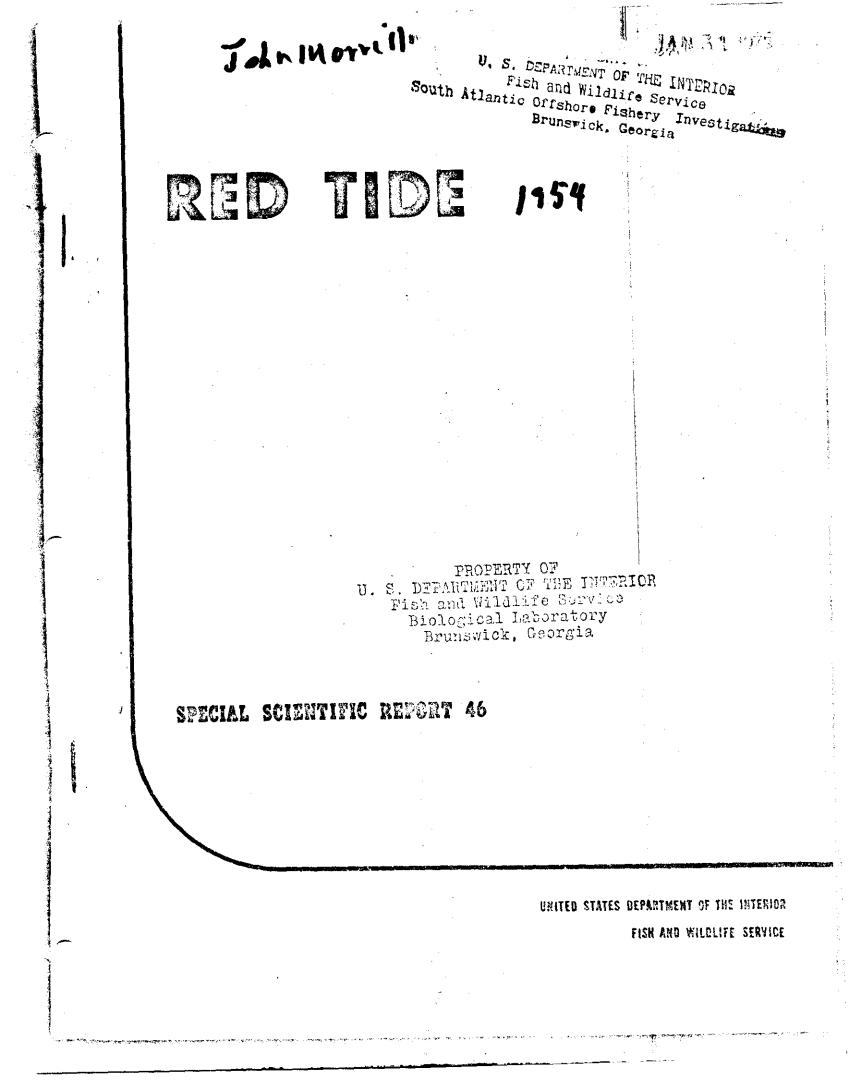
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Explanatory Note

The series embodies results of investigations, usually of restricted scope, intended to aid or direct management or utilization practices and as guides for administrative or legislative action. It is issued in limited quantities for the official use of Federal, State or cooperating Agencies and in processed form for economy and to avoid delay in publication.

United States Department of the Interior, Douglas McKay, Secretary Fish and Wildlife Service, John L. Farley, Director

RED TIDE

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Progress Report on the Investigations of the Cause of the Mortality of Fish Along the West Coast of Florida Conducted by the U. S. Fish and Wildlife Service and Cooperating Organizations

By

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Special Scientific Report No. 46

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This report was issued in a limited quantity in 1948, shortly after the occurrence of red tides off the Florida gulf coast in late 1946 and in 1947. The original supply was soon exhausted. Public interest in red tides has continued since the earlier outbreaks and has increased recently as a result of new outbreaks in the fall and winter of 1953-54. Because it contains information of general interest, this report is reissued, pending preparation of reports on the latest findings of research on red tides.

CONTENTS

	0-
Red tide	· 1
Blooming of the sea	• 3
A review of the literature	• 3
The mortality of fish and the red tide	• • 9
First outbreak of red tide: November 1946 - April 1947	• 9
Fish mortality: June 1947	. 11
Second outbreak of red tide: July - September 1947	. 12
Characteristics and effects of red water	. 15
Color	. 15
Plankton	. 16
Phosphorus content of red water	. 20
Poisonous "gas"	. 23
Toxic effects on fish	. 29
Toxic effects of alcholic extracts of red plankton	. 29
Effect on the rate of cleavage of Arbacia eggs	32
Toxicity of clams from Fort Myers area	• 333
Control measures	• 34
Economic losses	- 35
Conclusions	. 36
References cited	• 39

Page

TABLES

No.	Page
1	Salinity of water near Fort Myers, July 1, 1947
2	Plankton of the samples collected near Fort Myers on July 1, 1947
3	Red water plankton, July 24, 1947, 3 miles off Musketters Pass
4	Total phosphorus content of sea water samples collected in the areas of intense growth of <u>Gymnodinium</u> off the west coast of Florida
5	Chlorinity, pH oxygen content and buffer capacity of samples of Florida water, February 1947
6	Effervescence, drop size and irritating effect of red water and clear sea water
7	Effect of the concentration of 1:1000 of red water extract on <u>Fundulus</u> , August 29, 1947
8	Effect of the concentration of 1:250 of red water extract on <u>Fundulus</u> , fish kept in 500 ml. of water, salinity 31.48, temperature 22° C

