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By Paul S. Galtsoff, Herbert F. Prytherch
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EFFECTS OF CRUDE OIL POLLUTION ON OYSTERS IN LOUISIANA WATERS[1]

By PAUL S. GALTSOFF, Ph. D., HERBERT F. PRYTHERCH, Ph. D., ROBERT O. SMITH, and VERA KOEHRING, Ph. D., *United States Bureau of Fisheries*

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FOREWORD

The mortality of oysters in Louisiana waters in 1932–33, coincident with the development of oil wells in the coastal areas of the State, brought up again a question as to the possible effect of oil on marine life. In the spring of 1933, at the request of the Louisiana Conservation Department, Dr. H. F. Prytherch was detailed by the United States Bureau of Fisheries to make an investigation in the Terrebonne Parish and adjacent territory with the view of determining the probable cause or causes of the mortality. In 1934, in an attempt to carry out a more comprehensive study, the Bureau obtained from the Civil Works Administration approval of a project to investigate the oil-pollution problem in Louisiana and to carry out both laboratory and field experiments in order to determine the effect of oil on oysters. Unfortunately, out of the $42,000 allotted for this project only an amount of $3,000 was made available, and after the completion of a preliminary hydrographic survey of Timbalier and Terrebonne Bays and adjacent bodies of water the field work was discontinued. Laboratory experiments on the effect of oil on oysters and oyster food were carried out, however, at Beaufort, N. C., Woods Hole, Mass., and Washington, D. C.

Although the exact cause of the mortality of oysters has not been determined, the reports of the field and laboratory investigations throw considerable light on the conditions of oyster beds in Louisiana waters and on the possible effect of oil pollution on oysters.

INTRODUCTION

By P. S. Galtsoff and H. F. Prytherch

The fact that the discharge of oil into natural waters may be detrimental to aquatic life has been recognized for a long time and was a subject of lengthy discussion before the numerous governmental committees (Oil Pollution in Navigable Waters, 1926; Pollution of Navigable Waters, 1930) attempting to remedy the situation by proper legislative action. In view of the widespread oil pollution of coastal waters, especially in the vicinity of large cities and industrial centers, and the interest in this problem shown by many Federal and State agencies, it is surprising to learn that there has been very little direct experimentation on the effect of crude oil or its derivatives on fresh-water or marine organisms and that most of the statements appearing in the minutes of official hearings are based primarily on field observations and frequently represent opinions and assumptions not corroborated by direct evidence.

It is true that in the case of gross pollution conditions in the affected body of water may be such as to make any deeper investigation superfluous. However, when pollution is light or only temporary, the lack of knowledge of the toxic properties of oil and of the manner in which it may affect various organisms, constitutes a serious handicap in developing efficient methods of protection. In the case of the location of oil fields in the coastal area, the question arises as to whether or not the coastal fishery and the oil industry can coexist. The problem is of particular importance in south Louisiana waters where the production of oil has increased rapidly from a total output of 5,032,400 barrels in 1927 to 15,540,341 barrels in 1933, according to Dabney (1934). Drilling operations have been extended to within a few miles of the Gulf of Mexico, particularly in Terrebonne Parish, where, according to the statistics of the Louisiana Department of Conservation, there were 17 wells in opera-

tion in 1933, having a total average daily output of 6,961 barrels. In this parish most of the wells are not located on land, but are situated in the open waters of Lake Barre, Lake Pelto, and vicinity, which constitute one of the most important oyster-producing regions of the State. Pollution of the water has occurred as the result of oil-well operations, and coincident with this condition there has been a high mortality of oysters particularly during the winter of 1932–33 and to a lesser degree during the previous winter. The aggregate losses of the oyster planters, alleged to have been caused by oil-well operations, have been estimated at several hundred thousand dollars.

In January 1933 the Louisiana Department of Conservation received reports of an extensive and serious mortality of oysters in the Lake Barre and Lake Pelto region, which were corroborated by immediate field surveys of its Bureau of Research and the State Department of Health. The chemical studies of pollution and subsequent field and laboratory experiments conducted by these departments are reported briefly by Gowanloch (1934). Similar chemical and biological field investigations were also made by chemists of the Texas Co. and by Dr. C. E. Coates, Dr. A. R. Choppin, and Dr. W. H. Gates of the chemistry and zoology departments of Louisiana State University. In May 1933 the cooperation of the U. S. Bureau of Fisheries was requested by the Louisiana Department of Conservation, following which, field studies were made by Dr. H. F. Prytherch in May, June, and September of the oyster mortality in Terrebonne Parish in relation to oil-well operations in this region. At the Bureau's laboratories in Washington, D. C., and Beaufort, N. C., a series of experiments have been conducted by the authors to determine the effect of different grades of crude petroleum and accompanying brine waters on the survival, feeding, and food of the oyster.

There is no doubt that unavoidable pollution of water by oil and bleed water incidental to drilling operations constitutes a serious danger to local oyster, shrimp, and fishing industries from the point of view of a fisherman, it being immaterial whether pollution has actually destroyed the stock of fish and shellfish or rendered them unmarketable on account of oily taste and emaciated condition of the flesh. In both cases the industry sustains economic losses. Of course, in case of oyster or other mollusks there is a possibility of transplanting the stock to other areas unaffected by pollution. So far as the Louisiana coastal section is concerned, this appears to be only a palliative, for it is but a question of time until the development of oil fields will spread all along the coast and most of the oystermen in the State sooner or later will face the problem which at present confronts the industry in Timbalier and Terrebonne Bays and Lake Pelto, the sites of the present extensive drilling operations.

The questions to which the oyster industry desires to receive a competent answer can be formulated as follows: Whether the unusual mortality which occurred in Louisiana in 1932–33 is attributable to the discharge of oil and bleed water, and how further development of the oil resources of the coastal section may affect the cultivation of oysters. The marine biologist called to provide an answer finds himself in a difficult situation. As often happens in the case of an unusual mortality among fish or oysters, he is requested to investigate the cause or causes of it several weeks or months after death has occurred, and when the conditions responsible for the mortality have already changed or disappeared. There is a general and well understandable tendency on the part of a layman to attribute his troubles to unusual activities or events that occurred in the affected area. The concurrence of the two phenomena, however, does not constitute in itself a proof that one is the cause of the other.

There are two aspects in the study of mortality problems in relation to pollution of water. One is to determine the cause of the mortality, that occurred some time ago, the second is to find out how the pollutant agent may affect marine life and what are the expectations of the fishing industry if the pollution is permitted to continue. From the point of view of conservation, the second problem is of far greater importance, while the parties involved in the controversy regarding the causes of mortality are primarily interested in the first one. Although the present report fails to give a definite answer to the first question, it supplies sufficient data regarding the possible dangers of oil pollution to oysters.

One must bear in mind that actual conditions in the sea may be much more complex than they appear to a casual observer. There is a possibility, for instance, that because of unfavorable meteorological conditions, attacks of parasites, or other unknown factors, oysters already have become weakened or diseased. In that case an additional adverse factor, as for instance a small concentration of a toxic substance in the water, may have been responsible for the mortality, although under more favorable circumstances the oysters might have been strong enough to withstand it. Being ignorant of the past history of the case and not being able to duplicate conditions that existed at the time of greatest mortality, the biologist is unable to determine with certainty the cause of it. He can provide, however, sufficient evidence regarding the toxicity of the suspected pollutant, the manner in which it affects the organism, its fate in the ocean, and from all this information provide a substantial basis for outlining the methods and policies of future conservation.

These considerations and the lack of funds to carry out field observations on the large scale contemplated in the original project, made it necessary for the authors to concentrate their attention on the experimental studies of the effect of oil on the behavior of the oyster and on the growth of diatoms, which constitute the principal part of the oyster diet.

It was thought desirable, however, to present first the preliminary field observations made by H. F. Prytherch in 1933 and the result of the survey made by R. O. Smith in March and April 1934.

PRELIMINARY FIELD INVESTIGATIONS, 1933

By H. F. Prytherch

Terrebonne Parish includes practically the whole oyster-producing region between Barataria Bay and the Atchafalaya River and is the westernmost section in which good oysters are obtained in considerable quantities. According to the Bureau's fisheries statistics for 1930 (Fiedler, 1932), this parish exceeded all others in oyster production and furnished over 360 thousand bushels, valued at $156,213, or approximately 30 percent of the total State crop. Virtually all of the oysters produced here come from private beds, leased from the State, most of which formerly were natural beds that had become depleted through overfishing and the destruction of small seed (spat) by natural enemies. Through the leasing and cultivation of these areas production has been maintained and a better grade of oysters produced.

The usual procedure is to stock the leased areas with seed obtained from natural beds in other coastal sections such as Sister Lake, Bay Junop, Barataria Bay, etc. The seed are planted quite densely at reported concentrations of 700 to 900 barrels per acre which does not allow a great deal of room for subsequent growth and increase in volume. This practice, however, has been generally successful for many years and is apparently caused by the fact that the oyster beds are elevated to some extent

above the surrounding bottom and occupy but a very small percentage of the total acreage of those inshore waters. When the oysters reach marketable size they are removed from the growing areas, culled, and either shipped directly to market or temporarily stored in convenient protected areas near the oyster camps. It was during these operations that the oysters died in greatest numbers, as a result primarily of a weakened condition of the adductor muscle and its failure to maintain closure of the shell in air or water. During shipment the oysters opened quickly and spoiled because of evaporation and the loss of shell liquor, while a high percentage of those transplanted to storage areas soon succumbed to the attacks of crabs, small fish, etc., because of their inability to close the shell. The mortality was greatest during the winter months and occurred on beds located at distances ranging from approximately one-half mile to 9 miles from the Barre oil wells and 1 to 5½ miles from the Pelto wells as shown in figure 1.

During the periods from May 23 to June 1, and from August 31 to September 2, 1933, field observations were made of the oyster mortality in Lake Barre, Lake Pelto, and vicinity in relation to existing hydrographical and biological conditions and particularly in respect to pollution of these waters by oil-well operations. These studies were made several months after the mortality was at its peak and after pollution of the water by oil, hydrogen sulphide, and natural gas had been considerably reduced by action of the Louisiana Conservation Department.

Oil production at Lake Barre and Lake Pelto began in June and September 1929, respectively. The most important wells are those at Lake Barre, 14 of which were in operation on June 1, 1933, with a production on that day of 9,987 barrels of oil according to a report received from the Texas Co. These wells were also producing at that time from 6,500 to 7,000 barrels of brine or "bleed water" daily which after being combined and chlorinated (since February 1933) for removal of hydrogen sulphide was emptied into the bay. Samples of this effluent were found to have a salt concentration of 123 parts per thousand as compared with 15 to 22 per thousand for the surrounding waters. They became quite turbid soon after exposure to air or chlorine through the formation of colloidal suspensions of iron and sulphur, and gradually were covered with a thin film of oil after coalescence of finely divided particles. The daily output of the Lake Pelto wells was much less, amounting to only 57 barrels of oil and 650 to 750 barrels of brine, the latter having a salt concentration of 98 parts per thousand.

Studies were made at 27 stations throughout this region as to the condition of the water and its bearing on the reproduction, growth, and mortality of oysters on both natural and planted beds, located in the immediate vicinity and at varying distances from the oil wells. Particular attention was also paid to the abundance and activities of natural enemies of the oyster and to other marine organisms, particularly mollusks, which might be affected by oil-well pollution.

The following observations were made in respect to the oyster-oil well situation in Terrebonne Parish.

1. Random samples of oysters from planted beds in Lake Barre, Lake Pelto, Timbalier Bay, and vicinity, showed that a high percentage of the oysters had died previously on all but three of these leased areas. The mortality on the various beds ranged from approximately 50 to 95 percent as shown in figure 1 and in virtually every case included only the larger and older oysters of marketable size. Many of those surviving were in poor condition and exhibited retarded and abnormal shell growth during the preceding period.

2. No evidence of unusual mortality or retarded growth was found on the beds at stations 23 and 24 in Lake Felicity, on 2 adjacent plantings at station 16 in Lake Pelto, or on a small natural oyster bed, at station 25, which is located at a distance of approximately 500 yards from the Barre wells. Adult oysters were also growing on the piling of these wells and showed a general vertical distribution extending from 1 to 2 inches above the bottom to nearly the surface of the water. The shells of the latter measured from 2½ to 3½ inches and indicated continuous rapid growth over a preceding period of at least 1 year.

3. The oyster beds in Lake Barre and Timbalier Bay are located at distances varying from approximately 500 yards to 9 miles from the numerous Barre wells. Those in Lake Pelto lie within a radius of from 1 to 5½ miles from wells in that region. The previous observations failed to show any direct relation between the degree of oyster mortality on these beds and their distance from the oil wells.

4. Oysters in all localities contained a considerable amount of ripe spawn. A heavy spawning had already occurred several weeks prior to June 1 and was still in progress at that time.

5. Tow-net collections of microscopic marine life revealed an abundance of healthy oyster larvae as well as those of other bivalve mollusks. In the immediate vicinity of the oil wells the free-swimming larval stages of the oyster were plentiful at all ages from 1 day old to setting size.

6. Examination of old shells and those of live oysters showed that intensive setting or attachment of oyster larvae had already occurred (1) on the natural beds, (2) on the planted beds where the mortality occurred, and (3) on the piling of the oil wells and other submerged objects nearby. Heavy setting was still in progress at the time of the investigation as shown by maximum concentrations ranging from 200 to 500 spat per shell on those collected at the Lake Barre and Lake Pelto wells.

7. The spat or minute seed oysters found throughout Terrebonne Parish were of varying ages and size, ranging from 1 day old or recently attached specimens with a diameter of one seventy-fifth of an inch to those 2 or 3 weeks of age having a diameter of approximately one-fourth to one-half inch. It was evident that metamorphosis of the oyster from the larval to spat stages had been successfully completed and that the growth obtained subsequently under existing conditions was rapid and to all appearances normal. No indications of unusual mortality of spat were observed. Spat collected at the Lake Pelto oil well at depths varying from a fraction of an inch to 2 feet above the bottom were in good condition, as were those found on oysters in the test boxes at the Lake Barre oil wells and on the adjacent natural bed. Though setting and spat production is heavy throughout this region only a small percentage survive because of the attacks of natural enemies such as crabs and the borer (*Purpura haemostoma*).

8. Three natural enemies of the oyster, the borer, *Purpura;* the boring clam, *Martesia;* and the boring sponge, *Cliona,* were found to be abundant on many of the planted beds. There was no evidence of the destruction of adult oysters by the borer. The shells of approximately 50 to 75 percent of the dead oysters examined showed heavy infestation of the boring clam and boring sponge. The numerous small tunnels and perforations made by these organisms had caused partial disintegration and weakening of the shells, interfered with normal shell growth, and, apparently, had been a serious drain upon the vitality of the oyster. Serious sponge attack and perforation of the shell by the boring clam were found in many instances at the point of

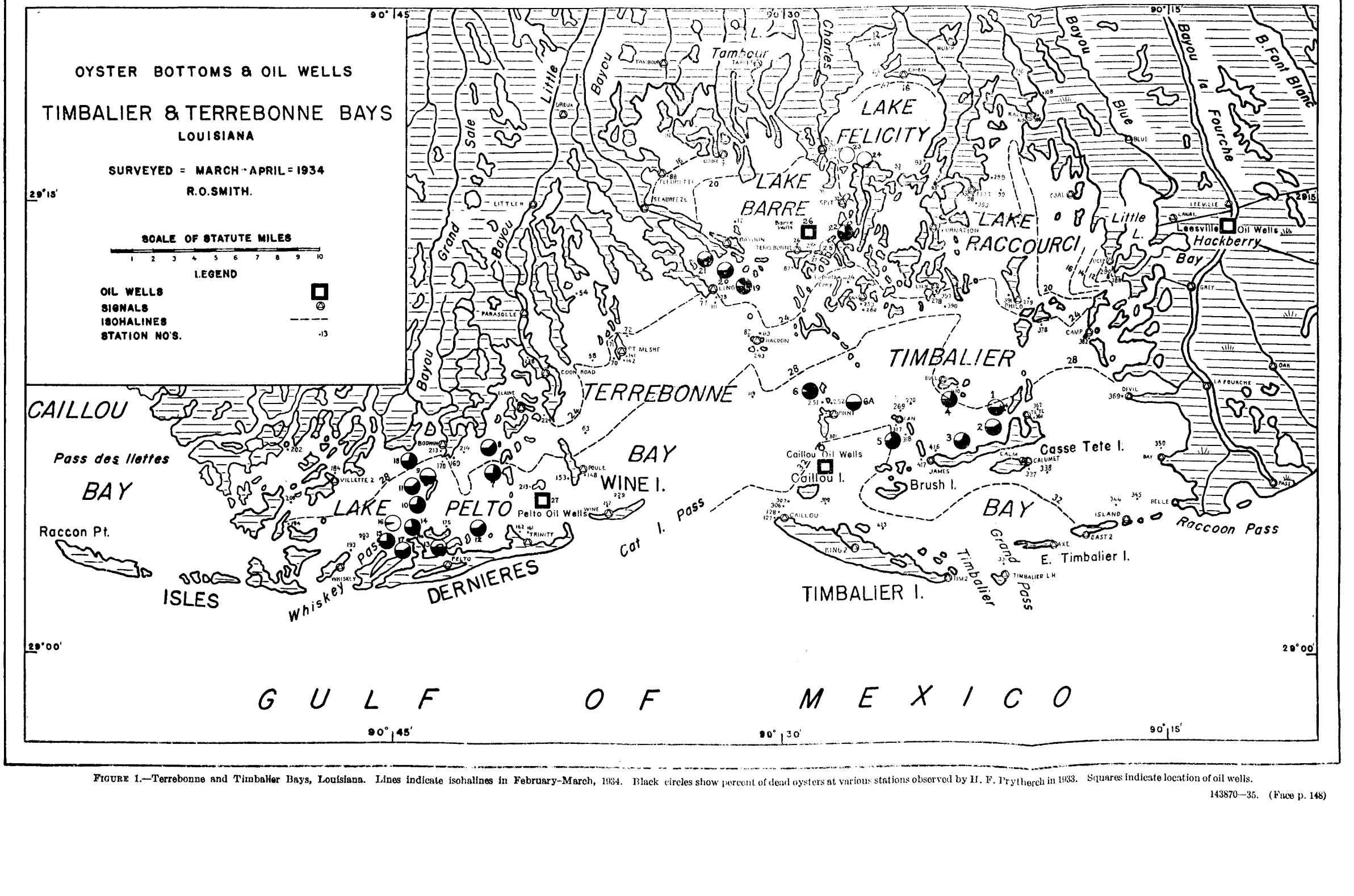

FIGURE 1.—Terrebonne and Timbalier Bays, Louisiana. Lines indicate isohalines in February–March, 1934. Black circles show percent of dead oysters at various stations observed by H. F. Prytherch in 1933. Squares indicate location of oil wells.

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muscle attachment. There was no evidence of a mortality of these natural enemies or of impairment of their growth and reproduction on many areas where a high percentage of the oysters had died previously.

9. A small percentage of the dead oysters and those in a weakened and dying condition showed no serious shell injury by the boring sponge and boring clam. There was one case in particular at station 6 where 95 percent of the oysters had died on a temporary storage bed and were free of shell infestations of these organisms. This bed is located approximately 5½ miles south of the Barre wells.

10. The field studies at the 27 stations in Terrebonne Parish failed to disclose any direct evidence of the destruction of marine animals or plants by oil-well pollution. As indicated previously, conditions throughout this region and in the immediate vicinity of the oil wells were found to be decidedly favorable for reproduction of adult oysters and for the development, attachment, and growth of oyster larvae and spat. Several other mollusks such as the boring clam, shipworm, mussel, *Crepidula*, *Anomia*, and the common borer showed no evidence of unusual mortality and were found to be reproducing and growing throughout this region in an apparently normal manner.

Barnacles and green algae were abundant on the piling of the oil wells and were thriving on submerged cross beams at the Barre well at a distance of 5 feet from the bleed-water discharge where they were subjected continually to a mixture of this effluent and sea water. Blue crabs were observed feeding on these forms at the latter location during the period that the effluent and oyster samples were being collected.

Many species of fish such as trout, croaker, alligator gar, bonnet head shark, channel bass, and bluefish were caught or observed at the Pelto well within 100 feet of the bleed-water discharge. At the Barre well many schools of immature fish, measuring from approximately one-half inch to 3 inches were actively swimming and feeding at distances ranging from 3 to 25 feet from the effluent discharge.

11. *Hydrographical observations.*—Determinations of water temperature, specific gravity, and hydrogen-ion concentration were made at all stations during the period, May 23 to June 1. The records of water temperature give an average of 28.1° C. (86.6° F.) and range from 27° to 29° C.

Measurements of specific gravity at surface and bottom, corrected to 17.5° C., range from 1.0118 to 1.0174 with an average figure of 1.0143. When converted into terms of salinity (grams of salt per 1,000 grams of sea water) the concentration of salts shows a variation of 15.41 to 22.77 per mille, with an average of 18.69 per mille. These salinities are favorable for oyster growth and reproduction and correspond to those found on some of the best oyster-producing areas in this country. The present records of specific gravity have been compared with those obtained in 1906 and 1907 by the United States Bureau of Fisheries (Moore and Pope, 1910) and the Gulf Biologic Station (Cary, 1907) and show that the salt content of the water in Terrebonne Parish was essentially the same in 1933 as at that time.

The observations of hydrogen-ion concentration (as expressed in pH) show an average pH of 8.3 and range from 8.2 to 8.6. These also compare favorably with the Bureau's records of this factor in other oyster-producing regions. Water samples, collected in the immediate vicinity of the Barre and Pelto wells (100 and 300 feet from brine discharge pipes) showed no appreciable difference in hydrogen-ion concentration and salinity at surface and bottom, and no noticeable increase in these factors as compared with conditions on the various oyster beds.

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SURVEY OF OYSTER BOTTOMS IN AREAS AFFECTED BY OIL WELL POLLUTION 1934

By ROBERT O. SMITH

METHODS

The survey vessel was a standard oyster and shrimp lugger, 36 feet in length, with 24 inches draft, and a maximum speed of 10 miles per hour. The vessel had been reconstructed to carry scientific equipment and to provide living accommodations for 4 persons.

A total of 423 stations approximately half a mile apart were made. At each station a sample of bottom water was taken for specific gravity; the depth, bottom temperature, and character of the bottom were observed. Occasional surface samples were taken for comparison. An average of 3 minutes was required for these observations at each station. The water samples were taken with a Galtsoff sampler, using a 1-liter Wolff bottle. Depths were measured with a 16-foot sounding pole marked in conformity with Coast and Geodetic Survey practice. Bottom temperatures were read from a Bureau of Fisheries surface thermometer in brass cup case. Bottom water samples at each station were placed in citrate of magnesia bottles and the specific gravities of all were measured by hydrometer at the end of the day's run. Conversion of specific gravity to salinity was made from Knudsen's table.

The stage of the tide throughout the hydrographic work is given as at Wine Island unless otherwise stated.

All locations refer to the Coast and Geodetic Survey progress sketch of Terrebonne and Timbalier Bays prepared February 1934, under the direction of Lt. W. D. Patterson, chief of party. Prior to this work no accurate chart of the region existed. Without this chart, and the signals erected in preparing it, this survey could not have been made, for there are no natural landmarks, no trees, and only occasional human habitation in the form of fishing camps.

GENERAL CONDITIONS

The area covered by the survey extended from Timbalier Bay on the east to Pelican Lake on the west, including approximately 400 square miles (fig. 1). The examination of beds and hydrographic survey began February 19, 1934, and continued to March 15. Two days, March 27 and 28, were spent in Barataria Bay and Lake Washington.

The bottom over this entire area is exceedingly level. There are very few gullies or reefs except where islands are in the process of being broken down. The bottom, composed of soft black mud and mixed with broken shell, was devoid of vegetation at the time of the survey. On account of the shallowness of the water, rarely over 6 feet, moderate winds churn the bays from top to bottom, so that the water is seldom clear except at the passes during flood tide. The oyster reefs are limited to sections where the bottom is comparatively firm. This condition occurs usually only about the margin of the bays or around islands.

The mean range of tide at Wine Island, near the center of the area, is 1.3 feet. Usually there is only 1 high and 1 low tide daily. However, the actual change in

water level is largely determined by the direction and velocity of the wind. Northerly winds drive the water out, while southerly winds pile it up inside. In either case the difference caused by wind may exceed 1 foot under ordinary conditions and as much as 10 feet during a hurricane. Since the land elevation above mean high water seldom exceeds 2 feet, it is evident that even moderate winds result in considerable wash from the marshes.

On many of the beds the oysters were found to be quite variable in fatness. In general, it appears that accumulation of glycogen occurred very late during the winter of 1933–34. Many oystermen stated that their oysters were in better condition during the latter part of March than at any time previously in the season. It is possible that the customary planting level of 600 to 800 barrels (1,500 to 2,000 bushels) per acre is excessive at times for the amount of food available.

Two previous surveys of the area made, by Moore in 1898 (1899) and by Moore and Pope from 1906 to 1909 (1910), were not sufficiently detailed to permit direct comparison of hydrographic data. However, it seems safe to say that except for the elimination of extraordinary fluctuations in density resulting from crevasses, the salinity observed in 1934 appears to be much the same as during the 1909 survey.

In 1898 the water in Terrebonne Bay was found to be fresher than in Timbalier Bay, an observation not borne out by our determinations, as the salinity now is approximately the same in both bays. It is said that owing to the freshness of the water no oysters were found in Terrebonne Bayou above Lake Lagraisse prior to 1883. It was also stated in 1898 that considerable changes in topography were occurring in Terrebonne Bay. Such changes consisted in the tearing down of large amounts of marsh land then present in Terrebonne and Timbalier Bays, and separating Terrebonne Bay from Lake Barre. It was at one time possible to go from Houma to Timbalier Island by land. Undoubtedly this continued destruction of land area has been one of the most important factors bearing on oyster culture in the region, for the absence of obstructing land permits the rapid mixing of Gulf water with the fresh water from the bayous and serves to maintain a relatively high salinity (16 parts per million, February 1934) to the upper parts of Lake Barre and Lake Felicity.

At the time of the 1898 survey market oysters were no longer produced in Barataria Bay, which includes Lake Washington (Grand Ecaille), and only a few dead reefs existed there as evidence of previous importance. The chief oyster-producing region was Terrebonne Bay, which included Lake Pelto. Since then the beds in Barataria Bay have been rehabilitated and extended so that at present some of the finest oysters are produced here. Although Terrebonne Parish still is foremost in quantity, the quality is in general inferior to Barataria Bay.

