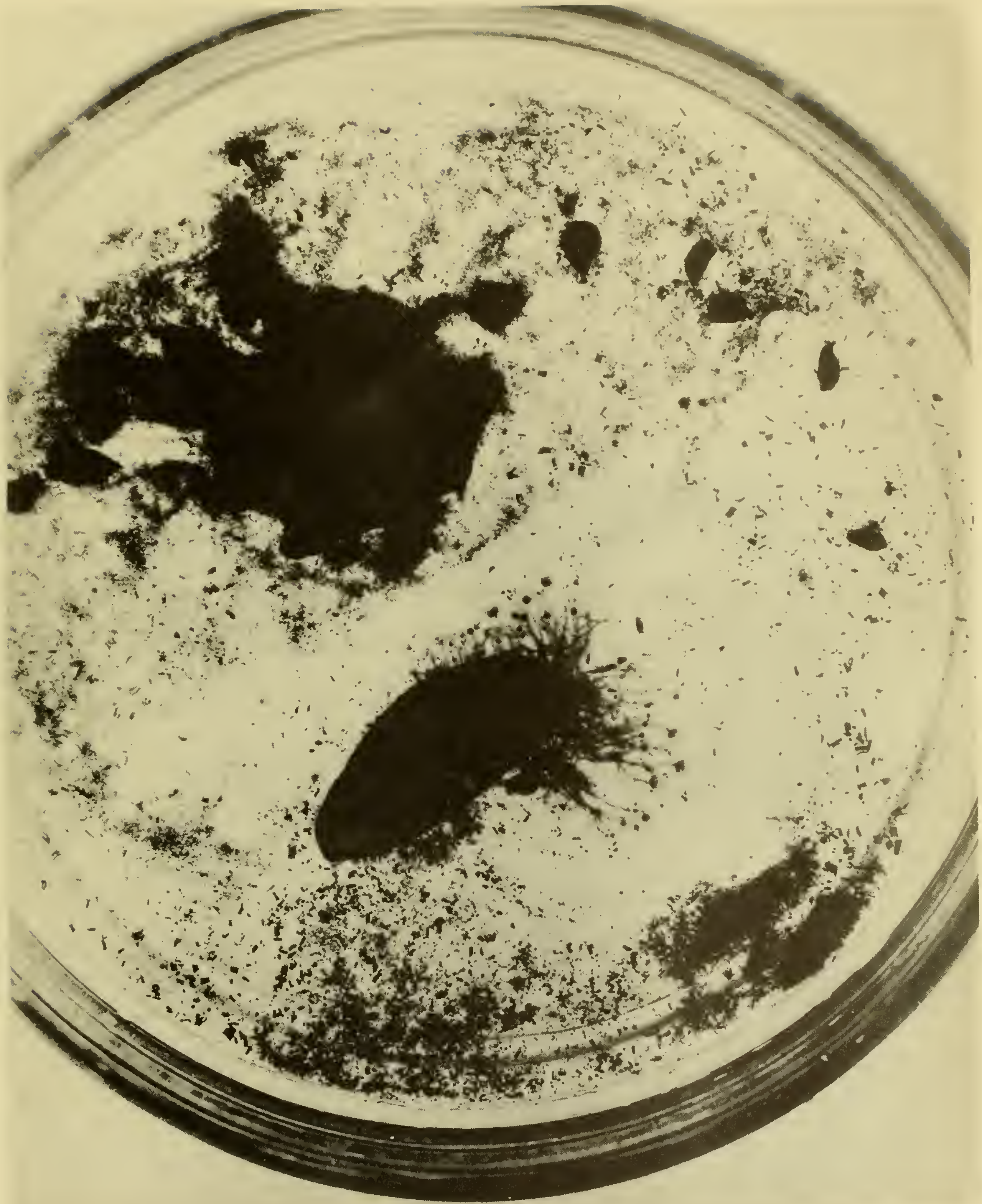

Figure 25. --- Tubularia crocea, attached to mussel shell, in running sea water with a mixture of carbonized sand and oil (5 ml. oil, plus 12.5 grams of sand); 50 hours after the beginning of the experiment. All the hydranths are separated from the stems and undergoing decomposition.