Insofar as shape, growth, and quality are concerned, observations just made are in almost complete agreement with the early survey, and such discrepancies as exist may be caused in part by confusion in identifying localities from the local names in use at that time.

An effort was made to obtain a definite record of sudden and unexplained losses of oysters in the past. No such occurrences were found other than the occasional killing of oysters said to have been caused by freshets due to breaks in the levee.

LAKE BARRE

The lake has an area of about 40 square miles and contains relatively few reefs, mostly on the southern side.

Mortality of oysters was reported in 1932–33 only from the southeast side of the bay at Lafont's camp (fig. 1), about a mile from Barre operations of the Texas Co. The loss was estimated by the fishermen to have been about 75 percent.

When visited during February and March 1934, oysters were still dying as evidenced by many clean paired shells and occasional dying or newly dead oysters. The dying individuals were very thin and watery as though they had slowly starved to death. The majority of oysters have the shells honeycombed by boring sponge, boring clam or worm, and the interior of the shell usually has from 1 to 6 mud inclusions covered by a thin layer of shell.

Out of 40 stations made on 2 days, February 19, from 11 a. m. to 6 p. m. and February 20 from 9 to 10 a. m., 14 stations were made on flood tide and 20 on ebb. Salinities varied from 16 parts per thousand at Signal Odor to 32 parts per thousand at the Barre wells of the Texas Co. Isohaline contours (fig. 1) indicate that fresh water from Bayou Terrebonne enters the lake at Seabreeze and is deflected down along the islands formerly paralleling the course of the bayou. Salt water from the Gulf is crowded somewhat to the western side of the bayou by fresh water from Lake Felicity.

Temperatures on the bottom at stations on February 19 ranged from 61° F. at 11 a. m. to 60° F. at 5:30 p. m. (16.1°–15.5° C.). On February 20, the temperature had fallen to 55° F. (12.7° C.).

LAKE FELICITY AND LAKE CHIEN

Depths in this section varied from 4 to 6 feet, with a depth of 7 feet in the channel at Seabreeze.

Lake Felicity has an area of about 20 square miles, and Lake Chien, which opens into it on the north, covers about 4 square miles. Although the reefs entirely surround these lakes, production of oysters is mostly limited to their northern and southern shores. Oysters were examined at three places: at the point where Lake Chien joins Lake Felicity; on the northwest shore of Felicity; and on the southeast side of Felicity where it joins Bay Jacko. The oysters observed were not high-grade shell stock, being more suitable for seed or steaming, according to size. No mortality was reported as having occurred in these waters, and samples taken in February contained no shells from recently dead individuals.

Owing to the low specific gravity, drills are not bothersome, so that the area is valuable for seed production. The entrance to the lake is about 4 miles from the nearest oil wells.

Eighteen stations were made in the 2 lakes, from 9:30 a. m. to 3:15 p. m. February 20. Although the survey was carried on mostly during flood tide, the prevailing north wind very probably prevented a normal high tide. The salinity varied from 10 parts per thousand at the head of Lake Chien, to 20 parts per thousand at Grand Pass Felicity on the southwest side of Felicity. The depth varied from 5.5 to 7 feet, but the depth over the reefs around the shore is 2.5 to 4 feet. A depth of 25 feet with a strong current was found in Grand Pass Felicity on the southwest side where Felicity joins Lake Barre. The bottom temperature varied from 54° to 57° F. (12.2°–13.9° C.).

A number of reefs are said to be present in Bay Jacko, but the water was too shallow for the survey boat.

TERREBONNE BAY

This bay covers an area of about 100 square miles, exclusive of bayous, small lakes, and bays. Natural reefs are limited by soft bottom to bayous and the shores of islands in the bay. The section affected by mortality lies between Lake Barre and Terrebonne Bay. The heaviest losses in this region occurred in the vicinity of Lafont's camp, as mentioned above. At the time of this survey, in February 1934, the better oysters were watery. Whether or not this is the usual condition during late winter could not be ascertained.

This area is said to have produced large numbers of high quality oysters in the past. At present (1934) most of the oysters are used for steaming.

At the 99 stations which were occupied on February 26–27 and March 5 and 8, salinities varied from 22 parts per thousand south of the Texas Co.'s wells, to over 32 parts per thousand near the west end of Timbalier Island, and less than 30 at the east end of Wine Island. The opening between these islands is known locally as Cat Island Pass and is by far the largest pass in this region, being about 5.5 miles across. Isohalines show that the western side of the bay is slightly fresher than the east side, due apparently to drainage from Bayous Terrebonne and Little Caillou.

Bottom temperatures on February 26–27 varied from 51° to 59° F. (10.6°–15° C.); on March 5 they averaged 64.5° F. (18.0° C.); on March 8 the average was 70° F. (21.1° C.).

The depth varied from 4 to 10 feet. The lower part of the bay will average 8 feet although there is a channel up to the Texas Co.'s wells through which 10 feet may be carried. The upper part of the bay is mostly 5 to 6 feet deep.

TIMBALIER BAY

This bay has an area of approximately 230 square miles, bisected by a string of islands lying in an east to west direction. Practically all of these islands have oyster beds around them. The bottom, except about Philobruis, is soft mud.

The quality of the oysters varies greatly in the different sections of the bay. On the eastern side, including Devils Bay north through Jacks Camp Bay to Little Lake, excellent shell stock occurs, though on March 24, at the mouth of Bayou Grey and approximately 5 miles from the Leesville wells, the oysters had a pronounced oily taste. At Philobruis, 9 miles from the Leesville wells, oysters are of good quality for shell stock, no oily flavor could be detected, and no mortality was reported or observed. Practically the entire area between Philobruis and the eastern shore of the bay is covered with oyster reefs, all leased. Sponge, boring clam, and conchs are common. Most of the marketable oysters are between 2 and 3 years of age.

The greatest mortality was reported in the vicinity of the islands Castete and Bull, about 14 miles from the Leesville wells, and 9 from the Barre wells. Dredgings made on March 13 consisted of about 90 percent shell. Numerous clean paired shells found at this station indicated that oysters continued to die. As a result of the mortality during the winter of 1932–33, no fresh plantings were made by the leaseholders in 1933. Local setting does not survive, so that seed must be brought from Lake Felicity or from the Louisiana marshes to the east of the Mississippi River.

One hundred fifty-five stations were occupied during the 5 days of March 8, 12, 13, 14, and 15. The salinity varied from 22 parts per thousand at Philobruis,

to 32 parts per thousand at Timbalier Lighthouse (fig. 1). All but 25 samples were taken on flood tide.

The bay is very shallow. On the western and southern sides, the depth ranges from 5.5 to 7 feet. The center and northeast sections are shallower, ranging from 3 to 6.5 feet, but much of it on the eastern side does not exceed 4.5 feet.

On March 8, the botton temperature averaged 71.5° F. (21.7° C.); March 12, 61° F. (16.1° C.); March 13, 65° F. (18.3° C.); March 14, 62° F. (16.6° C.); March 15, 64.5° F. (18.0° C.).

LAKE RACCOURCI

Lake Raccourci, which lies north of Timbalier Bay, has an area of approximately 25 square miles. The depth varies from 2.5 to 4.5 feet. The bottom is soft mud mixed with small clam shells. There are no reefs in the center of the lake. A few oysters are to be found about the entire shore line, but the chief beds are at the northern end and at Philobruis on the southern boundary. At the time of the survey, March 9, 1934, the beds at the upper end of Lake Raccourci were not being worked. No mortality was observed. The salinity varied from 18 parts per thousand in Bay Courant above Lake Raccourci, to 22 parts per thousand at Philobruis. Boring sponge and boring clam were present.

Thirty stations on flood tide and 8 on ebb tide were occupied on March 2, 9, and 14. The bottom temperature on March 2 averaged 63° F. (17.2° C.); on March 9 and 14, the average temperature was 61.5° F. (16.4° C.); and 64.5° F. (18.0° C.), respectively.

LAKE PELTO AND PELICAN LAKE

Lake Pelto extends along an east and west line from Wine Island on the east where it connects with Terrebonne Bay, to Pelican Lake on the west. It connects with the Gulf at the southwest through Whiskey Island Pass. The lake is about 5 miles wide and covers an area of 50 square miles. Oil wells are located at the eastern end of the lake about equidistant from the north and south shores. The few oysters found in this lake were of good appearance and flavor, and some from the western end were equal to any produced in the region. Extensive losses have been reported throughout the lake except along the northern shores and at the extreme western end. The greatest damage, amounting in certain cases to complete destruction of the beds, has occurred about the centrally located islands and along the southern shore within a radius of 5 miles of the oil wells. Of natural enemies, the boring sponge was most abundant, there were some boring clams and some drills. Most of the oysters in this region have very dark gills.

Sixty-two stations were made on Lake Pelto on March 6 and 7. Salinity varied from 26 parts per thousand along the northern shore, to 30 parts per thousand along the southern shore. Bottom temperature varied from 64° to 71° F. (20.0°–21.6° C.) on March 7. The average depth through the center of the lake is about 7 feet. The greatest depth measured was 12.5 feet at Wine Island Pass.

Pelican Lake is situated at the northwest end of Lake Pelto. The area is about 9 square miles. The oysters were fairly fat, well flavored and of medium size. No mortality had occurred here according to the oystermen, and no clean shells were found on the reefs. The bayous leading off from the lake are well populated with coon oysters. The planted beds are limited to firm bottom around the shore and about the islands. Boring sponge was abundant, boring clams common, and there

were a few drills, though the actual abundance of the latter is difficult to determine until they begin to assemble for the spring spawning around the first of April.

On March 7, 9 stations, all on flood tide, were made from Bay Round to the head of Pelican Lake. The salinity varied from less than 24 parts per thousand at the head of the lake to 28 parts per thousand where Bay Round joins Lake Pelto. The average depth in Pelican Lake at three-quarters flood was 3 to 4 feet. In Bay Round, depths of 7 to 9 feet were found. The bottom temperatures on March 7 in Pelican Lake varied from 67° to 69° F. (19.4–19.6° C.). These high temperatures ended March 8, and by the morning of March 9 the temperature was down to 61° F. (16.1° C.) and only rarely exceeded 65° F. (18.3° C.) during the remainder of the month.

Production of oil at Lake Pelto was discontinued April 1, 1934. The total production from this dome is not known, but at the time the survey began (February 1934) only about 40 barrels per day were reported. No oil was produced in Bay Saint Elaine until the first of April 1934, but gas was piped from here to the Lake Pelto wells for fuel.

Bay Saint Elaine lies to the north of Lake Pelto at the eastern end. The area is about 4 square miles, but the shape is so irregular and there are so many marshy islands within that the actual extent of the bay is difficult to determine.

Few, if any, oysters were being marketed from this bay at the time of the survey. Coon oysters were abundant in places and seemed to grow rapidly.

Four stations were made on March 7. Salinities varied from 18 parts per thousand, at the head of the bay where bayou Little Caillou empties into it above Coon Road, to 24 parts per million at signal Elaine. The bottom temperature ranged from 69° to 72° F. (20.5°–22.2° C.), an increase of about 2 degrees per mile from the lower to the upper end. The depth was generally about 5 feet, but at station 225 east of signal Elaine the depth was 16 feet.

EXAMINATION OF OYSTER BEDS AT MOUTH OF BAYOU GREY AND LITTLE LAKE

On March 24, 1934, a trip to the beds was made by oyster lugger from Leesville. Thirteen wells were then in operation along the bayou below Leesville. The surface of the bayou was covered with oil for a distance of 3 miles below the wells. There was a flood tide, with a strong wind from the southeast.

Adult oysters, which had been on the bedding grounds 3 months, had a strong oily flavor. There had been some mortality as evidenced by recently dead paired shells. All shells were covered with a brownish black coating of a tarry consistency. When the bottom was stirred by tongs, an oily patch appeared on the surface. The oysters were of good shape but watery. There was very little gonad development. It was stated that ordinarily the oysters were very milky at that time.

On March 28, 1934, an examination was made of water conditions in Lake Washington, with reference to their bearing on oyster culture. At the time of the survey, there were 44 sulphur wells in operation, each producing 12 tons of crude sulphur per hour. Very little effluent found its way into the lake. The bottom over a short radius around the point of entry of the effluent was covered with a thick brownish layer of diatoms of undetermined species. There was a strong odor of hydrogen sulphide in the air, but the mud samples had very little odor, though they were quite oily.

Based on conditions existing on March 28, there was no evidence that the operation of the sulphur wells had an injurious effect on oysters.

On July 4, 1933, an oil well was out of control for 36 hours, during which time it was estimated that some 3,000 barrels of oil flowed into the lake. No appreciable quantity of oil was lost subsequently. One of the leases was reported to have been heavily covered with oil, and until November oysters were unmarketable on account of the oily flavor.

On March 28, 1934, a combined sample of three-quarters bushel was tonged from these beds. Some of the oysters had a slight oily taste. The tonging caused patches of oil to appear on the surface of the water, indicating that some oil still was held by the mud.

CONCLUSIONS

The purpose of the survey described above was to supply a knowledge of the local and general factors in the environment of the oyster beds in Terrebonne Parish and adjacent territory which might have a bearing on the problem of pollution.

The hydrographic data show that conditions of salinity, current, and temperature were, at the time of the survey, suitable for growing oysters throughout the area covered, and it has not been possible to assign the mortality to any known disturbance of the natural conditions on the oyster beds. Bearing this in mind, special attention was given to several factors whose combined effect would tend to magnify the action of any polluting substance. Among these may be mentioned the shallowness of the water. Even moderate winds stir the bays from top to bottom so that the water carries much suspended matter. Any polluting substance is quickly and thoroughly mixed with the water and is adsorbed by suspended matter; it may be transported over wide areas and deposited on the bottom far from the source of pollution.

In general, mortality has been higher on soft, muddy bottom than on hard ground or reefs. The significance of this is not known, but there is no evidence that silting is directly responsible for the mortality observed. Probably because of the decomposition of the organic matter, a muddy bottom presents a less favorable habitat for oysters than that found on hard ground.

In 1934, oysters on many of the beds throughout the region did not become fat until February or March, which points to a possible scarcity of food organisms during the fall and winter, or to a disturbance in the functioning of the oysters' organs of feeding. Overcrowding would tend to aggravate this situation. The oystermen state that from 600 to 800 barrels, i. e., 1,500 to 2,000 bushels, are planted per acre. While this quantity may be supported so long as conditions remain normal and the food supply adequate, it is obvious that should mortality begin in such a concentration it is likely to result in the loss of a considerable number of the oysters.

No direct evidence was found that the mortality had been caused by any known natural enemies, although they may have an indirect effect by increasing calcium metabolism or competing for food, and, in some cases, possibly a direct injury by attack or the secretion of poisonous substances, for boring clams, sponges, and worms are abundant in parts of Lake Barre, Timbalier Bay, Terrebonne Bay, and Lake Pelto. The borer has not been included in the above consideration of enemies because its habits, range, and destructiveness are well known, the damage done by it is fairly constant, and its depredations can be eliminated as a cause of the unusual mortality.

Crude oil pollution has been suspected as the chief cause of the mortality. No information is now available upon which to base an opinion as to the validity of this belief. Light films of oil were observed to be generally present in the vicinity of the Lake Barre wells, and below Leesville to the mouth of Bayou Grey. Whether or not this oil was harmful to oysters could not be determined in the short time allotted to the survey. But regardless of any alleged toxicity of oil to oysters, two facts should be borne in mind. First, oil in water is quickly taken up by oysters, imparting an oily taste to the flesh which renders the meat unsalable. Second, the effect of oil pollution will last over a long period, for the oil is carried to the bottom by suspended mud particles and released from time to time by storms, tonging, or dredging.

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EXPERIMENTAL STUDIES OF THE EFFECT OF OIL ON OYSTERS

REVIEW OF THE LITERATURE

By PAUL S. GALTSOFF

So far as the author was able to ascertain, the literature on the subject is rather meager, comprising less than a dozen papers. On the basis of a small number of experiments with water-gas tar carried out in 1912 at the United States Bureau of Fisheries station at Woods Hole, Mitchell (1914) arrived at the conclusion that in constantly renewed sea water tar shows no noticeable effect on oysters. Fatal effects are produced however, when considerable quantities of water-gas tar are in intimate contact with oysters kept in stagnant water.

Orton (1924) when studying the causes of the unusual mortality among oysters in England during 1920 and 1921, made a few experiments with oil and arrived at the conclusion that the petroleum residue is not seriously poisonous to oysters, as all of them kept in a jar covered with a thick film of oil survived at least 7 weeks. Similar results were obtained in the laboratory of the Scottish Biological Association (Orton, 1924). Examination of water made by the Government chemist (Orton, l. c., p. 42) showed that the sample of water in which the oysters were kept contained traces of the original petroleum waste in solution and in addition small quantities of substances of an acidic character. The latter, probably naphtenic acids which exist in certain petroleums, may have been present originally in the petroleum waste used in Orton's experiments or may have been derived from the waste by some chemical or biological action of the sea water.

Contrary results were obtained by Leenhardt (1925) who showed that the mortality of both European and Portuguese oysters (*Ostrea edulis* and *Gryphea angulata*) kept in water covered with petroleum increases in proportion to the amount of the latter. Thus, no mortality was observed in the oysters kept for a month in water containing from 0.01 to 0.05 percent petroleum. When the quantity of petroleum was increased to 0.1–0.5 percent only 2 or 3 oysters out of a dozen survived. In the water containing 2 percent petroleum all oysters died within 1 week. Although Leenhardt states that water was changed and presumably the oysters did not suffer from lack of oxygen it is not clear how often the water was renewed. From the observations that the same mortality occurred in the oysters that never were in direct contact with petroleum as in those which were periodically covered with it, simulating the conditions on tidal flats, Leenhardt concluded that oil contains a substance soluble in sea water, and poisonous to oysters. Unfortunately both Orton's and Leenhardt reports are very brief and fail to give all the details of the experiments.

More comprehensive investigation on the effect of oil on fish was carried out by Roberts (1926) who demonstrated that oil extracts (gas oil, Diesel oil, 600 seconds and 1,500 seconds oil) prepared by shaking 100 cc of oil with 2,000 cc of boiled and filtered river water are toxic to brown trout. He attributed the toxicity to both soluble toxic substances and emulsions. Mention should be made also of the work of Stephen (quoted from Roberts, 1926) who described the adverse effect caused by a film of paraffin 0.03 mm in thickness on pelagic larvae of plaice and flounders; and of the experiments of Jee and Roberts (1923) with fresh-water shrimp killed by contact with fuel oil, and with caddis larvae and fresh-water shrimp killed by

immersion in a fuel oil extract. Gardiner (1927) found that the aqueous extracts of tar were completely without effect on freshly fertilized eggs and on "eyed" ova of trout, and that the resistance of trout alevins to the toxic action of phenol decreased with age. Alevins 60–66 days old were quite unaffected by 2-hour immersion in a solution of 40/100,000 phenol, whilst fry 110–115 days old were unable to withstand more than 15 minutes immersion.

Elmhirst (1922) found that oil in water had no effect on a number of bottom organisms (Purpurea, Serpula, Authozoa, Ascidia, Mollusca) and many planktonic forms although the latter were killed when they came in direct contact with oil.

According to James (1926), under conditions approaching those prevailing in nature, a 0.1 percent dilution of light or heavy bunker oil exerts no effect on the development of cod eggs, but toxic effect of the same oils becomes apparent under conditions of limited circulation and aeration. Slight injurious effect is exerted by the same oils upon larval flounders. He rightly states that further research should be based upon more specific knowledge of the physical and chemical phenomena accompanying oil pollution.

Considerable discrepancies in the results of the experiments mentioned in this brief revue may be attributed to various causes, namely, differences in the chemical composition of oils used, defects in experimental technique and small number of observations. It has been the purpose of the present experiments to obtain a better understanding of the effect of oil and brine on oysters and to carry them on a more comprehensive scale than has been done before. The experimental investigation comprises two distinct parts; a study of the effect of oil and bleed water on mortality, rate of feeding and behavior of the adductor muscle of the oyster; and a study of the effect of oil on the growth of a diatom. For the latter experiments the culture of a single species of *Nitzchia clostearia* E. was used. It is believed that the results obtained with this planktonic species which occurs in the normal habitat of the oyster, are applicable to other planktonic diatoms.

Most of the experiments on oysters were carried out at the United States Fisheries laboratory at Beaufort, N. C. Studies on diatom growth were made at the Bureau of Fisheries laboratory in Washington and Woods Hole, Mass. Since the experiments carried out by the authors were performed at different times and under different conditions, the results of their investigations are presented separately under their respective names.

SURVIVAL OF OYSTERS IN OIL-POLLUTED WATER

By Herbert F. Prytherch

The plan and purpose of these experiments was to expose oysters to high concentrations of the principal polluting substances from the oil wells in order to determine their effect on growth and survival. The oysters were subjected to crude petroleum, sludge, brine-effluent water, and hydrogen sulphide, samples of which, with the exception of H_2S, were collected previously at the wells by the author and stored in glass containers. These studies were conducted at the United States Fisheries Biological Station at Beaufort, N. C., and involved the use of several

different methods and apparatus, a description of which is furnished with each series of experiments. Special studies were also made of the changes in glycogen content of oysters after continuous exposure to three different grades of crude petroleum and varying concentrations of brine water from the Barre wells. The glycogen analyses were performed by P. S. Galtsoff.

EXPERIMENTS WITH SURFACE FILM OF OIL

The shell movements of the oyster indicate whether it may or may not be feeding and also reflect quite accurately its reactions to environmental conditions. Graphic records were obtained of the shell movements of one representative oyster in each of the experimental and control jars. For this purpose adult oysters of approximately the same age and size were immobilized in a horizontal position by cementing the lower valve to a brick or piece of slate. The upper valve was free to move, and, by means of a simple arrangement of levers and pen, its movements were transmitted and recorded continuously on a revolving smoked drum as shown in figure 2.

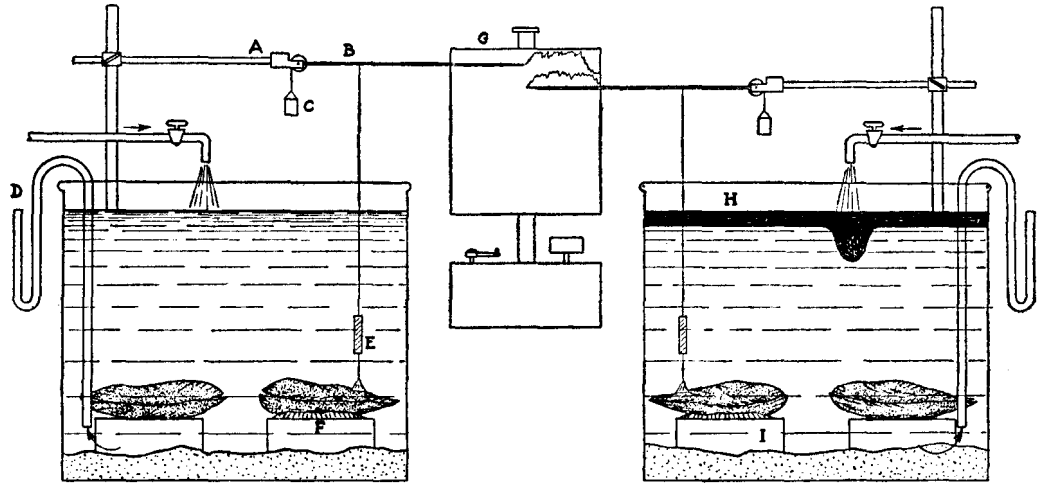

FIGURE 2.—Diagram showing arrangement of apparatus for obtaining comparative records of shell movements of oysters in sea water (control) and in sea water passed through oil (experimental). A, light muscle lever; B, aluminum pen; C, counterbalance weight; D, constant level arrangement; E, rubber universal joint and light rod connecting upper valve of oyster to recording pen; F, oyster cemented to brick (control); G, kymograph; H, layer of oil; and I, experimental oyster.

A series of experiments was arranged, employing 5 glass jars of 6-liter capacity, in each of which 2 small and 2 large adult oysters were placed, 1 of the latter being attached to the recording apparatus. Four of the jars were covered with a heavy surface layer (50 cc) of the following oils and sludge—grade A and grade B crude petroleum from the Barre wells, a composite sample of crude petroleum from the Pelto wells, and basic sludge collected from the storage tanks at the Barre wells. Each experimental jar was supplied with running sea water, which was introduced above the oil film at the rate of 8 liters per hour as shown in figure 2. This series of experiments was conducted from July 3 to September 4, 1933, during which time the surface layer of oil and sludge was renewed weekly. The temperature of the water ranged from 24.8° C. to 28.5° C., and the salinity from 32.7 to 33.4.

The results of these studies are shown in table 1. All of the large adult oysters survived, and those attached to the recorder and exposed to oil and sludge showed essentially the same behavior as the control in respect to shell movements and ability

to maintain closure when kept in air for over 72 hours after completion of the experiment. Records of shell movements of these oysters made for 2 days before exposure to oil and sludge showed no noticeable differences in comparison with those obtained during and at the end of the experiment. Examination of table 1 shows that the experimental oysters were open on an average of 10 to 13.6 hours daily as compared with 11.2 hours for the control specimens. This difference, however, is not significant if allowance is made for the individual variations in oysters in respect to duration of open periods. For example, in other experiments 16 control oysters in running sea water (temperature 22° to 30° C.) showed an average daily open period ranging from 7.5 to 14.2 hours. Hopkins (1931) states that oysters at Beaufort, N. C., averaged between 10 and 14 hours per day open in running water.

TABLE 1.—*Length of time oysters remained open in running sea water passed through surface layers of oil and sludge*

[July 3 to Sept. 4, 1933]

Specimen	Medium	Total hours open	Average number of hours per day open	Percent of time open
No. 1 (control)	Running sea water—no oil	719	11.2	46.8
No. 2 (experimental)	Running sea water—Barre oil A	783	12.2	51.0
No. 3 (experimental)	Running sea water—Barre oil B	664	10.3	43.2
No. 4 (experimental)	Running sea water—Pelto oil	641	10.0	41.7
No. 5 (experimental)	Running sea water—Barre sludge	870	13.6	56.6

During the last 2 weeks of the experiments 7 of the small adult oysters died, the losses occurring as follows: Control, 2; Barre oil A, 1; Barre oil B, 2; Pelto oil, 2; Barre sludge, 0. Similar losses have occurred in previous experiments not involving oil pollution and are believed to be due to the fact that these oysters were collected from a densely populated bed near the laboratory and consequently were not in as good condition as the larger oysters which were obtained from planted beds.

SURVIVAL OF OYSTERS IN SEA WATER PASSED THROUGH OIL

According to Gowanloch (1934) the toxicity of Louisiana crude petroleum can be demonstrated by continuously exposing oysters to sea water passed through a heavy layer of oil. A similar series of experiments was conducted at the Beaufort laboratory using a large wooden tank having 4 watertight compartments in each of which were placed 25 small, adult "coon" oysters from a bed adjacent to the laboratory and 25 large oysters obtained from planted beds in Newport River. The arrangement of the equipment in one of experimental compartments is shown diagrammatically in figure 3. One compartment was used as a control and the other 3 supplied with the same amount of running water at the rate of 20 liters per hour, which was passed through 1 liter samples of the following oils: Barre grade A, Barre grade B, and Pelto composite. The experiment was in operation from March 26 to July 27, 1934. At the completion of the experiment it was found that there was little difference in the survival of the large adult oysters in the control and experimental jars, though the small oysters exposed to oil showed a slightly greater mortality than the controls. The results of this series of experiments are given in the following table (table 2). In reviewing the results, consideration must be given to the possibility that certain compounds in the oil may reduce the oxygen content of the inflowing water and that the oil itself may

act as a filter and prevent many food organisms from reaching the oysters in the experimental tanks.

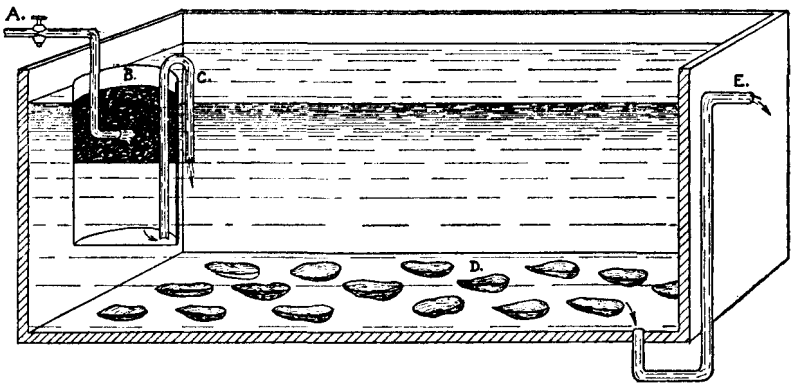

FIGURE 3.—Cross section of apparatus used to expose oysters to sea water passed through oil and smudge. A, sea water intake; B, glass cylinder containing 1 liter of oil; C, siphon tube; D, oysters; and E, overflow.

TABLE 2.—*Survival of oysters in sea water passed through oil*

	Total number of live oysters				Percent survival according to size	
	Apr. 30	May 30	June 30	July 27	Large	Small
Tank no. 1 (control—no oil)	50	39	29	23 (12 large, 11 small)	48	44
Tank no. 2, Barre oil A	50	37	24	18 (11 large, 7 small)	44	28
Tank no. 3, Barre oil B	50	38	29	21 (11 large, 10 small)	44	40
Tank no. 4, Pelto oil	50	36	25	do	44	40

IMMERSION OF OYSTERS IN OIL

The purpose of these experiments was to immerse oysters in crude petroleum at regular intervals in order to determine if they would be killed by direct exposure to oil or would subsequently recover from such severe treatment. By means of an artificial

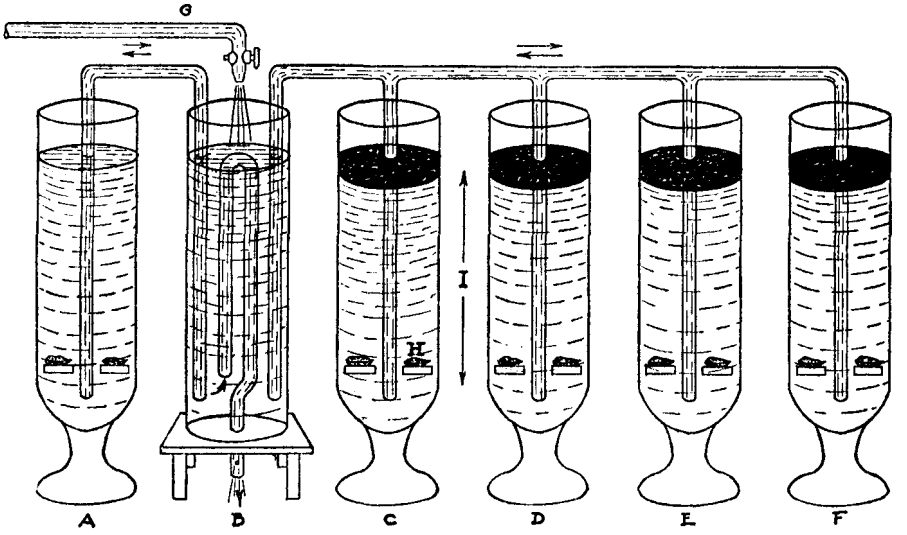

FIGURE 4.—Diagram of apparatus used to immerse oyster in oil at regular intervals. A, control jar; B, automatic siphon jar; C–F, experimental jars containing different grades of petroleum and sludge; G, sea water intake; H, oysters (4 per jar); I, vertical movement of oil layer during each artificial tidal cycle.

tidal arrangement a heavy surface film of oil was made to cover completely the oysters at intervals of 1 and 2 hours. The equipment used, as shown in figure 4, consisted of

5 tall hatching jars of 5 liter capacity, in each of which 4 adult oysters were elevated above the bottom by a 3-inch layer of clean oyster shell. An additional jar containing an automatic siphon arrangement was connected with each hatching jar by glass and rubber tubing so as to bring about the filling and emptying of these jars at regular intervals. One control and 4 experimental jars were used in each series of experiments, each of the latter containing a heavy surface layer (50 cc) of the following: Barre oil grade A, Barre oil grade B, Pelto oil (composite sample), and Barre sludge. After the experiments had been in progress for approximately 3 weeks, 50 cc of each of the above oils and sludge were added to each experimental jar, respectively.

Four separate and complete series of experiments were conducted during the period from September 20, 1933, to January 3, 1934, in which a total of 64 oysters were completely immersed in oil at intervals of 1 and 2 hours over a period of 6 to 8 weeks. The results of these studies are given in table 3. In the first series (1 hour interval), which was conducted in the early fall at fairly high water temperatures, there was a loss of 31.25 percent of the oysters exposed to oil and 50 percent of the control oysters. In the second series the losses in the control and experimental jars were the same amounting to 25 percent. In the third and fourth series (2 hours exposure interval) not a single specimen died in either the control or experimental jars or during the subsequent period of 3 months when they were returned to natural conditions in the harbor. During the course of the experiments it was frequently observed that the oysters kept the shell open when covered with oil and that the formation of new shell was much greater in the experimental oysters than in the controls.

In order to test further the toxicity of water contaminated with oil and sludge, the overflow from all jars used in series 1 and 2 was passed into a tank containing 22 seed oysters, 22 clams (3 species), 4 gastropods (2 species), and 6 anemones, none of which died during the course of the experiments. In the third and fourth series the overflow water was passed into a tank containing approximately the same number and kind of marine organisms as the former and in addition 6 small fish (Fundulus, Hypsoblennius), all of which survived and appeared to be in a healthy condition.

TABLE 3.—*Survival of oysters after immersion in oil and sludge at regular intervals of 1 and 2 hours over periods of 6 to 8 weeks*

Conditions	Series no. 1 (Sept. 20 to Oct. 31)		Series no. 2 (Sept. 20 to Oct. 31)		Series nos. 3 and 4 (Nov. 2 to Jan. 3)		Total results (Sept. 20 to Jan. 3)	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
Number of oysters used	4	16	4	16	8	32	16	64
Number of times oysters immersed in oil	0	1,008	0	1,008	0	720	0	720-1,008
Number oysters alive at end of experiment	2	11	3	12	8	32	13	55
Percent survival by jar:								
Control	50		75		100		81.2	
Barre oil A		75		75		100		87.5
Barre oil B		75		50		100		81.2
Pelto oil		50		75		100		81.2
Barre sludge		75		100		100		93.7
Percent survival, total	50	68.7	75	75	100	100	81.2	85.9

EFFECT OF OIL ON GLYCOGEN CONTENT OF OYSTERS

This series of experiments was conducted to determine the effect of 3 different grades of crude petroleum on the glycogen content of oysters over a period of 4 weeks (Dec. 6, 1933, to Jan. 4, 1934). Two hundred and ten oysters of uniform age and size were used, of which a representative sample of 10 oysters was taken for glycogen

analysis at the beginning of the experiment. The experiments were conducted in a specially constructed wood tank having 4 compartments of equal size in each of which 50 oysters were placed. Each compartment had a capacity of 135 liters and, by means of an automatic siphon arrangement similar to that shown in figure 7, received an equal amount of sea water, which was completely renewed every 3 hours during the course of the experiment. One compartment was used as a control and the other 3 covered with a heavy layer of crude petroleum (2 liters each) of the following grades: Barre oil, grade B; Barre oil, grade A; and Pelto oil composite sample. The oil completely covered the surface of the 3 experimental tanks and during each low-water interval was approximately one-half inch above the oysters. At the end of each week 10 oysters were taken from each compartment and analyzed for glycogen content by P. S. Galtsoff.

The results of this experiment, given in the following table, indicate a slight decrease in the glycogen content of oysters kept in oil-polluted water.

TABLE 4.—*Effect of heavy surface layer of oil on glycogen content of oysters*

[Experiment G 1]

Item	Percent glycogen, fresh basis					
	Dec. 6	Dec. 13	Dec. 20	Dec. 28	Jan. 4	Average
Control	1.72	2.81	2.68	2.67	1.62	2.30
Barre oil B	(1.72)	1.89	1.82	2.56	2.54	2.10
Barre oil A	(1.72)	1.62	1.57	1.98	2.22	1.82
Pelto composite	(1.72)	1.69	.....	1.42	2.29	1.80

EXPERIMENTS WITH BRINE

The principal effluent from the oil wells in Lake Barre and Lake Pelto is the brine water that is separated from the petroleum. The quantity discharged daily, at the time of the oyster mortality, varied from approximately 5,000 to 7,000 barrels for the Barre wells and from 650 to 750 barrels for the Pelto wells. The composition of petroleum brines, according to Clark (1924), has been found in many instances to be quite similar to that of ocean water although modified by local conditions and differing in concentration. Such waters have been variously interpreted, sometimes as fossil sea water which was entrapped in the original sediments, and sometimes as derived by leaching from beds of salt.

Analyses of the brines from the Barre and Pelto wells are given in table 5, together with those of 3 samples of sea water which were collected from the following localities: (1) Oyster bed of St. Pierre, west of Barre walls, where a high mortality of oysters occurred, (2) oyster bed at Sea Breeze where no mortality occurred, and (3) Gulf of Mexico in ship channel at Buoy No. 3. These analyses, prepared by the Port Arthur laboratory of the Texas Co., show that the brines are quite similar to the sea water found in this region in respect to the presence and relative amounts of the different salts but differ as to their concentration. The samples of Barre brine that were used in the subsequent experiments showed a salt concentration of from 122.8 to 123.5 parts per thousand, while those from the Pelto wells varied from 97.0 to 98.5 parts per thousand. The salt content of the water over the oyster beds is considerably lower and was found, during the field survey in May 1933, to vary from 15.41 to 22.77 parts per thousand, with an average of 18.69.

TABLE 5.—*Analyses of brines from oil wells at Lake Barre and Lake Pelto, and sea water from oyster beds and Gulf of Mexico*

Constituents	Barre brine	Pelto brine	Oyster bed, Lake Barre	Oyster bed, Sea Breeze	Gulf of Mexico
pH value	7.0	7.5	8.1	7.9	8.7
Specific gravity 60° F./60° F.[1]	1.109	1.0735	1.016	1.009	1.016
	Parts per 100,000	*Parts per 100,000*	*Parts per 100,000*	*Parts per 100,000*	*Parts per 100,000*
Suspended matter[1]	5.6	3.70	1.0	1.2	1.3
Ash of suspended matter[1]	1.5	.40	None	.4	.7
Iodine adsorption as hydrogen sulphide (H_2S)[1]	.29	5.34	.046	.039	.12
Alkalinity as calcium carbonate ($CaCO_3$)[1]	33.5	31.2	10.8	11.2	9.9
Silica (SiO_2)[2]	14.0	6.4	5.0	2.5	1.5
Iron and aluminum oxides (R_2O_3)[2]	20.9	68.0	8.3	4.9	1.7
Manganese oxide (Mn_3O_4)[2]	1.1	----------	.7	.6	.3
Calcium oxide (CaO)[2]	474.0	395.0	34.0	24.0	38.0
Magnesium oxide (MgO)[2]	225.0	320.0	123.8	86.4	125.3
Alkalies calculated as sodium oxide (Na_2O)[2]	7,190.0	4,460.0	735.5	570.6	873.4
Sulphur trioxide (SO_3)[2]	24.8	27.0	130.0	88.2	131.5
Chlorides as chlorine (Cl)[2]	9,831.5	9,526.0	1,151.3	786.5	1,150.9
Silica (SiO_2)[3]	14.0	6.4	5.0	2.5	1.5
Iron and aluminum oxides (R_2O_3)[3]	20.9	68.0	8.3	4.9	1.7
Manganese oxide (Mn_3O_4)[3]	1.1	----------	.7	.6	.3
Calcium chloride ($CaCl_2$)[3]	938.2	744.6	67.3	47.5	75.2
Calcium sulphate ($CaCO_4$)[3]	----------	44.9	----------	----------	----------
Magnesium chloride ($MgCl_2$)[3]	532.0	756.0	292.4	204.1	296.0
Sodium chloride (NaCl)[3]	13,523.7	9,526.0	1,197.2	947.3	1,455.0
Sodium sulphate (Na_2SO_4)[3]	36.3	----------	230.6	156.4	233.1
Additional chloride from alkali chlorides as chlorine (Cl)[3]	632.4	----------	164.4	29.5	----------
Total of calculated solids	15,698.6	11,145.9	1,965.9	1,392.8	2,062.8
Residue on evaporation (160° C.)	16,370.0	11,124.0	2,120.0	1,419.0	2,050.5
Residue after gentle ignition	15,804.0	10,238.0	1,908.0	1,281.5	1,914.5
Mineral matter as sulphates	19,484.0	12,380.0	2,427.0	1,672.5	2,471.5

[1] On sample as received.
[2] On sample after boiling, filtering, and making to original volume with distilled water.
[3] Probably combined as indicated.

The purpose of the present experiments was to determine the effect of high concentrations of brine on the shell movements and survival of oysters. Nine large adult oysters were used, each of which was cemented to a brick by the lower valve and connected with a graphic recording apparatus as shown in figure 2, in order that an accurate record of their reactions might be obtained. Each oyster was placed in a shallow glass jar and covered with 4,000 cc of either sea water or a brine-sea water mixture, which was renewed daily. The experiments were conducted for a period of over 3 months—from January 12 to April 16, 1934.

In the experimental jars the oysters were subjected to different mixtures of brine and sea water which contained 12.5, 25, 50, and 100 parts per thousand of Barre brine and 25 and 50 parts of Pelto brine. The former mixtures contained, 1.53, 3.07, 6.15, and 12.30 grams of brine salt per liter and the latter 2.45 and 4.90 grams. During the course of the experiments the average salinity of these solutions was respectively as follows: 33.30, 34.44, 36.71, and 41.25 for those containing Barre brine, and 33.81 and 35.46 for those containing Pelto brine. In the control jars the oysters were kept in normal sea water having an average salinity of 32.17. The temperature of the water in the experimental and control jars was the same and ranged from 4.2° to 23.6° C., while these studies were in progress. Temperature fluctuations of as much as 5 to 13 degrees occurred daily as each new supply of cold harbor water became warmed to room temperature.

The results of the experiments are shown in table 6. None of the oysters died while exposed to brine or during a subsequent period of 1 month when they were kept in running sea water. An examination of the data presented in table 6 shows that oysters remain open for longer periods of time in sea water to which brine has been

added and that this reaction can be correlated with the amount of brine present and salt content of the water. The three control specimens were open an average of 7.26 hours daily as compared with daily open periods ranging from 8.5 to 11.8 hours in experimental specimens subjected to brine in amounts varying from 12.5 to 100 parts per thousand.

In many instances the brine was added when the oysters were open and had been feeding for a short period, which caused one or two partial contractions of the adductor muscle but did not produce closure of the shell or appear to have any noticeable effect upon its subsequent movements. With the exception of specimen no. 7 all of the experimental oysters showed normal records as to the number of shell movements or muscular contractions per hour as compared with their respective records prior to the first addition of brine, and those of the control specimens. In the case of specimen no. 7 there were frequent, nearly complete, contractions of the muscle which might be attributed not only to the higher concentration of brine but also to the high salinity of the water which was considerably greater than that found in the natural environment of the oyster. In spite of such abnormal muscular activity this oyster was able to maintain shell closure for periods ranging from 24 to over 96 hours toward the end of the experiment and subsequently recovered completely in running water.

TABLE 6.—*Comparison of length of time oysters remained open in normal sea water and brine-sea water mixture*

Specimen	Medium	Average salinity	Total hours open	Average number of hours per day	Percent of time open
CONTROL					
No. 1	Normal sea water	32.17	648	7.2	30.0
No. 2	do	32.17	806	8.9	37.0
No. 3	do	32.17	513	5.7	23.7
EXPERIMENTAL	BARRE BRINE				
No. 4	12.5 parts per thousand	33.30	927	10.3	42.9
No. 5	25 parts per thousand	34.44	1,026	11.4	47.5
No. 6	50 parts per thousand	36.71	1,053	11.7	48.7
No. 7	100 parts per thousand	41.25	1,062	11.8	49.1
	PELTO BRINE				
No. 8	25 parts per thousand	33.81	765	8.5	35.4
No. 9	50 parts per thousand	35.46	819	9.1	37.9

One purpose of the experiments with brine was to determine its effect upon the holding power of the oyster muscle, since loss of this function was apparently the immediate cause of the oyster mortality in Terrebonne Parish. Consequently all of the specimens were kept in air for a period of 96 hours, at an average temperature of 19° C., after completion of the experiments during which time they were able to keep the shells tightly closed and later showed no serious effects of such treatment when returned to running water. This test was again repeated 10 days later with the same result.

The previous experiments indicate that the brine waters from the Lake Barre and Lake Pelto oil wells do not affect the muscular mechanism of the oysters in relatively high concentrations provided the quantity present does not increase the salinity beyond the limits favorable for the growth of this shellfish. Since these effluents are greatly diluted before reaching the oyster beds, and since no significant

increase in the salt content of the water was found on these beds during previous field investigations, it is probable that the oysters in this region were exposed to much lower concentrations of brine than those used in the experiments. This is also indicated by the fact that the Barre brine, in concentrations of 50 parts per thousand, was found to be toxic to the boring sponge, whereas a prolific growth of this organism was found on a high percentage of the oysters in the vicinity of the oil wells.

EFFECT OF BRINE ON GLYCOGEN CONTENT OF OYSTERS

During the period from February 7 to March 8, 1934, studies were made of the changes in glycogen content of oysters kept in sea water to which brine water from the Barre wells had been added in varying amounts. Two hundred and ten oysters were used, of which a representative sample of 10 oysters was taken to determine their glycogen content at the beginning of the experiment. For this experiment a 4-compartment wooden tank was arranged as follows: Compartment 1, 50 oysters as control in 135 liters of sea water; compartment 2, 50 oysters in a mixture of 130 liters of sea water plus 5 liters of brine; compartment 3, 50 oysters in a mixture of 125 liters of sea water plus 10 liters of brine; and compartment 4, 50 oysters in a mixture of 120 liters of sea water plus 15 liters of brine. The water in all compartments was changed once each week and continuously aerated during the course of the experiment. The salinity of the water in the different compartments was as follows: Compartment 1 (control), salinity 31; compartment 2, salinity 34.5; compartment 3, salinity 38.7; and compartment 4, salinity 42.8.

At the end of each week 10 oysters were taken from each compartment and analyzed for glycogen content by P. S. Galtsoff. The results obtained are given in the following table:

TABLE 7.—*Effect of different concentrations of Barre brine on glycogen content of oysters*

[Experiment G 2]

Date	Percent glycogen, fresh basis					
	Feb. 7	Feb. 14	Feb. 23	Mar. 1	Mar. 8	Average
Control	2.16	3.33	2.74	1.93	4.36	2.90
Brine, 5 liters	(2.16)	2.66	4.77	2.92	3.24	3.15
Brine, 10 liters	(2.16)	3.06	3.38	5.26	2.92	3.35
Brine, 15 liters	(2.16)	1.71	3.15	2.65	2.25	2.38

THE EFFECT OF OIL ON FEEDING OF OYSTERS

By PAUL S. GALTSOFF and R. O. SMITH

EFFECT UPON THE ADDUCTOR MUSCLE

It has been established that oysters have a well-developed chemical sense and are sensitive to a wide variety of chemical substances (Hopkins, 1932). When an irritating chemical solution is brought in contact with the tentacles, situated along the free border of the mantle at the edge of the shell, they retract sharply. The reaction may spread to the mantle, which contracts, and to the adductor muscle, the response of which to stimulation according to Hopkins (1932) "bears a relationship to concentration similar to that of the tentacular reaction, but the reaction time is longer."

Sharp contraction of the adductor muscle caused by a sufficiently strong concentration of an irritating substance produces a twofold effect: The oyster snaps its valves to expell the irritating substance from the inhalent chamber and then keeps the valves tightly closed to protect itself from further irritation or injury. Since feeding can take place only when the muscle is relaxed and the valves are open, the number of hours the oyster remains open determines to a certain extent the duration of feeding. One must bear in mind that the principal organs of feeding of an oyster are the gills, the function of which consists of filtering large quantities of water and in carrying microscopic food particles toward the mouth. (For a detailed description of the function of gills see Galtsoff, 1928.) Obviously the gills can function only when the valves are open.

The presence of a toxic or irritating substance may affect the adductor muscle which will cause the oyster to close, therefore reducing the number of feeding hours and the quantity of food consumed by it, or it may have direct harmful effect on the delicate ciliary mechanism of the gill. In both cases the feeding of the organism is impaired. The effect of oil on ciliary activity of the oyster is discussed later. At present we are interested only in its effect on the muscular activity.

Records were obtained of the behavior of the adductor muscle of oysters subjected to Pelto oil. Immobilized oysters were attached to 24-hour recording instruments in such manner that every shell movement was transmitted and reproduced graphically upon charts which were divided into hours. The number of hours of activity and the number of closed hours were counted for each day.

The running water, supplied to 4-liter dishes containing 2 mounted oysters, flowed through a layer of oil and out from a siphon. Fifty cc of Pelto oil was used to form the surface layer. As the force of the inflow caused globules to be constantly forced down into the water, much of the oil driven into the water gradually adhered to the sides of the dish and to the mounted oyster. Some oil was also lost occasionally through the outflow, and fresh oil, therefore, frequently was added so that a heavy surface film was always maintained. The oysters were so placed in the dishes of running water that the upper valve was less than an inch below the surface of the oil.

TABLE 8.—*Average number of hours oysters remain open*

IN RUNNING SEA WATER UNDER OIL

Oyster no.	Dates	Days	Hours	Temperature range	Oyster no.	Dates	Days	Hours	Temperature range
	1933			°C.		1933			°C.
13y	June 30–July 12	12	8.6	23–26	406	Aug. 18–25	7	10.8	25–28
Do	July 12–23	11	12.1	22–25	Do	Aug. 25–29	4	8.9	28–30
Do	July 23–Aug. 5	13	8.5	27–30	Do	Aug. 29–Sept. 9	7	11.0	26–29
Do	Aug. 5–12	7	12.2	24–27	Do	Sept. 9–15	6	11.6	28–30
13x	June 31–July 12	11	10.8	23–26	Do	Sept. 15–20	5	12.5	25–28
Do	July 12–19	7	9.5	22–25	Do	Sept. 20–Oct. 2	12	8.5	24–27
Do	July 19–Aug. 2	14	16.6	25–28	169	Aug. 8–17	9	8.2	26–29
354	July 10–23	13	7.3	23–26	Do	Aug. 17–24	7	10.5	24–27
Do	July 23–Aug. 4	12	9.9	26–29	Do	Aug. 24–Sept. 5	8	11.0	26–29
Do	Aug. 4–16	12	8.5	24–27	Do	Sept. 5–13	8	10.2	24–27
Do	Aug. 16–27	11	8.6	25–28	Do	Aug. 18–24	6	10.2	24–27
Do	Aug. 27–Sept. 14	14	10.3	26–29	Do	Aug. 24–Sept. 7	11	7.1	26–29
Do	Sept. 14–26	10	11.5	24–27	Do	Sept. 7–14	7	8.3	27–30
160	July 18–24	6	12.4	25–28	Do	Sept. 14–29	13	8.2	24–27
Do	July 24–Aug. 3	10	13.7	27–30					
Do	Aug. 3–17	14	13.1	24–27	7		292	[1] 10.5	22–30
406	Aug. 13–18	5	8.2	26–29					

[1] Average.

TABLE 8—*Average number of hours oysters remain open*—Continued

IN RUNNING SEA WATER

Oyster no.	Dates	Days	Hours	Temperature range	Oyster no.	Dates	Days	Hours	Temperature range
	1931			° C.		1931			° C.
119	July 19–27	8	11.2	28–30	84	Sept. 2–10	5	10.0	22–25
Do	July 27–Aug. 4	8	12.4	27–30	Do	Sept. 10–21	7	9.0	23–26
Do	Aug. 4–12	8	9.0	27–30	Do	Sept. 21–29	6	7.0	22–25
Do	Aug. 12–20	8	9.1	27–30	121	Sept. 2–10	8	12.0	22–25
Do	Aug. 20–26	6	6.2	27–30	Do	Sept. 10–18	8	11.5	23–26
Do	Aug. 26–Sept. 2	7	7.0	28–30	Do	Sept. 19–27	8	10.0	22–25
Do	Sept. 2–9	4	7.0	22–25	311	Sept. 2–10	8	10.0	22–25
Do	Sept. 9–18	8	6.0	22–25	Do	Sept. 10–18	8	6.4	23–26
Do	Sept. 18–26	8	6.0	22–25	Do	Sept. 19–27	8	6.0	22–25
170	May 31–June 5	5	14.0	22–25		1932			
Do	June 5–13	8	10.4	24–27	68	July 25–Aug. 1	8	12.0	27–30
Do	June 13–22	9	11.1	24–27	Do	Aug. 1–9	8	12.4	27–30
Do	June 22–27	5	9.2	26–29	Do	Aug. 9–17	8	12.1	27–30
Do	June 27–July 3	6	9.0	27–30	Do	Aug. 17–25	8	9.0	28–30
Do	July 3–9	6	12.1	28–30	121	June 20–27	7	8.3	25–28
P	Aug. 21–25	4	10.7	24–27	Do	June 27–July 4	7	9.3	27–30
R	do	4	11.9	24–27	Do	July 4–10	6	11.7	28–30
84	July 19–27	8	8.5	28–30	124	June 25–July 2	7	15.0	27–30
Do	July 27–Aug. 4	8	13.0	27–30	Do	July 2–10	8	14.3	28–30
Do	Aug. 4–12	8	14.0	27–30					
Do	Aug. 12–19	7	10.0	27–30					
Do	Aug. 19–26	7	10.0	27–30	10		292	[1] 9.6	22–30
Do	Aug. 26–Sept. 2	7	10.4	28–30					

[1] Average.

The behavior of the oysters left in running water under oil was compared with that of the specimens kept in clear sea water under the same conditions—mounting, attachments to recording apparatus, volume of dishes, rate of flow of water, and temperature.

In view of the finding by Hopkins (1931) that temperature is one of the factors determining the length of time during which oysters remain open, the comparison between normal and experimental oysters was made for the same ranges of temperature. In presenting the results of the experiments (table 8) the average number of hours of activity of oysters during June, July, August, and September of 1931 and 1932 was compared with the activity of oysters left under oil in July and September 1933. By examining table 8 one can detect no significant difference in muscle behavior of the two groups of oysters. The number of hours of activity per day varied between 8.9 and 12.2 in the oysters kept under oil and between 8.8 and 11 in the untreated oysters. No significant differences are apparent in the average of 292 days of untreated oysters compared with the averages of 292 days of oysters kept under oil. The average numbers of hours of activity per day for the whole range of temperature 22°–30° C. were 9.6 hours for the untreated and 10.5 hours for the experimental oysters.

The question may arise that the difference in the muscular activity of oysters kept under oil and under normal laboratory conditions was unnoticeable because of the wide temperature fluctuations during the experiment. That this is not the fact can be seen from table 9 in which the average number of hours the oysters were open are computed for various temperature ranges. On account of daily temperature fluctuations in the laboratory water supply it was necessary to group the results of the observations into six overlapping classes. The results indicate very clearly that under the conditions of the experiments oil had no effect on the number of hours the oysters were open.

TABLE 9.—*Average number of hours of activity of the oysters*

Item	Temperature ranges, ° C.					
	22–25	23–26	24–27	25–28	26–29	27–30
In running sea water, under oil	10.8	8.9	10.3	12.2	10.5	10.2
In running sea water	8.8	9.0	11.0	8.3	9.2	10.2

In another set of experiments, simultaneous observations were taken on oysters kept under oil and in the control tanks. In these experiments water was supplied at the uniform rate of flow of 6 liters per hour to each of the oysters kept in glass aquaria tanks. Experimental tanks contained enough oil to make a layer 1 centimeter thick. The amount of oil varied from 300 to 350 cc. Water was not permitted to pass through the oil layer, both the intake and out-take tube of the siphon being kept under it. The results presented in table 10 are similar to other experiments. There was no significant difference between the behavior of the oysters under oil and in the controls, the average number of hours of activity being 11.2 and 11.8 respectively.

TABLE 10.—*Average number of hours of activity of oysters under oil and in controls*

Experiment number	Date	Number of days	Temperature range (° C.)	Average number hours per day open		Kind of oil
				Experimental	Control	
1	Oct. 11–30	20	16–22	5.75	7.25	Pelto.
2	Oct. 19–24	6	18–22	11.9	9.5	Do.
3	Oct. 26–31	6	16–20	12.2	16.0	Do.
4	Nov. 1–7	7	18–21	11.6	12.8	Do.
5	Nov. 11–16	6	11–18	16.6	10.6	Do.
6	Nov. 19–25	7	11–17	9.6	10.6	Barre.
7	Dec. 7–16	10	10–15.5	13.8	17.2	Do.
8	Dec. 5–16	12	10–17	8.5	10.5	Barre sludge.
Average		[1] 74		11.24	11.83	

[1] Total days.

The results of these observations show that presence of oil in the water failed to interfere with the muscular activity of the oyster and did not reduce the duration of their feeding.

EFFECT OF OIL AND OIL WELL BLEED WATER ON THE RATE OF FEEDING OF OYSTERS

The purpose of the experiments here described was to determine the effect of various concentrations of the water soluble fraction from crude oil and oil well bleed water on the rate of feeding of oysters. The importance of this matter from a practical standpoint can scarcely be overestimated.

Growth and fattening of oysters depend on three major conditions: 1. Abundance of food organisms in the water; 2, percentage of time the shell of the oyster is open; and 3, rate of flow of food-bearing water through the gills. It is obvious that no matter how abundant food may be in the water, or how long the shell is open, very little food will be available to the oyster unless a current is maintained through the gills. Consequently, any substance which slows down the filtering activity of the gills acts to reduce the quantity of nourishment available to the oyster. In an extreme condition, a substance which reduces the rate of flow might eventually cause the death of oysters without ever being directly toxic.

All experiments reported below were made during November and December 1933 and from June through September 1934. It was thus possible to observe the reaction of oysters to the experimental fluids over a temperature range of from 10 to 30 degrees centigrade and water salinity varying from 27.5 to 36.6 parts per million.

The crude oil, collected by the Louisiana Department of Conservation from the Lake Barre and Lake Pelto wells of the Texas Co. in Terrebonne Parish, was shipped to the laboratory and kept in glass containers. The feeding activity of the oyster was determined by measuring the rate, and quantity of water pumped through the gills by the ciliated epithelium.

CARMINE CONE METHOD

The carmine-cone and the drop-counting methods developed by P. S. Galtsoff were used. The data obtained by these methods may be expressed as volume of water pumped or work performed. The carmine cone method, while not so accurate as the drop counting, has the advantage of being simpler and is better suited for experiments extending over several days. It has been fully described by Galtsoff (1928). Only a brief résumé follows.

The oyster is prepared by carefully forcing the valves apart and inserting a short piece of glass rod to hold them open. A 4-inch piece of gum rubber tubing of ⅛ inch inside diameter is pushed a short distance into the outlet of the gill chamber. Cotton is then used to close the opening between the valves so that no water can escape from the exhalent chamber except through the tube.

The oyster is placed in an enameled rectangular tray approximately 14 inches by 20 inches by 2½ inches, having a capacity of 7 l. A constant level arrangement maintains the level at 5 l (5.25 quarts). An inverted T-tube is supported so that one of the arms may be connected to the prepared oyster and the other with a 20 cm piece of glass tubing of 6 mm bore, marked off with a 10-cm interval. The shank of the inverted T is connected to a glass funnel by a short length of rubber tubing. The funnel contains a suspension of carmine in sea water.

In operation, laboratory supply sea water flows through the tray at a rate of about 200 cc per minute. This flow is stopped at the beginning of an experiment.

Circulation and aeration are accomplished by a stream of air bubbling through an inclined piece of glass tubing about 18 inches in length and ½ inch in inside diameter.

After a preliminary period of half an hour to allow the oyster to adjust itself to the new conditions, it is considered ready for the experiment. A small amount of freshly made carmine suspension is admitted from the funnel and forms at the axis of the tube a cone which is carried out by the current from the oyster. By means of a stop watch, a measurement is made of the time, in seconds and tenths, required for the tip of the cone to traverse the graduated 10-cm. distance. As the mean velocity of the whole cross-sectioned area of the tube is one-half of the velocity at the axis (Galtsoff, 1928), the rate of discharge of water can be easily computed. No such computation was regarded necessary for the purpose of the present experiments, the results of which are presented as velocities of current in millimeters per second.

Each time 10 or more readings were made and the average taken as representative of the velocity of current of water at the axis of the tube. Observations are made

at 30- to 60-minute intervals, and are continued for from 2 to 4 hours, the average rate of flow for this period being considered as the normal rate of pumping. The flow of sea water is then cut off, if it has not already been stopped, and a predetermined amount of sea water is siphoned off and immediately replaced by the same amount of oil extract, prepared in the manner described below. The solution is allowed to remain in the tray from 1 to 24 hours, during which time measurements of the rate of flow are made, first at 15-minute intervals, later at 30- and 60-minute intervals. Measurements on controls run for the same period of time. The total elapsed time for these experiments, from preparation of the oyster to removal from the tray, averages 26 hours.

Treatment with soluble fraction is followed by fresh sea water, measurements being made hourly to indicate the rate and extent of recovery. The plugs, tubing, and glass rod are then removed; and the oyster, suitably marked for future identification, is returned to the large laboratory aquarium.

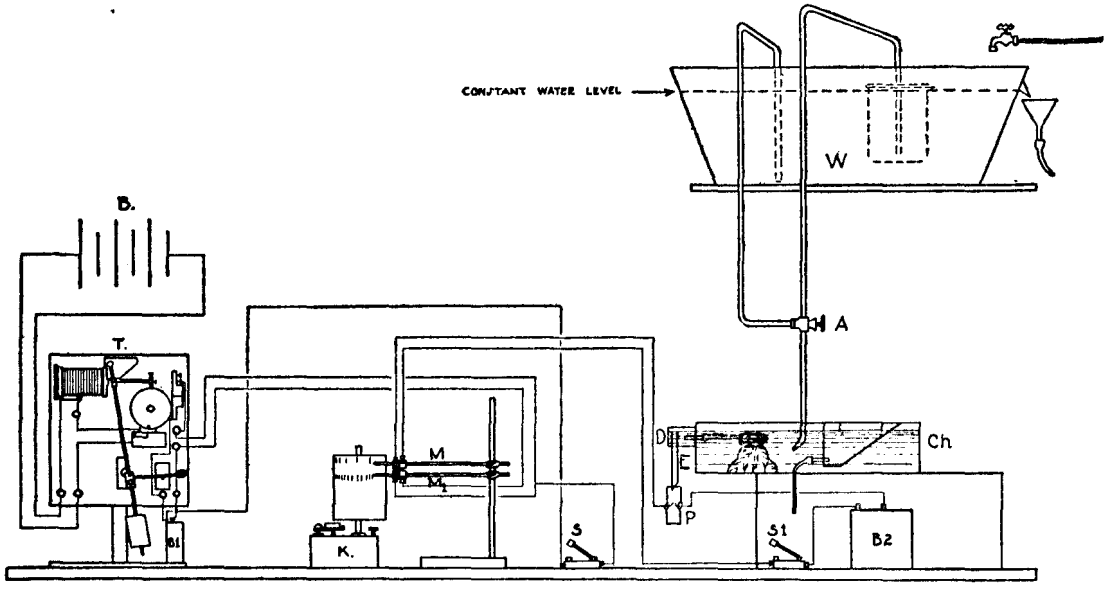

FIGURE 5.—Drop counting set-up for the determination of the rate of flow of water through the oyster gills. W, water supply tank; A, 3-way stop cock; Ch, experimental chamber (made of celluloid) provided with constant level arrangement; D, small cell connected to the chamber; E, overflow; P, drop counter; S, key switch connecting the signal magnet (M_1); S_1, key switch connecting the circuit in the drop counter and 45 volt "B" battery (B_2); K, kymograph; T, electric clock; B, storage battery; B_1, 2-volt dry cells for operation of the signal magnet (M_1); and M, signal magnet connected with a drop counter P.

DROP-COUNTING APPARATUS

The apparatus is illustrated in figure 5. There are three main parts:

1. Supply (*w*), experimental chamber (*Ch*) and recording apparatus (*k*). A three-way stopcock (*A*) supplies either laboratory sea water or a test solution. A constant level device maintains equal pressure for both liquids, delivering 200 cc per minute to the experimental chamber. Temperature fluctuations are kept within 1° C. of the laboratory sea water.

2. Experimental chamber (*Ch*) consists of a celluloid box of 1,500 cc capacity with a small cell (*D*) of the same material attached to its wall and connected through the side by a short piece of glass tubing. An overflow tube (*E*) in the center of the small cell drains through the bottom. The height of this tube must be so adjusted that when the two chambers are connected the water in both of them remains in

equilibrium. A constant level is maintained in the large chamber (*Ch*) by means of an overflow which is so adjusted that the water content of the chamber after the oyster is placed in it will be approximately 1 l. Before beginning an experiment the stopcock (*A*) supplying sea water is turned on and the levels are carefully checked. After the equilibrium has been established a single drop added to the small chamber immediately overflows through the tube (*E*). The oyster, prepared in the same manner as for the cone method, is then placed in the large chamber; and the rubber tubing introduced in the exhalent chamber of its gills is connected to the small horizontal glass tube. Water pumped by the gills will immediately overflow through tube (*E*). An electric stirrer and thermometer are kept in the large chamber.

3. Recording apparatus. Two platinum contact wires sealed in the glass tubing (*P*) are placed under the overflow tube (*E*) so that each drop makes a contact which activates a small signal magnet registering on the smoked revolving drum of a spring kymograph (*K*). Current for operating the magnet is supplied by a 45-volt radio B battery. An electric clock (*T*) operates another signal magnet which records on the drum time intervals of 1 second. Into this circuit is connected a key switch (*S*) and a 2-volt dry cell to mark the time of changing from laboratory supply to test solution and vice versa. Another switch (S_1) disconnects the circuit in the drop counter when the kymograph is not in operation. The kymograph was set to make 1 revolution in 5 minutes.

Since the pumping activity of the oyster transfers liquid (sea water or test solution as the case may be) from the large chamber to the smaller, it is necessary to have a continuous flow into the large chamber to maintain the level, otherwise additional work will have to be done by the gill cilia in raising water from the large chamber into the smaller. The change from sea water to test solution therefore is made instantaneously by a twist of the three-way stopcock (*A*). As soon as the shift is made the oyster is subjected to a gradually increasing proportion of test solution. This solution was allowed to flow into the experimental chamber for 5, 10, or 15 minutes as noted in the tables under the heading "Duration of test solution."

At the rate of 200 cc per minute, from 12 to 15 minutes were required to change the liquid completely in the experimental chamber, so that the oyster was rarely if ever subjected to the full concentration of test solution shown in the tables under the heading "Percent soluble fraction or bleed water." The percentage given in this column represents the concentration of the test solution, not the percentage to which the oyster was subjected. In experiment 55, table 17, for example, it should be understood that the figures do not show the effect of 10-percent bleed water remaining on the oyster for 10 minutes. It does show the effect of gradually replacing sea water with a 10-percent brine solution, the maximum concentration reached being unknown, but somewhat less than 10 percent. The same remarks apply to drop-counting experiments in which water soluble fraction of crude oil was used instead of bleed water.

In the experiments using the cone method the specific gravity of the test solution was brought, as nearly as possible, within ± 0.0002 of the laboratory supply sea water at the time of the experiment. This was not practicable when using the drop-counting apparatus, but in all cases the difference in specific gravity between the laboratory sea water and the test solution was kept as low as possible. Specific gravity determinations were made by Knudsen hydrometers certified by the National Bureau of Standards. Hydrogen-ion concentration was checked with a Hellige-Klett color disk.

No change in pH of sea water was found after stirring with oil, the average both before and after stirring being 7.6.

As in the case of crude oil, bleed water from the Barre and Pelto wells of the Texas Co. was furnished through the courtesy of the Louisiana Department of Conservation.

When received, the bleed water had a disagreeable oily odor, was slightly brown in color, had a pH of 7.1–7.6, and a specific gravity of 1.1064 (17.5°C). A small amount of brown flocculent precipitate present in the bottom of the bottle was left undisturbed when taking a sample of brine for the experiments.

The brine was mixed with distilled water, laboratory sea water, or both just before the beginning of an experiment. The percentage of dilutants was varied in order to keep the specific gravity of the test solution as near that of the laboratory sea water as possible and avoid confusing the results through change in density.

PREPARATION OF WATER SOLUBLE FRACTION OF OIL

The soluble fraction solution was made by placing 6 liters of crude oil and 3 liters of laboratory supply sea water in a glass jar and stirring violently for 30 minutes. The mixture was allowed to stand overnight so that the two fluids would separate as completely as possible. However, only 2,500 to 2,900 cc of soluble fraction solution were recovered from the 3 liters of sea water added. This preparation was regarded as a stock solution from which all other dilutions were made. In the following discussion and in the tables the undiluted solution is called 100 percent soluble fraction. The authors were not in a position to make a chemical analysis of the extracts they used, but due care was exercised in using exactly the same method of preparation. There is a possibility that because of the variation in temperature and salinity of water, the actual amount of substances extracted from the crude oil varied in different samples.

RESULTS OBTAINED WITH THE CONE METHOD

Experiments using the cone method were begun in the early part of June 1934, and completed in the middle of September of that year. The percentages of soluble fraction used were 1, 5, 10, 20, 40, 50, 80, and 100. Since the Pelto wells were no longer producing when this work began, Barre crude oil was used. During the course of investigations, 62 experiments were performed with the cone method. The results of these experiments are summarized and presented in table 11, which contains the essential information regarding the conditions under which the experiments were carried out. The hydrogen-ion concentration of the oil extract was slightly higher than that of the natural sea water. At Beaufort the pH of the water was 7.8. After dilution with distilled water to adjust the salinity, the pH of the sample was as low as 7.6. In Beaufort experiments the observed differences between the sea water and soluble fraction did not exceed 0.3 pH. In a test made at Woods Hole the pH value of the oil extract was 7.4 as compared with the pH 8.3 of the natural sea water. This extract was obtained by stirring oil and water for 24 hours and permitting them to separate in 3½ days. Sea water, the pH of which was reduced by the addition of 0.1 N HCl to 7.4, decreased the ciliary motion by 13.7 percent. The oil extract of the same pH completely inhibited the ciliary mechanism.

TABLE 11.—*The effect of water-soluble fraction of Lake Barre oil on the rate of pumping of water by the gills of the oyster*

[Cone method, Beaufort, 1934]

Experiment no.	Date	Average velocity, current		Effect of treatment (percent)	Duration of test in hours	Specific gravity, 17.5° C.		Increase in specific gravity due to washing	Number of times oil washed	Temperature (° C.)	
		Before	During			Laboratory sea water	Soluble fraction			Beginning	End
CONTROL											
92	June 5	27	38	140.0	59	1.0211				25.0	25.4
98	June 13	29	36	124.1	24	1.0243				25.4	23.7
115	June 27	44	43	97.7	25	1.0257				27.9	28.8
121	July 9	34	30	88.2	43	1.0272				27.6	25.8
139	July 25	51	54	105.8	25	1.0272				27.2	26.0
151	July 31	44	62	140.9	24	1.0270				27.2	26.4
161	Aug. 9	77	75	97.4	24	1.0274				27.4	25.6
179	Aug. 29	44	45	102.3	48	1.0270				25.7	23.9
Average		43.7	47.8	112.0							
1 PERCENT SOLUBLE FRACTION											
135	July 19	47	58	123.4	22	1.0279	1.0275	0.0002	2	27.7	28.9
146	July 27	36	42	116.6	24	1.0274	1.0281	.0009	5	26.9	27.3
154	Aug. 2	54	46	85.2	24	1.0273	1.0274	.0000	21	27.6	27.0
158	Aug. 7	54	48	88.8	24	1.0273	1.0275	.0003	17	26.1	26.7
164	Aug. 13	41	28	68.3	24	1.0277	1.0277	.0004	25	27.7	28.0
168	Aug. 15	58	57	98.3	18	1.0280	1.0281	.0002	19	28.4	28.1
174	Aug. 18	83	81	97.6	18	1.0275	1.0277	.0004	20	28.7	27.7
Average		53.3	51.4	96.9							
5 PERCENT SOLUBLE FRACTION											
106	June 21	67	71	105.9	24	1.0259	1.0259	.0002	4	27.4	27.5
107	do	41	33	80.5	24	1.0259	1.0259	.0002	4	27.2	27.6
138	July 23	46	49	106.5	24	1.0273	1.0275	.0003	1	26.8	27.4
144	July 26	36	41	113.9	18	1.0272	1.0279	.0003	4	27.2	28.0
150	July 31	46	34	73.9	18	1.0270	1.0274	.0006	16	26.8	26.1
157	Aug. 8	61	49	80.3	18	1.0273	1.0275	.0003	17	26.8	26.5
182	Sept. 5	38	45	118.4	18	1.0231	1.0232	.0003	29	25.5	25.3
Average		47.8	46.0	97.0							
10 PERCENT SOLUBLE FRACTION											
104	June 20	59	35	59.3	18	1.0258	1.0259	.0001		27.5	27.2
105	do	28	16	57.1	18	1.0258	1.0259	.0001		26.9	26.8
112	June 26	45	44	97.7	14	1.0259	1.0259			27.9	27.0
113	do	62	42	67.7	12	1.0259	1.0259			27.9	27.6
116	June 27	47	44	93.6	18	1.0257	1.0259		2	28.0	28.3
117	June 28	40	31	77.5	24	1.0249	1.0250			28.2	28.5
170	Aug. 15	59	50	84.7	24	1.0280	1.0281	.0004	19	27.9	27.4
176	Aug. 30	39	28	71.8	24	1.0269	1.0267	.0007	28	23.0	22.0
Average		47.4	36.2	76.2							
20 PERCENT SOLUBLE FRACTION											
99	June 14	47	39	83.0	20	1.0239	1.0251	.0004	1	25.1	26.4
122	July 9	64	42	65.6	18	1.0274	1.0270	.0004	15	27.6	26.7
123	do	52	10	19.2	18	1.0274	1.0270	.0004	15	27.7	27.1
137	July 23	43	13	30.2	24	1.0273	1.0275	.0003	1	27.5	25.8
163	Aug. 13	36	24	66.6	24	1.0277	1.0277	.0002	25	28.2	27.5
175	Aug. 17	40	13	22.5	18	1.0275	1.0277	.0004	20	28.6	27.4
178	Aug. 29	41	9	21.9	18	1.0270	1.0267	.0002	22	25.3	20.4
Average		46.1	21.4	45.6							
40 PERCENT SOLUBLE FRACTION											
125	July 12	74	22	29.7	24	1.0276	1.0274		16	26.4	27.9
126	do	46	21	45.7	18	1.0276	1.0274		16	26.5	27.7
129	July 16	37	13	35.1	23	1.0275	1.0276	.0003	17	28.4	29.6
142	July 25	51	26	51.0	12	1.0272	1.0281	.0005	4	26.7	26.3
153	Aug. 2	74	41	55.4	24	1.0273	1.0274	.0006	16	27.7	27.6
166	Aug. 13	52	11	21.1	18	1.0277	1.0276	.0003	10	27.7	27.9
Average		55.7	22.3	39.7							
50 PERCENT SOLUBLE FRACTION											
128	July 16	58	33	56.8	24	1.0275	1.0276	.0003	17	28.5	29.1
152	Aug. 1	58	22	37.9	24	1.0277	1.0275	.0006	21	27.8	26.3
160	Aug. 7	112	19	16.9	24	1.0273	1.0275	.0002	17	27.3	26.9
173	Aug. 17	74	15	20.2	18	1.0275	1.0277	.0004	20	28.1	26.9
183	Sept. 4	34	12	35.3	24	1.0230	1.0232	.0003	29	24.9	26.1
Average		67.2	20.2	33.4							

TABLE 11.—*The effect of water-soluble fraction of Lake Barre oil on the rate of pumping of water by the gills of the oyster*—Continued

[Cone method, Beaufort, 1934]

Experiment no.	Date	Average velocity, current		Effect of treatment (percent)	Duration of test in hours	Specific gravity, 17.5° C.		Increase in specific gravity due to washing	Number of times oil washed	Temperature (° C.)	
		Before	During			Laboratory sea water	Soluble fraction			Beginning	End
80 PERCENT SOLUBLE FRACTION											
143	July 26	63	0	0	18	1.0272	1.0271	0.0004	13	27.4	28.2
165	Aug. 13	66	27	40.9	24	1.0277	1.0276	.0003	10	27.4	27.0
171	Aug. 15	85	1	1.2	24	1.0280	1.0281	.0002	19	28.2	28.2
172	Aug. 16	53	2	3.8	18	1.0277	1.0279	.0001	26	28.7	28.2
177	Aug. 29	62	0	0	18	1.0270	1.0267	.0002	22	25.3	21.6
185	Sept. 6	115	11	9.5	24	1.0238	1.0241	.0003	24	25.1	22.8
Average		74.0	6.8	9.2							
100 PERCENT SOLUBLE FRACTION											
131	July 18	76	0	0	1	1.0277	1.0274	.0001	18	27.9	27.8
136	July 23	61	0	0	1	1.0273	1.0275	.0003	12	27.8	28.2
140	July 25	59	0	0	2	1.0272	1.0272	.0000	20	27.1	27.2
147	July 30	64	0	0	4	1.0275	1.0274	.0006	6	27.4	27.1
156	Aug. 3	75	0	0	4	1.0275	1.0275	.0000	7	27.7	27.0
162	Aug. 8	51	28	54.9	2	1.0274	1.0276	.0002	23	27.1	25.9
167	Aug. 14	54	11	20.4	6	1.0276	1.0278		18	28.6	28.0
181	Sept. 4	95	23	24.2	24	1.0230	1.0233	.0002	23	24.5	25.2
Average		66.9	7.8	12.4							

One of the difficulties encountered in these experiments lies in the seasonal fluctuations in the laboratory sea water. In preparing soluble fraction there was a slight increase in the specific gravity of water owing to washing with oil. However, the solution used in various experiments was not always heavier than the laboratory sea water used in the same experiment. This was caused by daily fluctuations in the salinity and the necessity of preparing soluble fraction solution in advance of the experiment. The specific gravity of the laboratory sea water used during each experiment and the specific gravity of soluble fraction solution are given in separate columns. They correspond to the measurements of velocity of current before and after treatment. In making up various concentrations of soluble fraction, efforts were made in each experiment to adjust the specific gravity of the solution, by diluting it with sea water of high or low salinity, as close as possible to that of the laboratory sea water. However, this was not always feasible. A complete record of several experiments with 20-, 50-, and 100-percent soluble fraction are given in figures 6 and 7. The vertical lines in these figures indicate time when the laboratory sea water was replaced with soluble fraction of oil.

The figures of the velocity of the current produced by the gills are the averages of 10 or more readings in millimeters per second. They all represent the velocity at the axis of the tube. In the case of the controls, there is no acutal distinction between the periods marked "Before treatment" and "During treatment." Separation has been made aribitrarily on a time basis. To provide a basis for comparison, at least 3 readings were made during a period of 2 or more hours before treatment. The change in efficiency of the ciliary motion, designated in table 11 as "Effect of treatment", is expressed by the ratio of the average velocity of the current during treatment divided by the average velocity before treatment and multiplied by 100.

The effect of changes in specific gravity on the activity of gill cilia has not been studied. However, there was relatively little change in the salinity of the laboratory

sea water from day to day, and this factor is not believed to have affected the results. In most experiments the specific gravity of the test solution was brought within ±0.0002 of the specific gravity of the laboratory sea water so as to avoid stimulation by salinity changes during the course of an experiment. Complete data of the specific gravity of the laboratory water and of the solution of soluble fraction used in the experiments are given in the table.

Fluctuations in temperature may be disregarded insofar as they might influence the results obtained with different concentrations of soluble fraction. The maximum difference in average temperature between the several groups of experiments is 0.9° C. This comparatively slight variation was made possible by using 4 sets of apparatus at a time so that 4 experiments could be run simultaneously. By arranging to have a different concentration of soluble fraction in each tray, the experiments at various concentration levels were carried along together so that seasonal changes in temperature did not affect one group of experiments more than another. Temperature fluctuations during the course of individual experiments were usually less than 2° C.

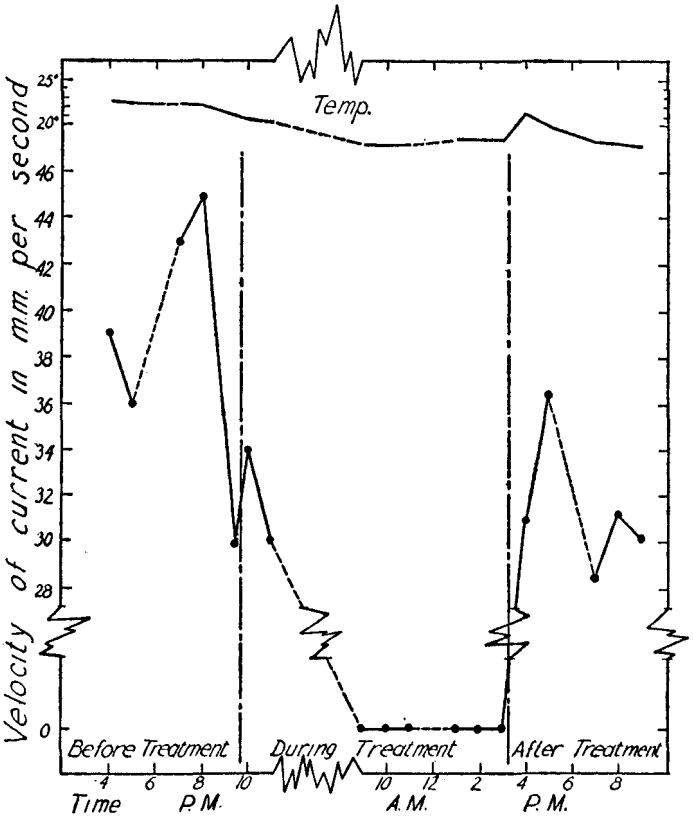

FIGURE 6.—Effect of water soluble fraction on rate of flow of water. Exp. 178.

In 5 of the controls (table 11) the rate of flow increased slowly during the course of the experiments. Two others showed a very slight decrease in rate, and in 1 experiment (no. 121) in which there was no aeration or circulation of water the decrease amounted to 11.8 percent. The characteristic gradual increase in rate of pumping is attributed to recovery from the mechanical stimulation of the plugging operation. The controls cover the period from June 5 to August 29, and except for the first experiment, no. 92, all were subjected to the same range of salinities and temperatures as the experimental oysters. On the average for all the 8 controls there was an increase in the velocity of current which amounted to 12.0 percent. The average duration of test for controls was 34 hours; for experimental oysters, 20.5 hours. The total elapsed time during which each oyster was plugged averaged approximately 6 hours more than the duration test.

In experiments extended from July 19 to August 18, 7 oysters were subjected to a 1-percent soluble fraction solution for from 18 to 24 hours. During this time the specific gravity of the laboratory sea water was very high, ranging from 1.0273 to 1.0280 (17.5°). Water temperature averaged 27.6° C.

The average rate of pumping during treatment, as compared with the rate before treatment, showed a reduction of 3.1 percent (table 11). This is insignificant by itself insofar as indicating any effect of 1-percent soluble fraction solution is concerned. However, this reduction in rate of flow is 15.6 percent lower than the control average during the treatment period.

The greatest decrease in rate of flow resulting from this treatment was found in experiment 164, in which the reduction in rate amounted to 31.7 percent. This maximum effect was obtained with soluble fraction from a sample of oil washed 25 times, the increase in specific gravity caused by washing being 0.0004. If the

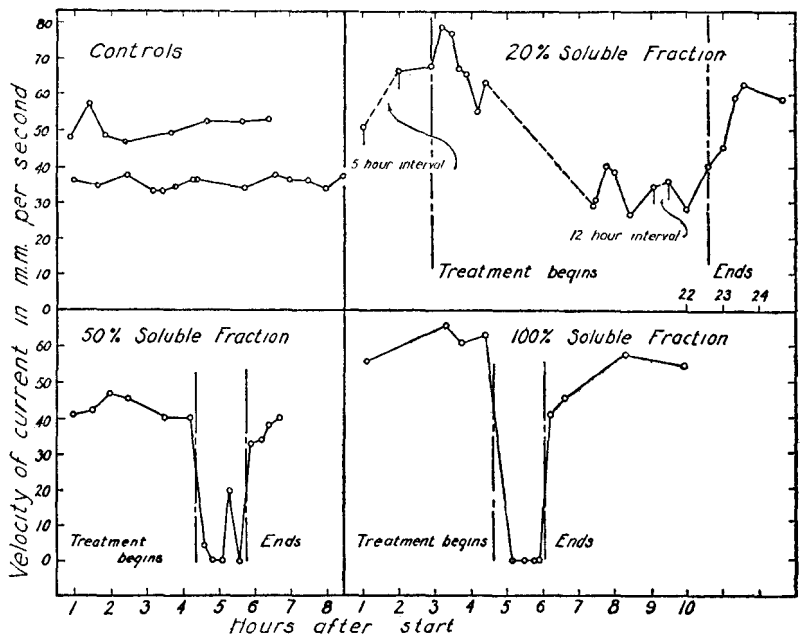

FIGURE 7.—Effect of water soluble fraction on rate of flow of water.

effect of the soluble fraction was owing to mineral salts taken up from the oil by the sea water, it would be expected that the inhibiting power of the soluble fraction on rate of flow would gradually diminish as the salts were exhausted, and that the increase in specific gravity of the sea water after washing with oil would likewise decline. However, this was not the case and the inhibiting effect of the oil extract did not diminish with subsequent washing of the sample.

Except for experiment 164, the effect of treatment with 1 percent soluble fraction gives percentages closely comparable with similar figures for the control experiments. However, considering the orderly decrease in rate of flow caused by treatment with increasing percentages of soluble fraction, it is believed that a 1 percent soluble fraction exerts a definite though slight inhibiting effect on the ciliated epithelium of the gills.

Seven experiments were made with 5 percent soluble fraction solution between June 21 and September 5. The average temperature was 26.8° C., and the specific

gravity ranged from 1.0231 to 1.0273 (17.5° C.). The average rate of flow during treatment is 97.0 percent of the rate before treatment. It would appear that 5 percent soluble fraction is no more toxic to oysters than 1 percent, over a period of 24 hours. However, on closer examination we find an interesting situation in this group. Of the seven experiments, four show an average increase of 11.2 percent during treatment, comparable to 12.0 percent shown by controls. The remaining three experiments have an average rate of 78.2 percent during treatment, comparable to the rate obtained with 10 percent solutions.

In considering factors which might be responsible for the effect, or lack of it, a comparison was made between the conditions of the two groups of experiments.

The average temperature of the four experiments in which an increased rate of flow resulted from treatment is 26.7°. The average for the three experiments whose flow was reduced during treatment is 26.9°. This difference of 0.2° between the averages for the two groups is less than the fluctuations generally found during the course of an experiment. It is obvious that temperature differences could have no connection with the disparity in effect of the soluble fraction solution.

Also, the effect of the soluble fraction has no apparent relationship with the specific gravity of the laboratory sea water. In experiment 182, the low specific gravity of 1.0231 was accompanied by an increase of 18 percent in rate of flow during treatment. In experiment 144, the high specific gravity of 1.0272 was associated with an increase in rate of flow of 13.9 percent during treatment. Consequently, there is no evidence that an increase or a decrease in rate of flow during treatment has any connection with a high or low specific gravity of the sea water, though this statement is intended to apply only to the limits of salinity occurring in these experiments.

The difference in specific gravity between the laboratory sea water and the soluble fraction was, in most experiments, not over 0.0002. Where the difference exceeded this figure, notably in experiment 144, in which the difference was 0.0007, the effect is negligible compared with fluctuations in experiments where differences in specific gravity were not present. For example, experiments 106 and 107 were carried on simultaneously. They received soluble fraction prepared from the same sample of oil and sea water, were treated for the same length of time and the soluble fraction was adjusted to the same specific gravity as the laboratory sea water. Yet oyster 106 increased the rate of flow during treatment by 5.9 percent, while oyster 107 reduced its flow 19.5 percent.

Since there is no intergrading effect between the two groups of oysters used in these experiments, the inference can be drawn that 5 percent solution exerted a definite effect on one group of them which comprised specimens more sensitive than the others. In other words, this concentration may be regarded as a threshold of inhibitory action.

These results are based on treatments extending only for 18 or 24 hours. It is possible that the same depression of the efficiency of ciliary motion may be reached with smaller concentrations acting over a longer period of time.

Ten percent soluble fraction solution very definitely inhibited ciliary activity and resulted in an average decrease in rate of pumping during treatment of 23.8 percent. (Table 11.)

There is no relationship between the increase in the specific gravity of the undiluted oil extract and the effect on rate of flow during treatment. In experiment

104, the increase in specific gravity caused by washing was 0.0001, and the rate of flow during treatment was only 59.3 percent of the normal rate before treatment, while in experiment 176, the increase in specific gravity was 0.0007, and the rate of flow during treatment was 71.8 percent of the normal.

The difference in specific gravity between the laboratory sea water and the test solution was held within close limits, not exceeding 0.0002 in any experiment in this group. The specific gravity of the sea water was high in all experiments, ranging from 1.0249 to 1.0280. There is no apparent relationship between a high specific gravity and effect of the soluble fraction, for the greatest reduction in rate of flow, 42.9 percent, occurred in experiment 105, in which the specific gravity was considerably lower than in experiment 170, specific gravity 1.0280, the reduction in flow during treatment for the latter experiment amounting to only 15.8 percent.

Fluctuations in temperature may be disregarded as factors influencing the rate of flow during the experiments, the maximum difference in temperature at the beginning and end of an experiment in the 10 percent group not exceeding 1.0° C. The time of treatment in this group varied from 12 to 24 hours. Within these limits the duration of treatment does not appear to be a factor in determining the effect. Three experiments, 104, 105, and 116, were carried on for 18 hours. The first two showed the greatest, the last one next to the least effect for the group.

Seven experiments were made with 20 percent soluble fraction solution. These were well distributed over the period from June 14 to August 29. The average temperature of the water in these experiments was 27.1° C. Specific gravities ranged from 1.0239 to 1.0277. The average reduction in rate of flow resulting from this treatment amounted to 54.4 percent.

In the experiments with 20 percent solution (figs. 6 and 7) the difference in specific gravity between the laboratory sea water and the test solution was not held to as low limits in all cases as was done in the experiments with 10 percent solution. The maximum difference in specific gravity occurred in experiment 99, where the test solution was higher by 0.0012. This increase in specific gravity of the soluble fraction solution apparently was not of serious proportions in this case, for the reduction in rate of flow during treatment was only 17 percent, the lowest for the group. Consideration was given to the possibility that the comparatively slight effect of soluble fraction in experiment 99 might be due, in part, to the relatively low salinity of the laboratory sea water, 1.0239, for the other experiments in this group were made when the specific gravity was at or above 1.0270. However, an examination of the control and other experiments does not support this contention. While most of our experiments were made with sea water of higher salinity than is found over many oyster beds, nevertheless it may be stated that within the range of specific gravities used the effect of the soluble fraction is independent of the salt content of the sea water.

Increase in specific gravity of the sea water after washing with oil has no bearing on the effect of the 20 percent soluble fraction. In experiments 122 and 123 (table 11), two oysters were treated with portions of the same soluble fraction for 18 hours. The increase in specific gravity following washing with oil was 0.0004. The rate of flow of oyster 122 was reduced 34.4 percent during treatment. The reduction in rate for oyster 123 was 80.8 percent. Oyster 99 was treated for 20 hours with a different sample of soluble fraction having the same increase in specific gravity after washing and the reduction in rate of flow was 17 percent, comparable to the effect in experiment 122. Oyster 163, treated for 24 hours with soluble fraction having an increase in

specific gravity of 0.0002, half that of experiment 122, showed a decrease in rate of flow of 33.4 percent, almost exactly the same as no. 122. Finally, oyster no. 178 (fig. 6), treated for 18 hours with soluble fraction having an increase in specific gravity of less than 0.0002, had the rate of flow reduced 78.1 percent, a figure of the same order of magnitude obtained in experiment 123.

Fluctuations in temperature are not significant insofar as their effect on the rate of flow is concerned. Experiments 122 and 123, as mentioned above, were carried on simultaneously. The temperatures differ little in the two experiments, but the effect of the soluble fraction is not at all comparable in amount. It might appear that the temperature drop of nearly 5° C. in experiment 178 is partially responsible for the relatively large decrease in rate of flow. Actually, this is not the case as can be seen by examining figure 6 which presents a complete record of the experiment.

Six experiments were made with 40 percent soluble fraction, from July 12 to August 13. The average temperature of the water in these experiments was 27.2° C. Specific gravities of the laboratory sea water were very high, with little fluctuation, the range being from 1.0272 to 1.0277 (17.5° C.).

Except in experiment 142 (table 11), in which the decrease in rate of flow during treatment was almost the smallest in the group, the difference in specific gravity between the sea water and the soluble fraction was kept within 0.0002. The greatest increase in specific gravity of the sea water due to washing (0.0006) occurred in experiment 153, which was least affected by treatment. The maximum reduction in rate of flow following treatment was found in experiment 166, and the soluble fraction used here was a mixture from two oils which had been washed 10 and 24 times respectively.

Five experiments were made with 50 percent soluble fraction solution from July 16 to September 4. The average temperature of the laboratory sea water during this period was 27.3° C. The specific gravity of the sea water varied but little in July and August, 1.0273 to 1.0277; but in the last experiment on September 4 it had fallen to 1.0230.

The average rate of flow during treatment dropped 66.6 percent. There is relatively little difference in the effect of 40 and 50 percent soluble fraction. The experiments in the two groups have been separated chiefly to emphasize the close agreement between them.

The main interest of the experiments with 50 percent soluble fraction is the lack of any definite relationship between the effect of the soluble fraction and specific gravity (table 11, fig. 7). The reduction in rate of flow was practically the same in experiment 152, with the high specific gravity of 1.0277, and in experiment 183, with the low specific gravity of 1.0230.

Six experiments were made with 80 percent soluble fraction solution from July 26 to September 6. The average temperature of the laboratory sea water during this time was 27.0° C. Five of the experiments were carried on during July and August, when the specific gravity of the laboratory sea water ranged from 1.0270 to 1.0280. At the time of the last experiment on September 6, the specific gravity had fallen to 1.0238. The maximum difference between the specific gravity of the laboratory sea water and the soluble fraction was 0.0003.

The effect of 80 percent soluble fraction was to reduce the activity of the ciliated epithelium 90.8 percent. The drop in pumping activity is 24.2 percent greater than was found with 50 percent soluble fraction.

In experiments 143 and 177 of this group we find, for the first time, the pumping immediately stopped on addition of the soluble fraction, and not beginning again until the test solution was replaced with fresh sea water. As in previous experiments, an extreme effect is probably attributable to poor condition of the oysters, for the difference between the specific gravities of the laboratory sea water and the soluble fraction was very small, 0.0001 in experiment 143 and 0.0003 in experiment 177. Nor can the stoppage of flow be attributed either to an increase in specific gravity of the sea water after washing with oil, or to the number of times the oil was washed.

The least effect of 80-percent solution occurred in experiment 165, in which the reduction in flow amounted to 59.1 percent. This relatively small effect is doubtless also due to the condition of the oyster, no. 165, being for some reason better able to withstand the test solution than any of the others in the group.

Eight experiments were made with 100-percent soluble fraction (table 11, fig. 7). The average temperature of the laboratory sea water for all experiments was 27.3° C. The specific gravity was high, 1.0272 to 1.0277, except in the last experiment, where it was 1.0230.

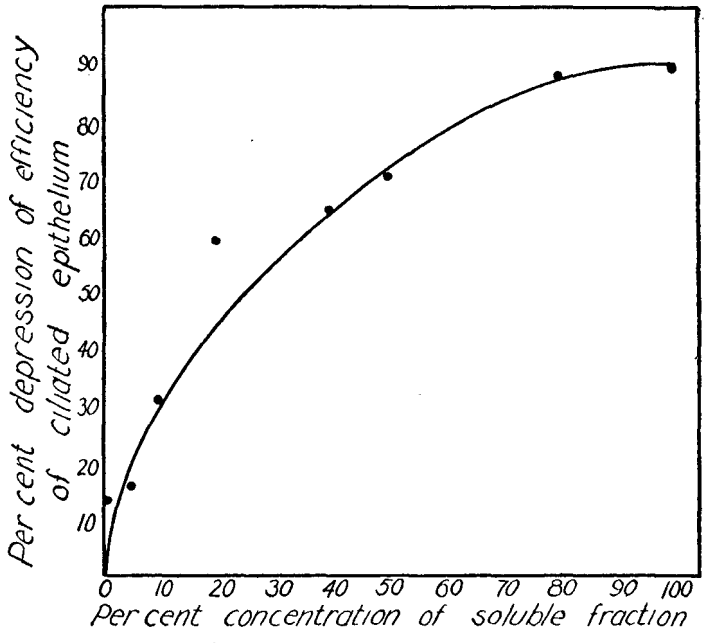

FIGURE 8.—Depression of rate of pumping of gills caused by various concentrations of water soluble fraction of oil.

The average reduction in ciliary activity amounted to 87.6 percent. In five experiments there was a complete cessation of pumping.

The results of all the experiments with the cone method show, without any doubt, that the presence of the water-soluble fraction of crude oil exerts an inhibiting effect on the efficiency of the ciliary motion of the gill epithelium, the work of which is interfered with in such a way that less water is pumped through the gills, and consequently the rate of feeding of an oyster is decreased. The decrease in efficiency of the epithelium is directly proportional to the increase in concentration of the soluble fraction. This is clearly seen from an examination of figure 8, which shows the percentage of depression caused by various concentrations. The values plotted in the figure are the averages, recalculated from the data given in table 11, based on the efficiency of the ciliary motion in the controls as 100 percent.

A relatively high degree of depression caused by the 20-percent solution may be considered as a breaking point in the curve, but so small a number of observations is not sufficient to determine this point with certainty. Regardless of the graphical interpretations that can be made concerning the shape of the depression curve, one

fact is clear: That a concentration between 20 and 30 percent soluble fraction will, on the average, reduce the rate of feeding of the oyster to one-half its normal value.

RESULTS OBTAINED WITH THE DROP COUNTING METHOD

Two series of experiments were made with the water-soluble fraction of crude oil, using the drop counting technique previously described.

The first group of 12 experiments (table 12) represents winter conditions, as they cover the period of November and December 1933. Two Louisiana crude oils were used, one from the Lake Barre wells, the other from the Pelto wells. Both fields are located in Terrebonne Parish and are operated by the Texas Co.

In the second series (table 13) are 19 experiments completed during May 1934, using only Lake Barre oil. The results of all experiments are summarized in these tables, which give the rate of pumping, in drops per minute, before, during, and after treatment. A column marked "Effect of treatment" gives the percent of the normal rate of flow obtained by dividing the average rate of flow during treatment by the average rate before treatment, making it possible to evaluate the inhibiting effect of oil extract on various oysters. In table 14 the results of the experiments are presented in a more detailed manner, showing for every 5-minute interval, the average number of drops of water per minute passed by the gills.

Heavy type indicates observations made when the soluble-fraction solution was running through the experimental chamber.

In both series of experiments a full-strength solution was used. There was a slight increase in the specific gravity of the sea water after it was stirred with oil, but there was no correlation between the specific gravity of the extract and its toxicity.

TABLE 12.—*The effect of soluble fraction of crude oil of Lake Pelto and Lake Barre wells on the rate of pumping of water by the gills of the oyster*

[Drop counting method, winter experiments, Beaufort, 1933]

Experiment no.	Date	Drops per minute			Effect of treatment	Duration test in minutes	Specific gravity, 17.5° C.		Temperature, ° C.		pH soluble fraction	Increase in specific gravity of soluble fraction	Recovery time		Source of oil
		Before treatment	During treatment	After treatment			Laboratory sea water	Soluble fraction	Beginning	End			Percent	Minutes	
					Percent										
60	Nov. 28	92.0	109.6	95.2	119.1	5	1.0261	1.0277	13.6				128	5	Barre.
60A	do	65.8	41.3	69.7	62.8	5	1.0261	1.0262	15.0	15.1	7.3	0.0001	116	30	Do.
62	Dec. 1	31.2	19.0	27.0	61.1	5	1.0261	1.02638	15.4	15.2	7.3	.00028	93	15	Do.
63A	Dec. 4	112.2	92.0	108.2	82.0	5	1.0262	1.0262	17.4	17.3	7.3		107	15	Do.
64A	Dec. 5	77.2	30.0	71.5	38.9	5	1.0260	1.0260	16.5	16.3			97	15	Do.
65A	Dec. 7	41.4	46.6	39.0	112.7	5	1.0265		14.8	14.8			123	5	Pelto.
66	Dec. 11	36.0	35.0	39.5	97.2	10	1.0267		13.2	13.5			145	5	Do.
67	do	60.5	54.7	58.2	90.4	10	1.0267		13.8	14.1			103	20	Do.
68	Dec. 12	67.2	52.0	57.2	77.4	10	1.0267		11.8	11.6			77	10	Do.
69	Dec. 15	77.0	60.4	79.6	78.4	10	1.0267	1.0279	12.3	12.5	7.5	.0012	109	15	Do.
70	Dec. 21	[1]120.7	0	36.5	[1] 30.2	15	1.0256	1.0260	15.6	16.5	7.6				Do.
70A	Dec. 22	[1] 83.5	0	64.9	[1] 77.7	15	1.0256		15.0	15.4					Sea water.
Average		66.0	54.06	62.2	82.0				14.48				109.8	13.5	

[1] Numbers omitted in making average.

TABLE 13.—*The effect of soluble fraction of Lake Barre oil on the rate of pumping of water by the gills of the oyster*

[Beaufort, 1934, spring experiments]

Experiment no.	Date	Average number drops per minute			Effect of treatment	Duration of test in minutes	Specific gravity, 17.5/17.5° C		Increase in specific gravity of soluble fraction	Temperature, ° C.		Number of washings	Recovery time	
		Before treatment	During treatment	After treatment			Laboratory sea water	Soluble fraction		Beginning	End		Percent	Minutes
CONTROLS					*Percent*									
73	May 9	44	40	38	91	78	1.0234			21.7	22.0			
74	May 10	121	128	134	106	71	1.0237			21.6	21.5			
84	May 16	139	141	137	101	92	1.0242			20.9	21.15			
86	do	115	114	115	99	55	1.0242			21.3	21.45			
SOLUBLE FRACTION														
71	May 7	109	49	73	45.0	5	1.0223	1.0177	0.0007	21.5	21.5	1	72	35
72	May 9	128	71	119	55.0	4	1.0234	1.0177	.0007	20.9	20.9	1	87	30
75	May 10	132	69	64	52.0	9	1.0237	1.0177	.0007	21.6	21.7	1	42	10
76	do	124	98	122	79.0	10	1.0237	1.0122	.0115	21.7	21.9		92.3	20
77	do	129	85	99	66.0	8	1.0237	1.0244	.0007	21.9	22.1	1	62.9	10
78	May 14	141	13	129	9.2	8	1.0242	1.0247	.0008	22.1	22.4	1	96	30
79	do	136	19	106	14.0	8	1.0242	1.0249	.0007	22.4	22.7	2	102	30
80	do	146	27	105	18.5	8	1.0242	1.0247	.0005	23.3	23.3	3	75	30
81	May 15	156	94	132	60.3	8	1.0242	1.0250	.0008	22.9	22.5	3	87.9	20
82	do	133	85	119	64.0	8	1.0242	1.0246	.0004	22.5	22.4	4	91	15
83	do	126	33	100	26.2	20	1.0242	1.0245	.0003	22.3	22.6	4 and 5	93	10
85	May 16	133	31	107	23.3	23	1.0242	1.0244	.0002	21.1	21.3	5 and 6	79.1	20
87	May 17	87	27	55	31.0	24	1.0241	1.0243	.0001	19.1	19.1	6 and 7	64.1	30
88	May 21	154	42	103	27.3	38	1.0242	1.0245	.0005	24.2	24.3	8 and 9	70	20
89	May 22	126	38	93.5	30.2	12	1.0230	1.0237	.0007	24.8	24.8	9 and 10	68	15
Average		130.6	52.0	101.6	40.0					22.1			78.7	21.6

NOTE.—Averages do not include controls or experiment no. 76, which was made with dilute sea water.

The column in table 13, marked "Number of washings" refers to the number of times the same oil has been stirred with sea water. In the winter experiments, the oil was used only once, then discarded. In the spring experiments as many as 10 extractions were obtained from a single sample of oil without exhausting its toxicity.

One must bear in mind that because the oysters in these experiments are kept in running water, which is gradually replaced by the solution, the duration of each test lasted only a short period of time, varying from 4 to 38 minutes, depending on the amount of solution available. In figure 9, representing the results of three experiments, the time when the treatment began is marked with a vertical line. It has been estimated that the sea water in the experimental chamber (fig. 5, *Ch*) was completely replaced in 12 minutes, and that the same period of time was required to replace the oil extract with normal sea water, but there was no means of checking the exact concentration of solution in the chamber at the beginning and end of the test.

A decrease in the rate of pumping of water, caused by the oil extract, is noticeable almost immediately upon the beginning of treatment, and continues as long as the toxic substances are present in the water. There was a wide difference in the sensitivity of various oysters. While in some of them the pumping was completely inhibited, in others it was only slightly depressed (table 14).

The average rates of flow of water, in drops per minute, were computed from the kymograph data. The time during which the record was taken varied for the period "Before treatment", from 20 to 45 minutes; "During treatment", from 15 to 45 minutes; and "After treatment", from 15 to 90 minutes. It is believed that these periods are of sufficient duration to eliminate the effects of accidental stimulation and other possible disturbances.

Although both the winter and spring experiments show a depression in the rate of flow following treatment, the effect is much greater in the spring series. The average depression in rate of flow resulting from treatment for the winter series is 18 percent. For the spring series it is 60 percent. This difference in the levels of depression for the two groups is presumably a function of temperature, and is due to the direct relationship existing between temperature and rate of motion of the lateral gill cilia. It will be noted (table 12) that the average rate of flow before treatment in the winter series is 66 drops per minute, while in the spring series the average is 130 drops per minute. The average temperature at the beginning of the experiments for the winter series is 14° C., while the spring average is 22° C. Thus an average increase of 8° C. in temperature approximately doubles the rate of pumping while the depressing effect of the soluble fraction solution is trebled.

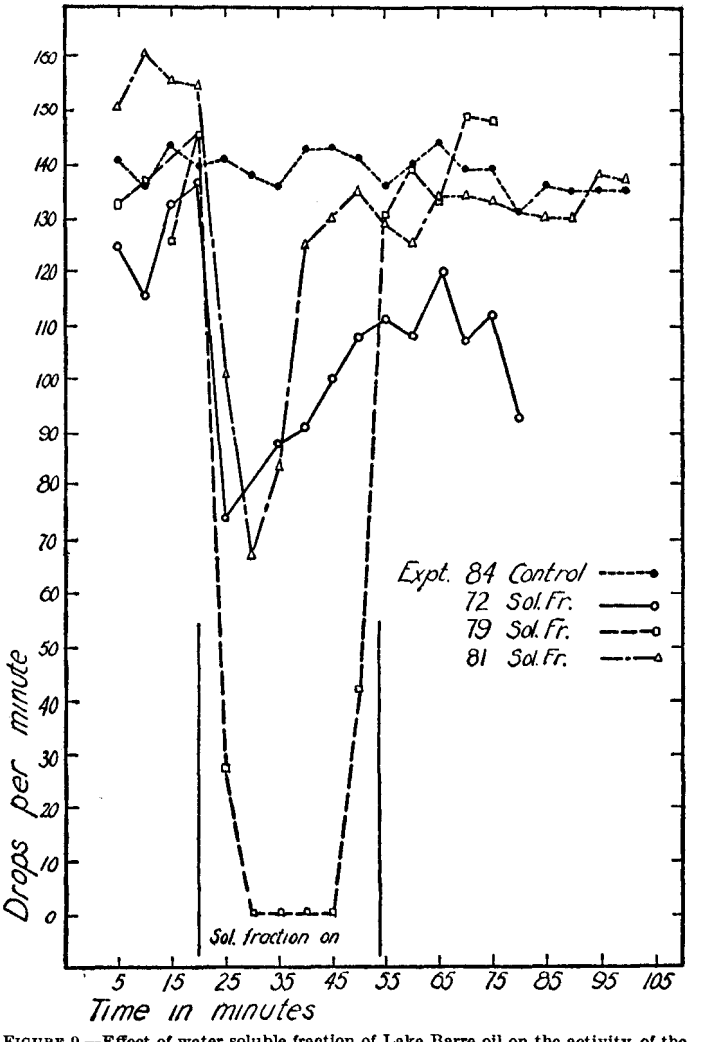

FIGURE 9.—Effect of water soluble fraction of Lake Barre oil on the activity of the ciliated epithelium. Drop counting method.

TABLE 14.—*The effect of soluble fraction of crude oil from Lake Barre and Lake Pelto wells on the rate of pumping of water by the gills of the oyster*

[Detailed record of the experiments. Figures printed in heavy type represent observations made when oysters are in solution of soluble fraction]

Experiment no.	Date	Crude oil	Time of treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
				Drops per minute, average by 5-minute intervals																	
				5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90
	1933		Min.																		
60	Nov. 28	Barre	5	94	92	91	87	**96**	**110**	118	101	104	98	91	88						
60A	do	do	5	61	64	64	65	**75**	**29**	37	58	61	69	73	76						
62	Dec. 1	do	5	34	35	31	30	**24**	**13**	18	26	29	27	25							
63A	Dec. 4	do	5	115	114	113	118	**101**	**85**	90	101	120	106	100	107						
64A	Dec. 5	do	5	80	81	74	75	**76**	**14**	14	62	75	75	68	68						
65A	Dec. 7	Pelto	5	44	42	40	39	**42**	**42**	51	47	44	40	34	38						
66	Dec. 11	do	10	35	35	40	34	**39**	**22**	**27**	52	45	38	38	37						
67	do	do	10	63	59	64	56	**57**	**53**	**52**	57	61	62	54	56						
68	Dec. 12	do	10	70	72	59	68	**81**	**31**	**44**	52	51	54	56	68						
69	Dec. 15	do	13	65	81	80	82	**81**	**73**	**40**	35	73	84	83	72						
70	Dec. 21	do	15	119	122	121	121	**1**	**1**	**1**	0	0	29	42	39	52					
70A	Dec. 22	Sea water	15	80	83	85	86	**2**	**2**	**2**	25	25	28	75	91	91					
	1934																				
73	May 9	Control		46	49	46	36	45	42	42	43	40	36	35	32	42	38	41	39		
74	May 10	do		127	128	118	126	106	127	126	120	134	117	142	144	116	141	126	144		
84	May 16	do		141	136	144	140	141	138	136	143	143	141	136	140	144	139	139	131	136	135
86	do	do		123	122	104	113	117	117	109	112	113	114	116	117						
71	May 7	Barre	5	118	115	111	107	104	103	**49**	40	58	63	64	67	71	78	70	77		
72	May 9	do	5	125	116	133	137	**74**	32	88	91	100	108	111	108	118	107	112	93		
75	May 10	do	10	**122**	**54**	45	55	54	45	43	47	52	56	48	44	44	56	65	69	107	
76	do	do	12	124	**129**	**106**	**71**	88	104	114	105	136	114	138	140						
77	do	do	10	123	**125**	**72**	64	80	76	83	67	86	94	66	100	107	110	120	110	118	
78	May 14	do	10	148	136	149	123	**53**	**0**	0	0	119	220	131	136	129	114	140	133	137	
79	do	do	10	146	**77**	**0**	0	0	0	42	130	139	133	149	148						
80	do	do	10	153	**103**	**4**	0	0	0	6	78	109	105	133	139	147	143	144	149		
81	May 15	do	8	151	161	156	155	**101**	**67**	84	125	130	135	129	125	134	134	133	131		
82	do	do	8	133	131	135	132	**61**	**44**	117	120	121	117	117	120						
83	do	do	20	120	127	126	130	**80**	**27**	**36**	**21**	**1**	108	117	102	89	100	98	95	97	
85	May 16	do	24	137	133	129	134	**104**	**39**	**31**	**12**	**0**	0	71	102	105	99	104	112	119	
87	May 17	do	24	87	87	87	85	**64**	**45**	**32**	**21**	**1**	2	37	52	54	55	56	45	60	
88	May 21	do	38	151	155	154	156	**120**	**69**	**54**	**52**	**49**	**34**	**2**	**0**	**0**	29	82	107	108	
89	May 22	do	12	118	130	131	128	127	127	121	125	**85**	**15**	**16**	37	84	86	84	90	95	

The effect of temperature on the rapidity of recovery is equally striking. The oysters in both winter and spring experiments show, after treatment with soluble fraction solution, a fairly steady rise in the rate of pumping but this initial rise is followed by a slight drop (fig. 9). The significance, if any, of this peak in the recovery curve is not known. However, it is useful in demonstrating the relation of temperature to rate and extent of recovery. In table 12, the column headed "Recovery time", shows the percent of recovery at the peak as compared with the average rate before treatment. The time in minutes extends from the moment of turning off the test solution to the point where the peak occurs. For the winter series, the average recovery at the peak was 109 percent in an average time of 13.5 minutes. The spring series averaged only 78 percent recovery at the peak in an average time of 21.6 minutes (table 13).

Results of the experiment carried out at Woods Hole, Mass., in which a solution of water-soluble substances present in Pelto oil was prepared by allowing them to diffuse through a collodion sack suspended in the sea water are similar to those obtained at Beaufort with soluble fraction prepared by shaking oil with the sea water. The sack, about 1 inch in diameter and 7 inches high, was made by dipping a glass test tube of corresponding size into a solution of Merck's collodion (reagent quality) and drying it for 1 minute. The membrane was carefully examined under a microscope and tested against leakage. Fifty cubic centimeters of oil were poured into the sack and the latter was kept suspended for 4 days in 4 liters of sea water which was gently agitated by means of an electric stirrer with glass rod. The specific

gravity of the water (at 17.5° C.) at the beginning and at the end of this period was 1.02455 and 1.02461, respectively. This slight increase in the specific gravity may be attributed either to the evaporation of water or to the dialysis of substances present in the oil.

An oyster was mounted in the usual manner and placed in a celluloid tank of 1,830-cc capacity used for measuring the rate of flow of water through the gills. Water was supplied at the rate of 100 cc per minute and the entire volume of the tank could be renewed in 20 minutes. Drops of water filtered by the oyster gills were recorded by means of an electric drop counter. The record of this experiment is presented in table 15 which shows the average number of drops per minute delivered by the gills before, during, and after the addition of the water soluble fraction of the oil. During this period the temperature varied between 21.5 and 21.8° C.

TABLE 15.—*The effect of water soluble fraction of Pelto oil obtained by dialysis through a collodion membrane on the rate of flow of water through the gills of the oyster*

Time	Drops per minute	Remarks	Time	Drops per minute	Remarks
12:05–2:25	[1] 208	Sea water.	4:07–4:08	105	Sea water replaced by solution—Continued
2:30		Solution turned on.	4:08–7:10		Left in solution.
2:50	96	Sea water replaced by solution.	7:10–7:11	53	Solution turned on.
2:53–2:54	96	Do.	7:11–7:12	52	Do.
3:20–3:21	90	Do.	7:12–7:13	60	Do.
3:22–3:23	66	Do.	7:15		Sea water turned on.
3:23–3:24	90	Do.	7:50–7:51	99	Do.
3:24–3:25	108	Do.	NEXT DAY		
4:00–4:01	10	Do.			
4:01–4:02	14	Do.			
4:03–4:04	72	Do.			
4:05–4:06	106	Do.	9:15–9:16	66	
4:06–4:07	107	Do.			

[1] Average.

By examining this table one notices that the presence of the water soluble fraction of Pelto oil greatly interferes with the normal activity of the gill epithelium. The rate of flow of water becomes less regular and decreases about 50 percent. A 3-hour exposure in this solution causes further inhibition of the ciliary motion of the epithelium, the activity of which was not fully restored even when the oyster was placed back in natural sea water.

On account of the limited amount of crude oil available in the laboratory experiments, it was necessary in preparing the water soluble fraction to use the same sample of oil several times. It was noticed that subsequent 30-minute stirrings of 2 volumes of crude oil with 1 volume of sea water did not exhaust the potency of the sample in inhibiting the work of the ciliated epithelium. Table 15 shows the percentage of depression in rate of flow of water caused by 10-, 20-, and 40-percent solutions of the extract. It will be observed that after 28 washings, there was no decrease in the toxicity of the extract.

TABLE 16.—*Showing no decrease in toxicity of a sample of oil due to repeated washings with sea water*

Number of washings	Percent depression	Number of washings	Percent depression	Number of washings	Percent depression
10 PERCENT		20 PERCENT		40 PERCENT	
2	6.7	1	17.0	4	50.0
19	10.3	1	69.5	9	54.3
28	29.1	8	34.0	9	70.5
		8	80.6	10	79.0
		13	68.0	16	45.0
		22	77.0		
		25	35.0		

This observation has an important bearing on the problem of oil pollution of natural waters, indicating that oil floating on water or absorbed in mud will, for a long time, remain a source from which toxic substances diffuse into the water. It seems probable that the toxic effect is not due to the mineral salts which occur in the crude oil and which doubtless will be leached out by subsequent washings, but to the organic compounds which gradually dissolve in the sea water.

TABLE 17.—*The effect of oil well bleed water on the rate of pumping of water by the gills of the oyster*

[Drop counting method]

Experiment no.	Date	Drops per minute			Effect of treatment	Bleed water on—	Percent bleed water	No current for—	Specific gravity 17.5/17.5		Temperature, °C.		pH bleed water solution	Source bleed water
		Before treatment	During treatment	After treatment					Sea water	Bleed water	Beginning	End		
						Min.		*Min.*						
55	Nov. 24	61.0	58.2	65.7	95	10	10	------	1.0258	1.0261	15.5	15.8	7.3	Barre.
63	Dec. 4	125.8	113.0	108.7	90	5	10	------	1.0262	1.0267	17.3	17.0	-------	Do.
63B	Dec. 5	41.2	30.3	40.0	73	5	10	------	1.0260	1.0265	15.2	15.6	-------	Do.
64	---do----	66.0	73.0	80.7	111	5	10	------	1.0260	1.0264	16.2	16.6	-------	Do.
65C	Dec. 8	90.7	95.6	87.3	106	10	10	------	1.0268	1.0263	14.8	15.2	7.5	Pelto.
Average		-------	-------	-------	95	-------	-------	------	-------	-------	-------	-------	-------	-------
54	Nov. 23	78.7	57.5	69.0	73	10	20	------	1.0255	1.0259	16.4	16.6	7.3	Barre.
64B	Dec. 6	77.5	80.2	82.5	103	5	20	------	1.0260	1.0257	16.2	16.6	-------	Do.
64C	---do----	95.0	74.8	96.3	79	10	20	------	1.0260	1.0258	17.1	17.3	-------	Do.
65D	Dec. 8	97.0	89.6	91.6	91	10	20	------	1.0268	1.0268	15.2	15.2	-------	Pelto.
Average		-------	-------	-------	86.5	-------	-------	------	-------	-------	-------	-------	-------	-------
65	Dec. 7	74.2	70.2	89.0	94	10	33	------	1.0268	1.0302	15.0	15.0	7.3	Barre.
65B	Dec. 8	86.2	35.0	100.5	41	10	40	3	1.0268	1.0397	14.1	14.4	7.3	Do.
53	Nov. 21	71.2	55.0	68.0	77	10	33	------	1.02535	1.0379	12.8	13.2	7.3	Do.
53A	---do----	97.0	70.7	96.0	81	10	33	------	1.02535	1.0379	--------	15.9	-------	Do.
53B	Nov. 22	130.0	56.5	97.4	44	10	40	------	1.02559	1.0436	--------	16.6	7.7	Do.
Average		-------	-------	-------	67.4	-------	-------	------	-------	-------	-------	-------	-------	-------
67A	Dec. 11	57.0	16.0	36.0	28	14	50	10	1.0267	1.0532	14.1	14.3	-------	Barre.
68A	Dec. 12	71.0	10.0	32.0	14	10	60	12	1.0267	1.0638	11.6	11.7	-------	Do.
68B	Dec. 13	91.0	21.5	18.0	24	10	80	29	1.0267	1.0851	12.0	12.0	7.5	Do.
69A	Dec. 15	84.0	4.5	24.5	5	10	100	120	1.0267	1.1064	14.4	15.4	7.5	Do.

EFFECT OF BLEED WATER ON THE RATE OF FEEDING

Eighteen experiments were made with "bleed water" or brine from the Barre and Pelto wells during November and December 1933, using the drop counting technique. An adjustment period of from 10 to 30 minutes preceded the beginning of the measurements. A summary of all the experiments is given in table 17. Column 3, "Before treatment," shows the rate of pumping in drops per minute, averaged by 5-minute intervals, from the time the kymograph was started until the test solution was turned on. The elapsed time for this period is usually 20 minutes.

Column 4, "During treatment", shows the average rate of flow in drops per minute while the test solution was flowing over the oyster, and for 10 minutes after the test solution was turned off, as this time is required to replace it with sea water. The test solution was on for 5 or 10 minutes in most cases (col. 7).

Column 5, "After treatment", shows the average rate of pumping in drops per minute for a period about 20 minutes immediately after treatment. The value is a measure of recovery occurring during this period. There was not always a sufficient time for the rate of flow to return to the level established before treatment, but a

study of recovery was not considered to be of sufficient importance to the problem to warrant additional time.

The effect of treatment obtained by dividing the average rate during treatment (col. 4) by the average rate before treatment (col. 3) multiplied by 100 is a measure of the toxicity of the test solution. However, this value sometimes masks the actual effects of the test solution, and for this reason each experiment is presented completely in table 18, in which the average rate of flow in drops per minute is shown for each 5-minute interval. The intervals in which the test solution was flowing are indicated in bold face type, the figure in the last column giving the actual number of minutes during which the bleed water was running into the experimental chamber.

An example of the masking of brine effects as shown in column 6, table 17, is experiment 55. In this table the reduction in rate of flow due to a 10-minute treatment with 10 percent bleed water solution is 5 percent. An examination of the actual figures in table 18, shows that during the first 5 minutes of treatment there was an accelerating effect of about 16 percent; during the second 5-minutes of treatment the rate fell to nearly normal; in the 5-minute period immediately succeeding treatment, the flow decreased 36 percent, and returned to normal in the next 5-minute interval. The effect of bleed water in experiment 55, therefore, consists of an initial acceleration in ciliary activity followed by a considerable inhibition.

Of the 5 experiments using approximately 10 percent bleed water solution, 3 (nos. 55, 63, and 63B) show a reduction in rate of flow during treatment, the other 2 having an increased rate of pumping. It was to be expected that similar to the action of various poisons the low concentrations of bleed water may have a stimulating effect at least in some of the individuals.

Owing to large individual differences in condition and resistance of oysters, it is impossible to establish an exact concentration level for bleed water solutions, above which the rate of pumping would always be reduced, and below which an increase in rate would occur. However, the rate of pumping in the 10 percent bleed water group is reduced an average of 5 percent, which seems to indicate that this concentration slightly exceeds the limit of tolerance.

Four experiments were made using 20 percent bleed water. These show a decidedly greater depressing effect, the average rate of flow during treatment for the group being only 86.5 percent of normal. This increase in depressing effect of the 20 percent bleed water probably is due to a greater proportion of ions affecting the gill cilia, for there was no increase in the specific gravity of the solution as compared with laboratory sea water. On the contrary it will be seen (table 17) that the specific gravity of the test solution in experiments with 20 percent bleed water is closer to that of the laboratory sea water than is the case in the experiments with 10 percent bleed water.

The effect of 33 percent bleed water solution is shown by three experiments (nos. 65, 53, and 53A). In two experiments (65B and 53B) 40-percent bleed water was used. The average reduction in rate of flow resulting from a 10-minute treatment with these concentrations of bleed water was 32.6 percent.

One experiment each was made with bleed water concentrations of 50, 60, 80, and 100 percent. There is comparatively little difference in the effects of these concentrations during treatment for 10 minutes. However, the length of time during which no water was pumped through the gills increases steadily, from 10 minutes in the case of 50 percent bleed water to more than 2 hours for 100 percent. The

exact extent of the nonflow period in the latter experiment is not known as the oyster began recovery sometime during the night.

TABLE 18.—*The effect of oil well bleed water on the rate of pumping of water by the gills of the oyster*

[Drop-counting method. Records of two experiments made in Beaufort, 1933]

Experiment No.	Date	Drops per minute, average by 5-minute intervals															Time treatment (minutes)
		Percent bleed water	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	
55	Nov. 24	10	65	59	57	63	71	63	39	62	66	70	61	66				10
63	Dec. 4	10	130	130	123	125	121	119	101	119	104	109	115	107				5
63B	Dec. 5	10	41	43	41	41	45	35	29	27	43	42	36	39				5
64	do	10	61	60	70	74	65	63	79	76	83	83	72	85				5
65C	Dec. 8	10	96	92	89	86	88	101	105	96	88	85	86	91				10
54	Nov. 23	20	74	81	77	83	84	54	25	67	71	68	68					10
64B	Dec. 6	20	74	76	82	78	89	83	78	71	78	91	77	84				5
64C	do	20	99	95	96	90	88	81	57	69	79	85	92	112				10
65D	Dec. 8	20	90	95	92	91	88	89	86	87	98	94	94	91				10
53	Nov. 21	33.3	67	76	73	69	64	59	38	59	60	70	70	71				10
53A	do	33.3	88	89	91	86	87	91	80	90	96	98	102	92	21	68	96	11
53B	Nov. 22	40	119	116	126	116	123	135	56	81	114	130	120	21	32	53	59	10
65	Dec. 7	33.3	69	75	76	77	77	65	62	77	85	87	90	94				10
65B	Dec. 8	40	89	88	80	88	84	37	1	18	86	99	111	106				10
67A	Dec. 11	50	52	61	56	59	58	19	20	0	2	18	34	39	41	47		14
68A	Dec. 12	60	52	69	77	81	76	38	2	0	0	9	26	31	37	37	38	10
68B	Dec. 13	80	93	89	77	8	1	0	0	0	0	0	0	0	11	19	22	10
69A	Dec. 15	100	79	89	85	9	7	2	0	0	0	0	0	0	0	0	0	10

Undiluted bleed water as it comes from the wells, has, as stated above, a specific gravity of about 1.1064 (17.5° C.). A 20-percent solution of brine has approximately the same specific gravity as the laboratory sea water during November and December. Higher concentrations of brine undoubtedly owe part of their inhibiting action on the gill cilia to an increase in density, but this effect is probably much less pronounced than that caused by its chemical composition. It has been found, for instance, that in sea water, the specific gravity of which was increased by the addition of salt to 1.0361, the reduction in rate of pumping was 2 percent, much less than the depression caused by a 20-percent solution of brine. Sea water having a specific gravity of 1.0422 (increased by adding NaCl) caused a decrease in the rate of flow by 20 percent, almost equal to that of the 20-percent solution of brine (specific gravity 1.0258). An inference from these observations is that the inhibitory effect is due to the chemical composition of brine rather than to the increased density of water.

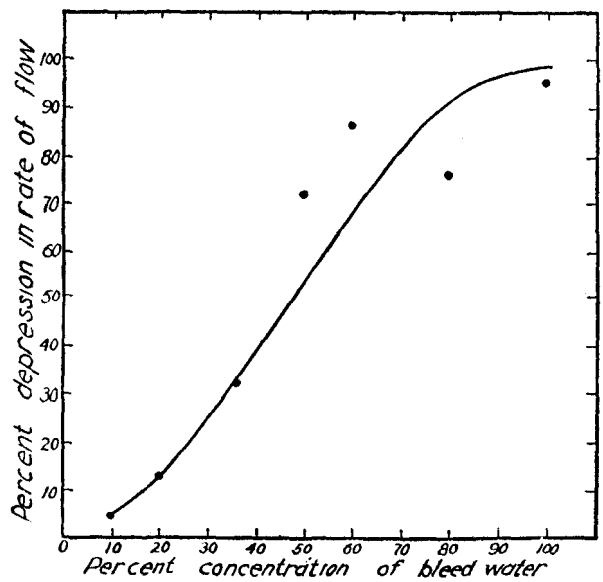

FIGURE 10.—Depression of the rate of pumping of the gills caused by various concentrations of "bleed water" (brine).

The percentage of brine in these experiments is beyond any possibility of occurrence in nature, except under most unusual conditions, and the oyster itself is unable to endure so high a salinity for more than a few hours, so that the results of the experi-

ments are not considered to be significant insofar as the oil pollution problem is concerned, and are included here only for the sake of completeness.

The experiments with bleed water show that it contains a substance or substances inhibiting the work of the ciliated epithelium. The action becomes apparent at a concentration of 10 percent brine in sea water. The toxicity increases in proportion to the increase in concentration. This is apparent from an examination of figure 10, in which the average depression in rate of flow of water through the gills, expressed in percentage of the normal, is plotted against the concentrations.

EFFECT OF CONSECUTIVE TREATMENTS

In the experiments described above the oysters were treated for a very short period of time lasting only a few minutes. The question arises as to whether or not as a result of a more prolonged exposure, they may become adapted or develop greater tolerance to the toxic substances present in crude oil and bleed water.

As stated previously, the drop counting technique is not adapted for experimentation over a period of more than 2 or 3 days. Several experiments were made in which the same oyster was treated with oil extract or bleed water several times within this period.

Five oysters were used in a total of 16 experiments. The results are summarized in table 19. Thirteen of these experiments have been analyzed to determine the effect of 2 or more treatments.

TABLE 19.—*The effect of consecutive treatments with soluble fraction of oil and bleed water on the rate of pumping by the gills of the oyster*

Experiment no.	Date	Test solution	Percent of solution	Effect of treatment	Length of treatment	Temperature
				Percent	*Minutes*	° *C.*
60	Nov. 28	Soluble fraction	100	119.6	5	13.6
60A	do	do	100	62.8	5	15.0
63	Dec. 4	Bleed water	10	90.0	5	17.3
63A	do	Soluble fraction	100	82.0	5	17.4
63B	Dec. 5	Bleed water	10	73.0	5	15.2
65	Dec. 7	do	33	94.0	10	15.0
65A	do	Soluble fraction	100	112.7	5	14.8
65B	Dec. 8	Bleed water	40	41.0	10	14.1
65C	do	do	10	106.0	10	17.1
65D	do	do	20	91.0	10	15.2
78	May 14	Soluble fraction	100	9.2	8	22.1
79	do	do	100	14.0	8	22.4
80	do	do	100	18.5	8	23.3
81	May 15	do	100	60.3	8	22.9
82	do	do	100	64.0	8	22.5
83	do	do	100	26.2	20	22.3

Oyster no. 60 was used in two experiments, 60 and 60A. The duration of treatment with soluble fraction from Barre oil was 5 minutes in both. Five hours elapsed between treatments. The difference in temperature at the beginning of the 2 experiments was 1.4° C. In experiment 60, the rate of pumping was accelerated almost 20 percent above normal, while in 60A there was a reduction in rate of about 37 percent.

Oyster no. 63 was used in experiments 63, 63A, and 63B. Bleed water was used in 63 and 63B, and soluble fraction from Barre crude oil in 63A. Two hours elapsed between 63 and 63A. 63B was made the following day, 18 hours after 63A. The duration of treatment was 5 minutes in each.

Comparing the effect of the two treatments with bleed water, it will be noted that the second treatment reduced the rate of flow 27 percent as compared with a 10-percent reduction for the first treatment. The interval between these treatments was more than 20 hours. The difference in temperature at the beginning of these experiments was 2.1° C.

Oyster no. 65 was used in 5 experiments, 4 with bleed water (65, 65B, 65C, and 65D), and 1 (65A) with soluble fraction from Pelto crude oil. Nos. 65 and 65A were made on December 7, with a 3-hour rest period between. The other three experiments were made the following day. The intervals between these experiments are 65A to 65B, 18 hours; 65B to 65C, 2.5 hours; 65C to 65D, 50 minutes. Comparison of the effects of several treatments in this case is difficult because a different concentration of bleed water was used in each. However, it will be observed that the final treatment (65D) with 20 percent bleed water was of the same order of effectiveness as the first treatment with 33 percent bleed water. The time of treatment was 10 minutes in both experiments, and the temperature at the beginning of each was practically the same.

Experiments 78, 79, and 80 were made with the same oyster, using the soluble fraction from Barre crude oil. The duration of treatment was 8 minutes for each and the temperature difference was negligible. Twenty minutes elapsed between 78 and 79; 3 hours elapsed between 79 and 80. The average reduction in rate of flow during treatment as shown in table 19 is 90.8 percent for experiment 78, 86 percent for experiment 79, and 81.5 for experiment 80. Thus it would appear that succeeding treatments were not so effective as the first. However, it will be remembered that the figures in this table are prepared to show only the average effect during the period of treatment, which is considered to be the actual interval during which the test solution flows over the oyster plus an additional 10 minutes for replacing the test solution with fresh sea water. A detailed analysis of the records shows that while the initial reduction in rate of flow is greater in the first experiment, and consequently the average reduction appears to be greater, yet the period of no current is 20 minutes in the second experiment and only 15 minutes in the first. Actually, the second treatment exerted a retarding action on the pumping activity of the cilia for a longer period than was the case in the first experiment. These remarks apply also to the third experiment, no. 80. The initial depressing effect is not so great, as in no. 78, and the period of no current is only 15 minutes, but this period begins after the test solution has been turned off, and subsequent recovery as evidenced by the average rate of flow will be seen to take place more slowly than in either of the preceding two experiments.

Experiments 81, 82, and 83 were made the same day with the soluble fraction from Barre crude oil. The interval between treatments is approximately 2 hours for experiments 81 and 82. Two and a half hours elapsed between treatments in 82 and 83. The duration of treatment was 8 minutes in the first two and 20 minutes in the third experiment. The latter, therefore, cannot be compared directly with the others.

Oyster no. 81 used in these experiments had the highest rate of pumping in the series and had a high resistance to treatment with soluble fraction. The reduction in rate of flow caused by the test solution was 39.7 percent in experiment 81 and 36 percent in 82. The average rate at 5-minute intervals as given in table 14 does not show an appreciable difference in effectiveness of the soluble fraction for the two experiments.

The results of 13 experiments may be summarized as follows:

1. Oysters subjected to several treatments of oil extract or bleed water do not develop higher tolerance to oil extract or bleed water in concentrations used, or within the time limits studied.

2. Particularly in the case of oil extract, the second and succeeding treatments have a less immediate but more prolonged retarding effect on the rate of pumping.

3. There is no evidence of permanent injury caused by this treatment. Recovery is usually complete.

EFFECT OF CRUDE OIL ON DIATOMS

By Paul S. Galtsoff and Vera Koehring

Since diatoms invariably appear to be constituents of the oyster diet, their rate of growth in the medium to which various polluting substances were added, may be taken as an index of the effect of pollutant upon the food supply of the oyster. In the regions where oysters grow, diatoms are usually distributed throughout the water, in the surface layers as well as on the bottom. Oil in water may affect their growth by forming a surface film which may interfere with the gaseous exchange between air and water or by the toxic action of water soluble constituents of the oil. It has been demonstrated by Lord Raileigh (1923) and Langmuir (1916, 1917) that the spreading of an oil upon water is due to the active carboxyl group of the oil molecule which because of its great affinity to water readily goes in solution, whereas the hydrocarbons having far greater attraction for one another than for the water, remain insoluble. By spreading into a monomolecular film the carboxyl groups combine with water without causing the separation of the hydrocarbon chains. Hence, a pure hydrocarbon oil, as has been demonstrated by Hardy (1912, 1919) for benzene and cymene, fails to spread. In case of oil in the natural waters the problem is more complex because we are usually dealing with mixed oils which, upon being exposed to air, change their chemical properties. Oxidized samples of oil, according to Hacker (1925), have an increased spreading power, and chemically inert hydrocarbons upon acquiring certain radicles, $-OH$, $-O$, $-COO$, and $—NH_2$, have an attraction for water. For a detailed discussion of this problem the reader must consult the original papers of the above-mentioned authors, whose findings are briefly mentioned here only as an illustration of the possible changes in the solubility and behavior of crude oil in water, which may account either for the formation of blobs or for the unchecked spread of oil film over a great area of the sea. A comprehensive investigation of the problem would require extensive chemical studies of the oil before and after its discharge into the sea, which the authors were not in a position to undertake although they fully realized the necessity of such a study for the solution of the oil pollution problem.

Oil discharged into the sea does not remain on the surface. Part of it is absorbed by the colloidal particles of clay present in the water and is gradually settled on the bottom. This can be seen in both the aquaria tanks in which oil-polluted sea water is kept and in many sections of the coastal waters affected by pollution. In a comparatively short period of time considerable quantities of oil floating in the water is found absorbed and deposited on the mud bottom from which it can be separated by squeezing and decanting. Mud contaminated with oil may be considered as a possible source from which water soluble constituents of the oil gradually go into solution even after a complete disappearance of oil from the surface.

Experiments carried out with diatom cultures and discussed in the present paper were devised to test the effect of oil (1) as a heavy layer covering the surface of the water; (2) absorbed by some neutral substance and held on the bottom of the culture flasks; and (3) as a soluble extract.

METHOD

Cultures of the single diatom species, *Nitzschia closterium*, E., were grown in the laboratory in solutions prepared according to the Miquel formula. (Solution A, potassium nitrate 20.2 g in 100 cc distilled water; Solution B, calcium chloride 4 g, sodium phosphate (secondary crystals) 4 g, ferric chloride 2 g, and 1 cc concentrated hydrochloric acid in 80 cc of water. Add 2 cc solution A and 1 cc solution B to one liter of carefully filtered sea water; sterilize by bringing just to the boilding point, then cool and filter.) Eighty cc of this solution were poured in round pyrex flasks of 150-cc capacity and inoculated by adding 2 or 5 cc of Nitzschia stock culture. Flasks covered with inverted small beakers were placed in front of the laboratory window where they were protected from direct sunlight. In some of the experiments instead of Miquel solution plain filtered sea water was used. Sea water used during winter experiments was received from Woods Hole, Mass., and stored in paraffined oak barrels. It contained noticeable amounts of hydrogen sulphide. Before using, the water was aerated for at least 24 hours and the precipitated sulphur filtered off. During the summer months the sea water from the laboratory supply was used. The salinity of the water varied between 31 and 32 parts per thousand. Twice a day temperature readings were made by means of a thermometer kept in one of the flasks filled with water. Fluctuations in the light and temperature conditions which were not controlled, are undoubtedly responsible for certain variations in the growth rates. To avoid this difficulty a comparison was always made between the experimental and the corresponding control flasks which stood by its side. The relative position of each pair of flasks was changed every other day to compensate for a possible difference in illumination.

Diatom counts were made by means of a photoelectric set-up (fig. 11) which consisted of Weston photronic cell (*Ph*) connected to a Weston D. C. microammeter (*A*) of 0–100 ranges, accurate within 0.5 percent of the full scale value at any part of it. For measuring the turbidity of a sample, 50 cc of it were poured in a cylindrical glass container *C*, having a diameter of 27 mm, 90 mm high, with fused bottom made of optical glass. The cylinder rested on a diaphragm, the opening of which was slightly smaller than the inside diameter of the cylinder. Both the diaphragm and the cylinder were placed directly over the photoelectric cell the surface of which was covered with thick black paper in such a manner as to cut all the light except that which passed through the column of water in the cylinder. A 21-candlepower, 6-volt Mazda bulb (*L*) fed by a 108 ampere-hour storage battery served as a source of light. The photoelectric cell was placed in a wooden box 4 by 4 by 12 inches. The bulb was mounted at the lower end of an adjustable arm which could be moved up and down and fixed in a desired position by a set screw. A piece of fine ground glass (*Gl*) separated the two compartments of the box. The front part of the box had a door through which the glass container could be placed in position. The box was painted inside with black paint. Great care was exercised in avoiding the fluctuations in the voltage and in insuring uniformity in the intensity of light. It was noticed that there was a drop in voltage during the first 10 minutes following the

turning on of the battery and small fluctuations occurred every time the current was turned off and on. To avoid this difficulty a second Mazda bulb (*L*) of the same candle power was introduced into the circuit. By means of a double switch (*S*) the current from the storage battery could be turned either through the bulb mounted in the box or through the second one located outside. During the observations the current was continuously passing through the spare bulb except during brief moments when measurements of the samples were made. By this arrangement the overheating of the box and difficulties due to the fluctuations in the voltage of the storage battery were overcome. The battery was kept well charged.

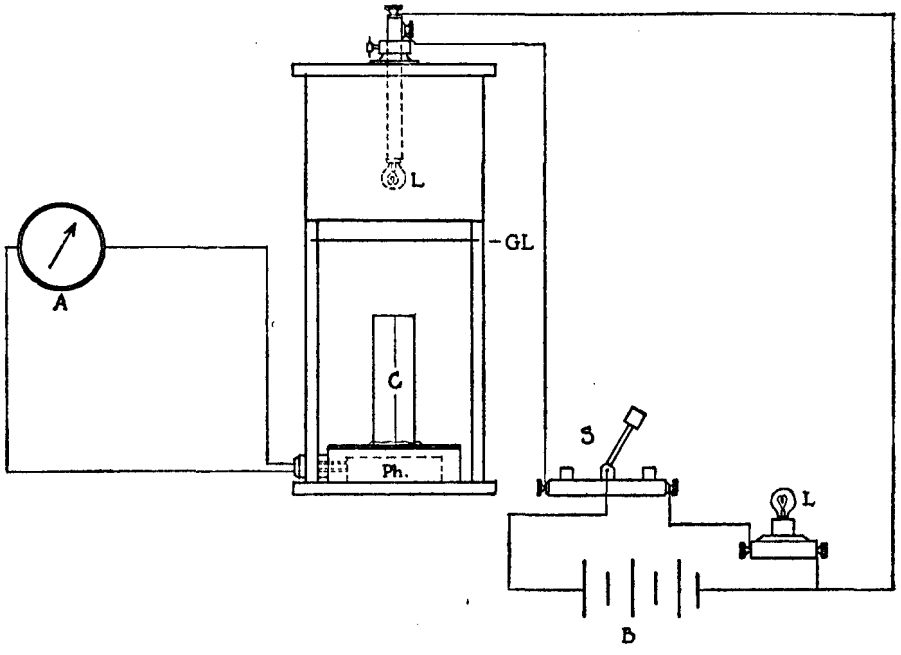

FIGURE 11.—Photoelectric set-up used for counting number of cells in Nitzschia culture. Ph, photoelectric cell; A, microammeter; C, glass container with fascil bottom made of optical glass; L and L_1, electric bulbs; Gl, ground glass; S, double key switch; and B, storage battery.

For the standardization of the apparatus the bulb was so adjusted that the light passing through the container filled with 50 cc of filtered sea water caused the needle deflection of the microammeter to stand at 30. This represented the zero point of the calibration curve. Other points were obtained by making readings with various concentrations of Nitzschia and counting by means of Sedgwick-Rafter chamber the corresponding number of cells. Altogether 35 samples were counted and from the data obtained, a curve was plotted which permitted the conversion of microammeter readings into number of diatoms per cubic centimeter.

Before withdrawing a sample for measurement each culture flask was thoroughly shaken for 2 minutes then 50 cc were taken by means of a certified volumetric pipette, and poured into container *c*. Care was taken to avoid air bubbles, the presence of which affected the ammeter readings. Upon measurement, which required about 30 seconds, the sample was returned to the same flask and both the container and pipette carefully washed in sterile distilled water and dried. Because of the simplicity and quickness of the operation it was possible to experiment with several dozens of flasks simultaneously.

An inconsiderable bacterial population was always present in the cultures. The bacteria were examined at intervals by plating them in agar medium prepared according to Waksmann's formula (1,000 cc sea water, 1 g peptone, 1 g glucose, 0.5 K_2HPO_4, 2–3 drops of 10 percent $FeCl_3$, and 15 g agar; sterilized in autoclave at 15 lbs. for 20 minutes).

When the diatoms and the surrounding medium are relatively free of bacteria the Nitzschia cells stay in suspension. When they tend to fall to the bottom and form loosely aggregated masses, microscopical examination shows them to be weighted with adherent bacteria. Plating under such conditions invariably shows a high bacterial count. All subcultures from the stocks were made, therefore, by carefully decanting only top portions containing diatoms in suspension.

At the beginning of each experiment all the flasks were inoculated with equal volumes of stock culture and 1 or 2 of them measured to determine the initial diatom population. Thereafter the cultures were measured every other day. At least 3 control and 3 experimental flasks were used in each test. In many experiments this number was doubled. All the figures given in tables or graphs are averages of three or more samples.

EFFECT OF HEAVY SURFACE LAYER OF OIL ON NITZSCHIA CULTURE

In this set of experiments Nitzschia was grown in 500 cc Erlenmeyer flasks containing 250 cc of culture covered by 25 cc of oil. The oil was sterilized in a boiling water bath for 1 hour.

There was no indication that the presence of oil kills the Nitzschia and no immediate effects on its growth were discernible even when the oil was thoroughly shaken up with the cultures several times a day. Microscopic examination showed no signs of oil sticking to the surface of the diatoms. For approximately a week the experimental cultures showed no significant difference in their growth as compared with the controls. Their further propagation was, however, markedly inhibited. Photoelectrical measurements of the cultures presented considerable difficulties as it was some times impossible to withdraw a portion of the sample free from oil globules. In two experiments, however, this difficulty was overcome. The first experiment, lasting 18 days, began on March 13 and was discontinued April 4, 1934. The second one, started on April 7, continued for 25 days until May 4. During the last experiment tests were made with purified mineral oil (Russian oil) and cod-liver oil. Cultures covered with cod-liver oil perished on the fourth day, those with mineral oil continued to the end of the experiment. The retarding effect of oil was noticeable in both groups, although in the cultures under Russian oil it was somewhat less pronounced than in those kept under crude Pelto oil (Fig. 12.) The adverse effect of oil can be measured by determining the percent of retardation of growth in the experimental flasks as compared with their growth in the controls. In the first experiment (March 13, Pelto oil) the retardation at the eighteenth day amounted to 35 percent. In the second experiment (lasting 25 days) retardation due to presence of Russian oil and Pelto oil was 37 and 42 percent, respectively.

An inhibitory effect of a heavy surface layer of oil on the diatom culture was quite visible in many other samples which could not be measured. This can be seen in the photographs (fig. 13) taken 30 days after the beginning of one of the experiments in February 1934. The control culture (left) had numerous diatoms and therefore

appears dark, whereas the flask on the right containing culture covered with oil had few diatoms, thus appearing much lighter.

All the experiments indicate that a heavy, unbroken surface layer of oil inhibits diatom growth when oil remains on the surface for a week and longer.

EFFECT OF OIL HELD ON THE BOTTOM

In attempting to devise a method whereby oil could be held at the bottom of the flask and would not interfere with the photoelectric measurements the following procedure was developed. Four grams of paraffin, melting point 52° to 55° C., was mixed with 6 drops of oil. The resulting mass, oily to the touch, and having a strong odor,

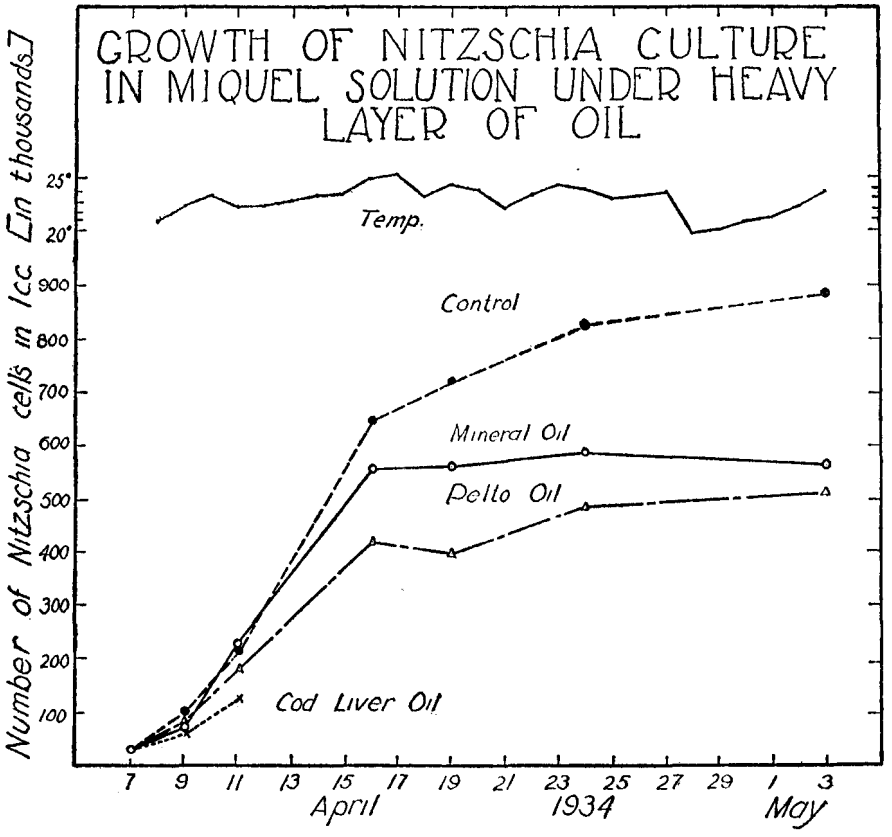

FIGURE 12.—Growth of Nitzschia culture under heavy layer of Pelto oil.

was sterilized at 150° C. and allowed to cool on the bottom of each flask and strongly adhering to it. Pure paraffin controls were prepared in the same manner. The flasks were filled with the standard Miquel medium, inoculated with Nitzschia and measured at regular intervals. The results of the experiments were, unfortunately, inconclusive. In three experiments the growth of Nitzschia in experimental flasks was retarded from 4 to 16 percent (fig. 14), as compared with their growth in pure sea water. In three other experiments there was a noticeable stimulating effect varying from 17 to 27 percent. Pure paraffin controls also produced inconsistent results. In one experiment in 13 days the number of diatoms reached exactly the same number as in the plain sea water. In other experiments the paraffin cultures

showed a retardation by 8–9 percent, while in one experiment the growth in pure paraffin and paraffin-oil cultures was increased by 17 percent (fig. 15).

Indefinite results of the experiments should probably be attributed to the action of bacteria which, as has been shown by Hopkins and Chinbal (1932), Buttner (1926), Tausson (1927), and Haas (1926), in the absence of more suitable material can grow on paraffin and utilize it as their only source of carbon. A great part of our paraffin and paraffin-oil cultures showed very abundant bacterial growth.

Other attempts to incorporate oil in some suitable substance at the bottom of culture flasks were unsuccessful.

EFFECT OF WATER SOLUBLE FRACTION OF OIL

Two different methods were used in preparing water soluble extracts of oil. The first method consisted in stirring together oil and filtered sea water and allowing the mixture to stand for various lengths of time. In the second method measured amounts of oil were poured in collodion bags suspended in flasks containing Nitzschia culture and the water soluble constituents of the oil gradually diffused through the membrane.

The proportions of oil and water used in preparation of the extracts according to the first method as well as the duration of stirring and standing are given in table 20.

TABLE 20.—*Preparation of oil extracts in sea water*

Proportions oil to water	Hours of stirring	Days of standing	Specific gravity of sea water (17.5° C./17.5° C.)	Specific gravity of extract (17.5° C./17.5° C.)
1:1	12	12	1.02433	1.02400
1:2	2	2	1.02461	1.02380
1:2	2	3	1.02435	1.02434
1:2	12	10	1.02463	1.02434
1:2	3	7	1.02445	1.02448

The oil was subsequently filtered off and the resulting clear aqueous extract was heated to boiling to allow for sterilization. Before sterilizing the specific gravity of the extract was determined. In most cases it was slightly lower than that of the sea water used.

The results of these experiments are summarized in table 21 which shows the percent of retardation (−) or stimulation (+) of growth caused by the addition of various concentrations of five different extracts. All the experiments were carried out with Lake Pelto oil. The figures are the averages of three or more samples.

TABLE 21.—*Effect of water soluble fraction of oil on the growth of Nitzschia culture*

[Figures in the body of the table represent percent of retardation (−) or stimulation (+)]

Extracts	1	2	3	4	5	*Extracts*	1	2	3	4	5
IN SEA WATER						IN MIQUEL					
12 percent	+48	+23	0	+11	−4	12 percent	−2	+27	−20	------	−14
25 percent	+32	+10	−18	−17	−25	25 percent	−8	−7	−75	------	−61
50 percent	------	−36	−51	−100	−46	50 percent	------	−62	−79	------	−90

The figures given in table 21 represent the end results of experiments which ran from 9 to 21 days. Of the 25 experiments with various dilutions of oil extract 6

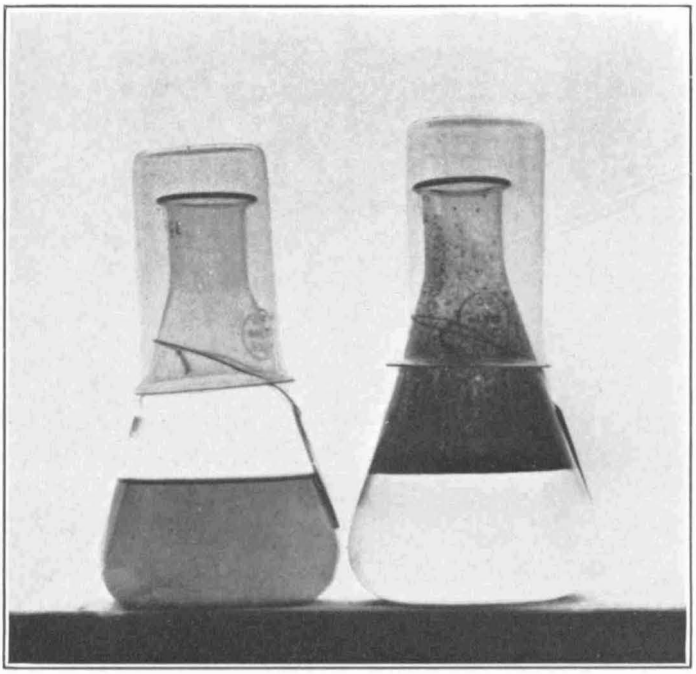

FIGURE 13.—30-DAY OLD NITSCHIA CULTURE IN SEA WATER (LEFT), AND IN SEA WATER UNDER HEAVY LAYER OF OIL (RIGHT).

(February 1934.)

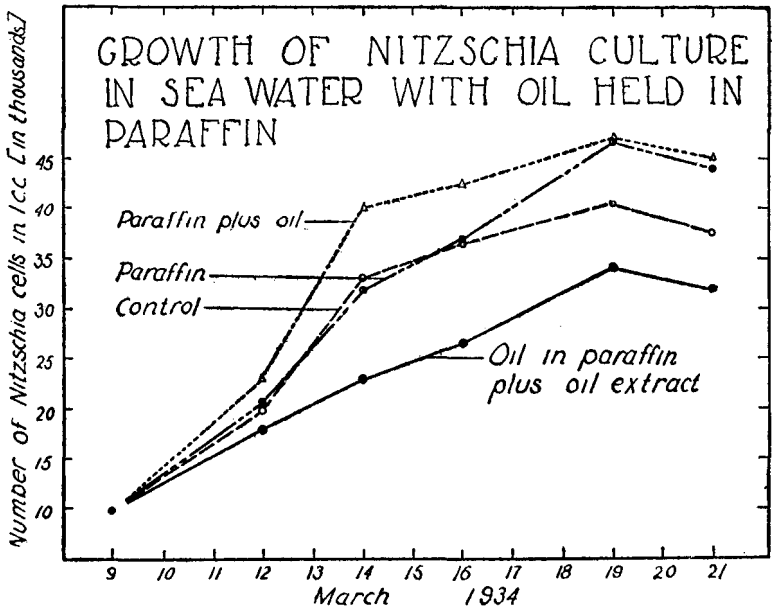

FIGURE 14.

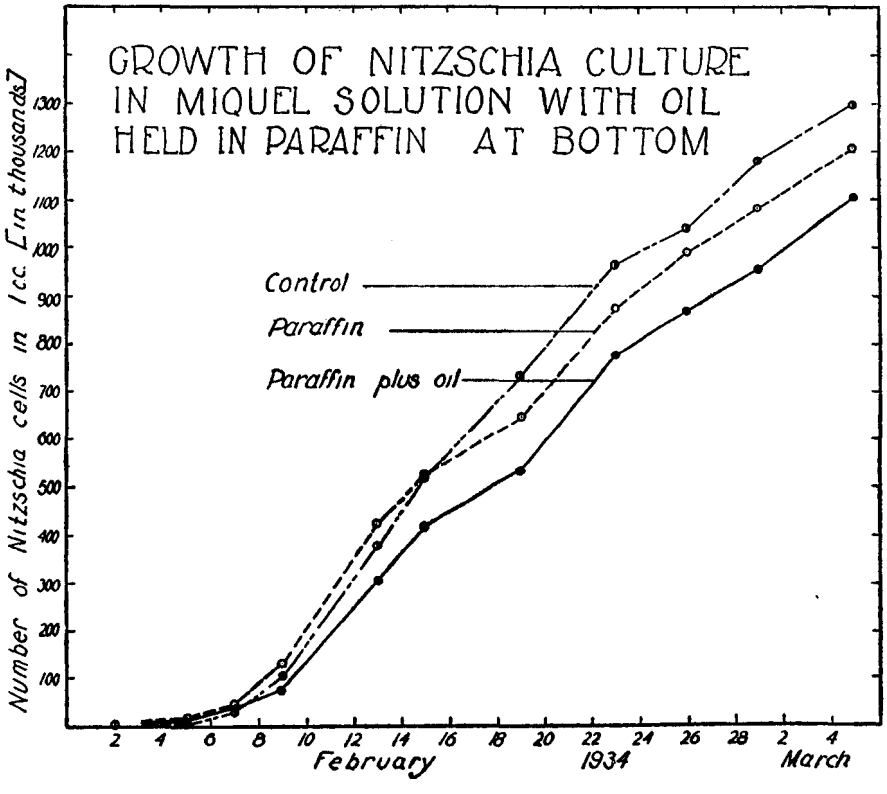

FIGURE 15.

showed increased growth as compared with the controls, while in 19 cases there was a retardation of the propagation or great mortality, accompanied by tremendous development of bacteria. In 14 experiments the population during some period of growth was greater than in the controls. These periods of increased rate of propagation occurred usually during the first few days of growth. Thereafter the controls continued to increase more rapidly and by the end of the experiment attained greater populations. These relationships may be noted by examining figure 16 representing the results of 3 experiments.

In all the experiments with oil extracts, the retarding effect of the latter is apparent in concentrations of 25 percent and higher and when the extract is permitted to act over a considerable period of time.

EFFECT OF OIL HELD IN COLLODION BAGS

The method used in these experiments consisted in suspending in a flask containing 80 cc of culture medium, a collodion bag with 2 cc of crude Pelto oil. Bags were made by dipping a test tube in a thin solution of collodion (Merck's reagent) and drying it for 45 seconds. Freshly made bags were sterilized by boiling in distilled water. Each bag remained suspended in a culture medium throughout the experiment. A more or less continuous dialysis through the collodion membrane was noticed, for water gradually collected in the bottom of the bag. The colorless and apparently somewhat volatile dialyzed substances have not been identified. Their presence was readily detected, however, by odor and irritating effect on the mucous membrane of the mouth while pipetting the sample for measurement. All the controls contained collodion bags filled with the Miquel solution or sea water. The end results of the experiments are given in table 22 the examination of which discloses that in all the cases there was a noticeable retardation in Nitzschia growth caused by oil held in collodion bags. Figure 17 presents a more complete record of one of the experiments.

TABLE 22.—*Retardation of growth of Nitzschia culture caused by oil held in collodion bags*

[Each figure is an average of measurements of 6 different flasks]

Beginning of experiment	Duration, days	Retardation of growth, percent	Medium
July 26	22	20	Miquel.
Aug. 6	12	24	Do.
Do	12	12	Sea water.
Aug. 22	14	32	Do.

EFFECT OF BRINE ON NITZSCHIA

Untreated brine (bleed water) separated from the oil as it comes out of the well was filtered from the heavy sediments accompanying it. The filtrate was carefully brought to the boiling point for sterilization. Sometimes this caused precipitation and the sample had to be discarded.

In a series of experiments lasting from 14 to 31 days tests were made with various concentrations of brine added to sea water or Miquel solution. As can be seen by examining figures 18 and 19 representing two typical experiments, the retardation

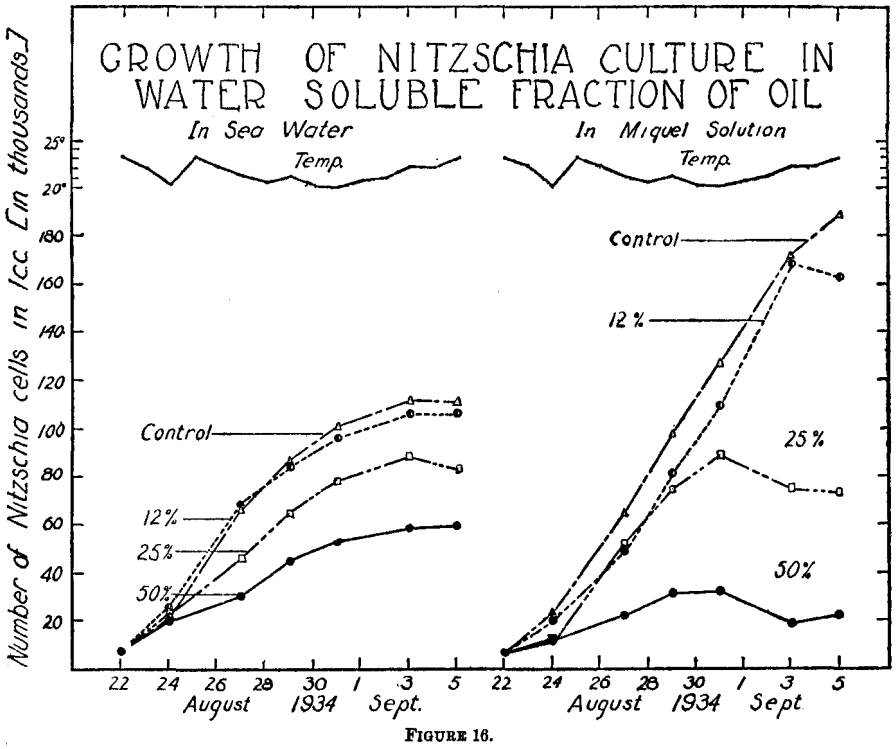

FIGURE 16.

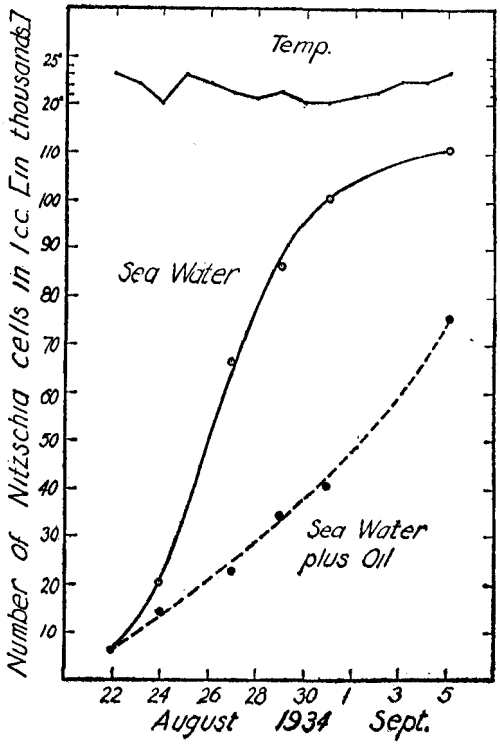

FIGURE 17.—Growth of Nitzschia in sea water with oil kept in collodion bag suspended in flask.

of Nitzschia growth is apparent in both media. An analysis of the results of all the experiments with brine summarized in table 23, shows that the retarding effect is more apparent in the sea water than in the Miquel solution. A 12-percent concentration of brine causes only 10 percent retardation of Nitzschia growth in the Miquel solution, whereas in the sea water retardation averages 41 percent.

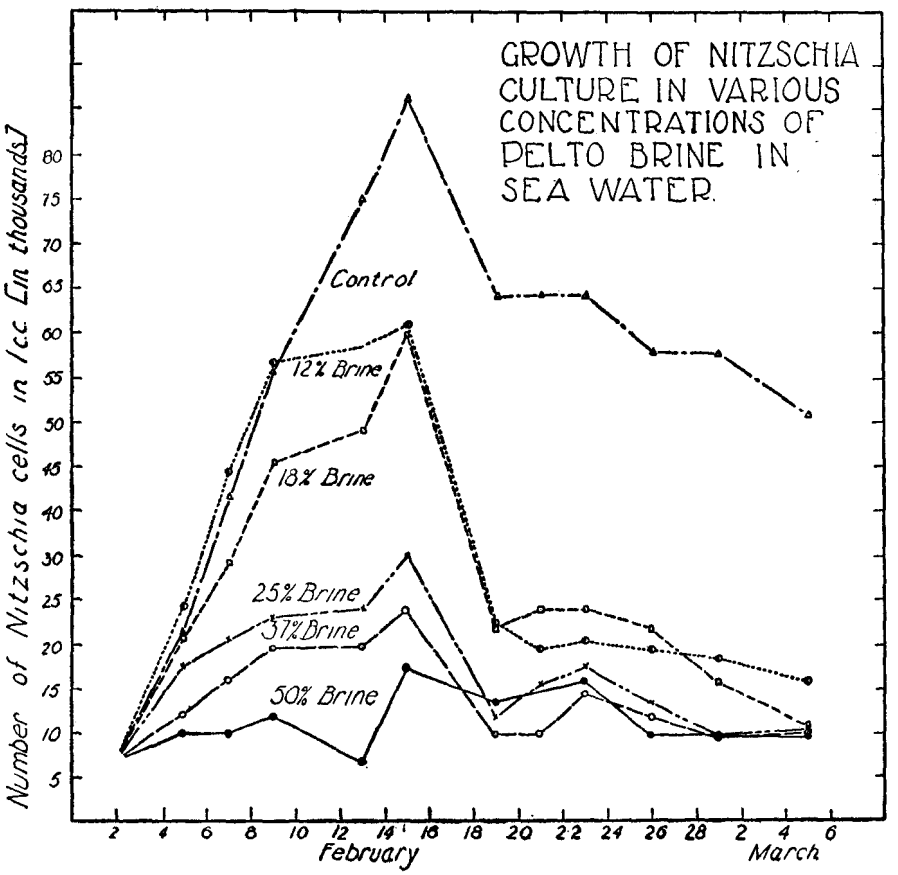

FIGURE 18.—Effect of brine on the growth of Nitzschia culture in sea water.

TABLE 23.—*Retarding effect of brine on the growth of Nitzschia culture*

[Figures in the body of the table indicate percent of retardation]

Concentration of brine	Dec. 23 (16 days)	Jan. 8 (22 days)	Feb. 2 (31 days)	Feb. 2 (31 days)	Apr. 2 (14 days)	Apr. 2 (14 days)	Average percent of retardation
Percent in Miquel solution:							
12	23	11	0	12	8	8	10
18.8	48	3	36	17			26
25	58	28	40	38	35	30	38
37.5			75	63			69
50			93	90			92
Percent in sea water:							
12		13	66	40		46	41
18		36	74	61			57
25		31	75	79		80	66
37			76	87			82
50			77	99			88

Both in the sea water and in Miquel solution the retarding effect is directly proportional to the concentration of brine (fig. 20).

From the results of the experiments it becomes apparent that brine contains substances which interfere with the growth and rate of propagation of the diatom.

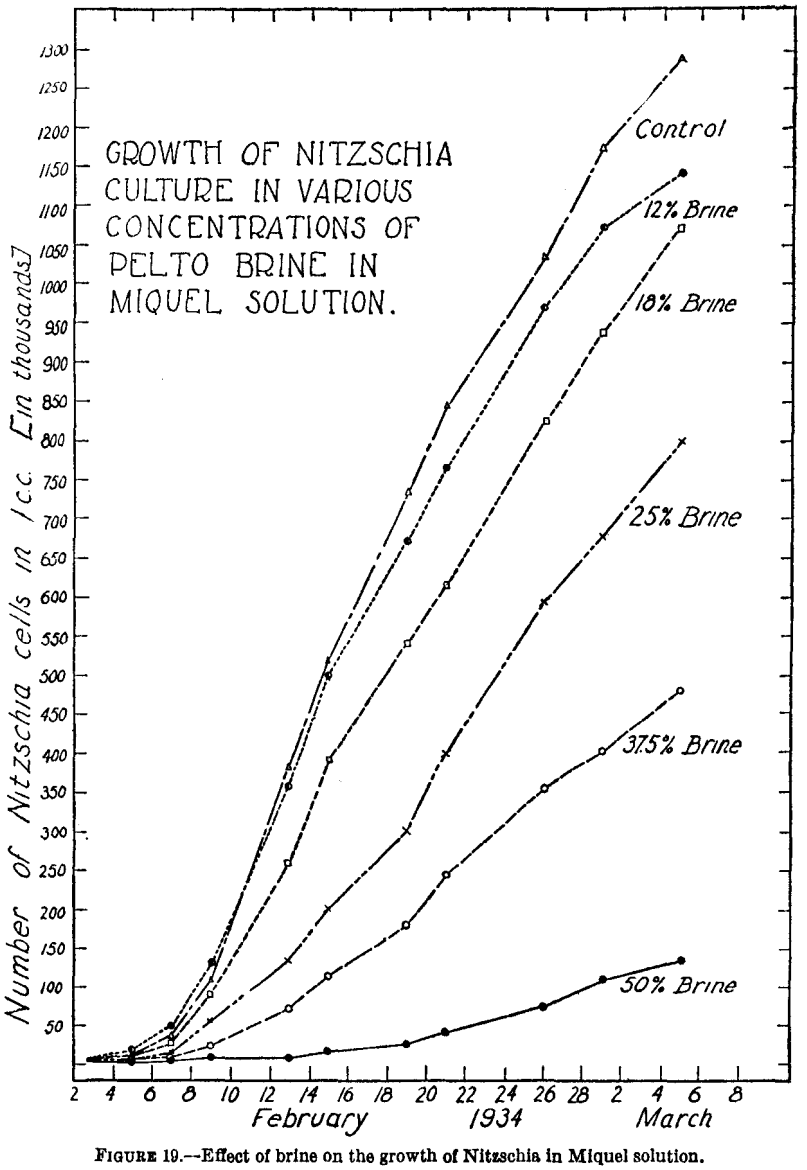

FIGURE 19.—Effect of brine on the growth of Nitzschia in Miquel solution.

The experimental work reported in this part of the report provides sufficient evidence of the possible adverse effect of crude oil and bleed water on the food supply of oysters and other plankton feeding animals of the sea. Probably the retarding effect of crude oil on the rate of diatom growth is primarily due to the toxic action of its water-soluble constituents. The extraction of this substance or substances is undoubtedly facilitated by the action of wind and current. Oil absorbed by mud

and deposited on the bottom may be therefore regarded as a source of potential danger to the microscopical algae. When the bottom is stirred by passing boats or by a strong wind action, a certain amount of oil may again become released and float in

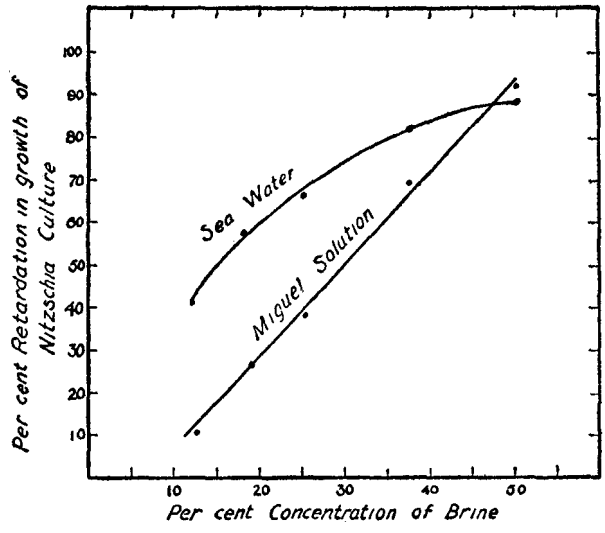

FIGURE 20.—Retardation in growth of Nitzschia culture in sea water and Miquel solution caused by various concentrations of brine.

the water giving off water-soluble toxic substances. The adverse effect of brine is even more pronounced than that of the oil.

DISCUSSION AND CONCLUSIONS

By PAUL S. GALTSOFF

Ecological and hydrographical observations presented in the first half of the report describe the conditions of the oyster bottoms affected by the oil-well pollution. Preliminary investigations carried out by Prytherch in 1933 failed to reveal the existence of a direct correlation between the intensity of mortality and the distance between the affected oyster bottoms and oil wells. This is evident from an examination of the chart (fig. 1), showing the intensity of mortality on various oyster beds (black circles) and the location of active oil wells. A number of oysters, barnacles, and green algae were found growing on the piling of oil wells, and no unusual mortality was observed among other organisms. The presence of small numbers of oysters on piling of oil wells was also observed in 1934 by Galtsoff and Smith. Examination of oysters and plankton showed that apparently there was no interference with the development of gonads, spawning, and setting of the larvae. The diseased condition of oysters was evidenced, however, by the loss of muscular tonus and the failure of the adductor muscle to maintain closure of the shell. It is known that if such a condition continues for a long time it results in a stunted growth and abnormal shape of the shell. No unusual changes in the salinity of water and other hydrographic conditions, which might account for a great mortality, were disclosed by these observations.

The oyster enemies, the borer, the boring clam, and the boring sponge, are rather abundant in Louisiana waters. Many dead oysters examined in 1933 showed heavy perforations caused by the boring clam and sponge, but on the other hand, at least

at one station (no. 6, fig. 1), 95 percent of dead shells were not infested by these pests. Oyster growers have not noticed an unusual increase in abundance of oyster enemies, and no evidence has been obtained which would indicate that such an outburst occurred at the time of the mortality in the winter of 1932–33. It is significant that in 1933 the mortality affected chiefly the larger and older oysters of marketable size and in several instances was especially severe among the recently transplanted oysters. Undoubtedly, the practice of overcrowding the beds by planting from 700 to 900 barrels of oysters per acre may be one of the contributing factors which aggravates the situation and in case of adverse environmental changes or the poor condition of the oysters, may materially increase their mortality.

A more detailed survey of the oyster bottoms, made by R. O. Smith in 1934, failed to assign the mortality to any known disturbance of the natural conditions on oyster beds, as for instance, temperature, salinity, current, and invasion of enemies. It has been noticed that in general mortality has been higher on soft, muddy bottoms than on hard ground. At the time this survey was carried out, pollution was noticeable at the mouth of Bayou Grey where the surface of the water was covered with oil for a distance of 3 miles below the wells and there was some mortality on the oyster beds of this section. All shells were covered with a brownish-black coating of tarry consistency and the meats were unpalatable because of the strong oily flavor. Considerable quantities of oil were held by mud, and oily patches appeared on the surface when the bottom was stirred. Light films of oil were observed also in the vicinity of the Lake Barre wells. In 1934 oysters on many beds throughout the region did not become fat until February or March, which points to a possible scarcity of food or to a disturbance in the functioning of the organs of feeding.

The shallowness of the water throughout the oyster-producing region in Louisiana must be regarded as a factor which tends to magnify the action of any polluting substance. Due to stirring by wind, the water carries much suspended matter which may absorb the pollutant, transport it over wide areas, and deposit it on the bottoms far from the source of pollution. Observations in the polluted areas show that on account of the absorption by suspended clay particles, oil quickly disappears from the surface and after being deposited on the bottom, remains there for a long time.

No information was obtained by the two surveys upon which to base an opinion as to the direct cause of mortality, but ample experimental evidence has been accumulated to show that the presence of crude oil in water produces conditions inimical to oysters.

The first series of experiments designed to determine whether oysters could be killed by the presence of oil in the water or by direct contact with oil, gave negative results. Unfortunately because of the circumstances over which the investigators had no control, these experiments were carried out not in Louisiana, but in a different environment at Beaufort, N. C., with uncultivated oysters taken from oyster reefs. Samples of crude oil collected by the State Conservation Department from Louisiana oil wells were shipped to Beaufort and used throughout the experiments. It is quite possible that the results might have been different had Louisiana oysters been used.

In a series of experiments lasting from 2 to 3 months, the mortality of oysters kept in running sea water under a surface layer of oil, and those kept in sea water that passed through oil was not greater than that in the controls. In the experiments carried out under similar but not identical conditions, Gowanlach (1934) observed considerable mortality among the oysters kept in oil-polluted water. The

discrepency between the two sets of experiments may be due to the difference in technique or to the better condition of North Carolina oysters used in Prytherch's experiments.

In another set of laboratory experiments no higher mortality than that in controls was observed among the oysters which, over a period of 6 to 8 weeks, were immersed at regular intervals in oil (table 3, p. 163). In some of the experiments the mortality among the controls was as high as 50 percent, indicating unfavorable laboratory conditions under which the animals were kept. It is possible that these conditions beclouded the effect of oil on oysters.

The fact that oysters survived the treatment with oil does not indicate that they were not affected by it. Analyses made by Galtsoff show slight decrease in glycogen content of oysters kept in the laboratory in the oil-polluted water (table 4). The result may be due either to the disturbance in the functioning of the feeding apparatus of the organism or to the decreased supply of food.

A regular operation of the muscular mechanism involved in closing and opening of the shell is prerequisite for the normal feeding of the oyster. Two sets of experiments, carried out by Prytherch in 1933 and Galtsoff and Smith in 1934, gave identical results showing that the presence of oil has no effect on the mechanism of the adductor muscle.

In the first set of experiments, continuous kymograph records were obtained of 5 oysters which were kept under observation for 3 months. The average number of hours per day the oysters were open was 11.2 for the controls and varied between 10.0 and 13.6 for the experimental oysters. In the second set of experiments, 6 control oysters kept under observation from 4 to 14 days, were open on the average of 10.5 hours daily, whereas the average figure for 10 experimental oysters kept under observation from 4 to 8 days, was 9.6 hours. In both cases the difference is insignificant.

Although the presence of oil in the sea water does not reduce the number of hours the oyster keeps its shell open, and therefore the duration of feeding of the mollusk is not decreased, the rate of feeding is easily affected by the presence of polluting substance. As the feeding of the oyster is primarily dependent upon the amount of water passed through the gills, the rate of pumping of water can be used as a measure of the rate of feeding. The results of the experiments in which the cone method, previously described by Galtsoff (1928) was used, and of those in which the drop counting technique was employed (fig. 5), show that crude oil contains substances soluble in the sea water which produces anaesthetic effect on the ciliated epithelium of the gills. The inhibiting action is not due to the mineral salts that may be leached out in preparing the water soluble fraction of the sample of oil by shaking it with sea water. It is apparent that certain organic compounds of oil are slightly soluble in sea water. This conclusion can be drawn from the observations that after 28 washings with water, the sample of oil did not lose its toxic property and yielded extract, the anaesthetic potency of which was equal to those obtained with the first washings. The inhibiting effect of the water-soluble fraction is proportional to its concentration (figs. 6, 7, 8, and 9). From a large number of experiments summarized in these figures and tables 11, 12, and 13, the inference can be drawn that a concentration between 20 and 30 percent of the soluble fraction will, on the average, reduce the rate of feeding of the oyster to one-half of its normal value (fig. 8).

Under the conditions of the experiments, the recovery of the ciliary motion following the removal of the oil extract, was almost complete. Inasmuch as the

experimental oysters were kept in the extract only for a limited period of time, the result of the prolonged exposure remains to be determined. There was no indication in the present experiments of an increased tolerance in oysters due to repeated treatment.

As it can be seen in tables 11, 12, 13, and 14, there was a large variation in the percentage of depression caused by a given concentration of the soluble fraction on individual oysters. Two explanations suggest themselves. First, there is a possibility that in spite of the precautions taken in preparing the soluble fraction, the toxicities of individual samples were different. Second, the oysters used in the experiments may have different sensitivity and tolerance. The second explanation seems to be more plausible, for the wild oysters used in the experiments at Beaufort, coming from exposed flats, greatly varied in appearance, glycogen content, and other characteristics.

From a large number of experiments with the water-soluble fraction the inference seems to be inevitable that crude oil discharged in the sea, regardless of whether it floats on the surface or, having been absorbed by mud particles, is deposited on the bottom, continuously yields water-soluble substances which narcotize the ciliated epithelium of the gills, thus reducing the rate of pumping of water and, therefore, materially decreasing the amount of food obtained by the organism. This should lead to gradual starvation and weakening of the oyster. The chemical nature of the substances and their concentration in the oil-polluted areas remains to be determined by future investigations.

The effect of brine or so-called "bleed water", which accompanies oil discharged by the wells and is usually dumped into the sea, has been studied by using the same technique as was employed in the experiments with oil. It has been found (table 6), that bleed waters of Lake Barre and Lake Pelto do not affect the muscular mechanism of the oyster in relatively high concentrations, provided the quantity present does not increase the salinity beyond the limits of tolerance. A 10-percent concentration of bleed water may exert a stimulating effect on the ciliated epithelium at least in some of the individuals. The depressing effect occurs at the concentration of 20 percent and higher. A 33–40 percent solution reduces the rate of pumping of water by the gills to 32.6 percent of its normal rate. The percentages of brine which cause this or greater depression are beyond any possibility of occurrence in nature.

Experiments reported in the last section of the paper attempt to throw light on the possible effect of oil and bleed water on the production of the food of the oyster. It has been assumed that the results of the laboratory experiments with Nitzschia, which occurs in the normal habitat of the oyster, and constitutes an important element in its diet, are applicable to other species of diatoms. It has been found that the presence of a heavy layer of oil on the surface of culture flasks inhibits the growth of Nitzschia (fig. 13) when oil remains on the surface for a week or longer. The soluble fraction of oil exerts a retarding effect on the growth of Nitzschia in concentration of 25 percent and higher and when the extract is permitted to act over a considerable period of time. Low concentration may have a slightly stimulating effect. In many instances the addition of the oil extract stimulated the growth of bacteria, small numbers of which were always present in cultures, and caused the death of diatoms.

Water-soluble substances obtained by dialysis through a collodion membrane also exerted a retarding effect on Nitzschia, both in natural sea water and in Miquel solution (table 22).

Bleed water retards the growth of Nitzschia, the inhibiting effect being pronounced in concentrations of 10 percent and higher. The retardation of growth is directly proportional to the concentration (fig. 20).

The experimental evidence presented in the report shows that the discharge of oil into the sea produces profound changes in the normal environment of the oyster. The substances which gradually dissolve from oil in the sea water irritate the delicate ciliated mechanism. In a very dilute solution they may act as stimulants, but in higher concentrations they inhibit the activity of the ciliated epithelium and may bring about complete stoppage of the current of water through the gills. The same substances which reduce the rate of feeding of the organism affect its food supply by retarding the rate of propagation of diatoms. Obviously the presence of oil creates adverse conditions.

In the light of the present investigation, it is easy to conceive that when the constitution of the organism is weakened by unfavorable meteorological conditions, natural changes in the environment or attacks of enemies, the pollution of water with oil may become a deciding factor which may cause irreparable injury and death of the oyster. It is obvious that from the point of view of conservation, the natural oyster resources of the sea must be protected from this danger.

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