FIGURES

No.	· · · · · · · · · · · · · · · · · · ·	Page
l	Dead fish of various kinds and turtles litter Madeira Beach, Fla., at 145th Street, August 1947	45
2	Line of dead fish as Red Tide invades the inshore waters	46
3	Bodies of dead fish (grunts, tarpon, redfish, grouper, and jewfish) floating on surface of water in Gulf of Mexico 8 miles southwest of St. Petersburg Beach	47
4	Mass of dead fish deposited at edge of water at high tide	48
5	Typical view of surface of water during mortality of fish in Gulf of Mexico, August 1947	49
6	Dead fish and horseshoe crabs on Pass-a-Grille Beach, Fla	50
7	Light absorption curve of the red water extract	51
8	Spraying dead fish with DDT and deodorants, Madeira Beach, Fla. August 1947	52
9	Bulldozers used in burying dead fish in sand	53

RED TIDE

Beginning in the latter part of November 1946, the fishermen operating along the Gulf Coast of Southern Florida near Naples reported large numbers of dead and dying fish floating in the area extending from 10 to 14 miles offshore. The mortality of fish increased in intensity and moved northward, reaching Boca Grande early in January. Late in January 1947, it extended as far north as Sarasota. The bays and beaches at Fort Myers, Captiva, Sanibel Island, and other places were littered with millions of dead and dying fish which presented (Fig. 1) a serious problem of disposal.

The catastrophic destruction of fish coincided with the appearance of large streaks of reddish-brown water, popularly known as "red tide" (Fig. 2), which extended several miles offshore. Fish entering these discolored patches were reported to be killed quickly.

Lacking funds and personnel needed for an immediate and comprehensive study of this phenomenon, the U. S. Fish and Wildlife Service asked the cooperation of the Marine Laboratory of the University of Miami and the Woods Hole Oceanographic Institution. It was fortunate that Doctors F. G. Walton Smith and Gordon Gunter of the Miami Laboratory, upon receiving information from the Washington office of the Service, were able to visit the West Coast of Florida about the middle of January, collect samples of water, and make observations which throw light on the mysterious mortality. Their preliminary report has been published in Science (Gunter, et al, 1947) and a more detailed account of their observations was submitted to the Service (Gordon Gunter, Robert H. Williams, Charles C. Davis, and F. G. Walton Smith, 1947, Catastrophic Mass Mortality of Marine Animals and Coincident Phytoplankton Bloom on the West Coast of Florida, November 1946 to May 1947; manuscript in files of the U.S. Fish and Wildlife Service.)

The Woods Hole Oceanographic Institution agreed to make chemical analysis of water from the affected area. The samples were not collected, however, until the end of February when the sea was already returning to normal conditions. Chemical analysis revealed nothing abnormal in these samples. In March 1947, the discoloration of the water disappeared and no dead or dying fish were found floating on the water or cast on the beach. Apparently the sea returned to normal conditions.

During the summer of 1947 the red tide reappeared in the same area along the coast of Florida. The violence of the associated fish mortality was even greater than during the winter. The water was deeply discolored and millions of pounds of dead and dying fish were washed ashore or carried away by tides. Observations and collection of samples of water, plankton, and fish were made by the Fish and Wildlife Service, the Miami Laboratory, Woods Hole Oceanographic Institution, and the Food and Drug Administration of the Federal Security Agency. Large samples of plankton and water were obtained and forwarded for chemical, microscopical, and toxicological studies by these agencies.

The destructiveness of the red tide inflicted serious monetary losses upon local communities, and, as will be shown later, constituted a hazard to public health.

Various aspects of this disastrous and mysterious phenomenon require detailed studies for our present knowledge of the red tide, based on preliminary and incomplete observations made since January 1947 and on a study of available literature, is too fragmentary to permit drawing definite conclusions regarding the origin, chemical nature, and mode of action of the red water toxin. Several leads discovered by these observations may be helpful, however, in formulating plans for a more comprehensive study which may be undertaken as soon as the Service has funds for its execution.

The present report attempts to summarize and evaulate the preliminary data assembled during the past winter and summer and provides a background for a special research project on the red tide and its effect on marine populations, which it is hoped will be undertaken in the near future.

BLOOMING OF THE SEA

A review of the literature

Under favorable conditions some of the aquatic microorganisms may multiply so rapidly as to cause noticeable discoloration of the water. This "blooming" may take place in fresh water ponds and lakes, in the reservoirs supplying drinking water, in bays and estuaries, and in the open ocean. The exact conditions which favor rapid propagation of one species, so etimes almost to the exclusion of others, are not known; neither has it been ascertained what causes the end of such an outburst of propagation and the return to normal conditions.

The list of microorganisms responsible for the "blooming" comprises bacteria, diatoms, blue-green algae, flagellates, and crustacea. Depending on the color of the pigment of these forms the sea water acquires yellow, green, blue-green, or red hues. A bluegreen or green discoloration of water is such a common occurrence in our fresh water ponds that it fails to arouse the layman's curiosity but the incidents of so-called "red water", "red tide", or "bloody seas", never fail to attract public attention. Furthermore, the "red water" or "red tide" is often associated with extensive mortality of fish and other marine animals.

One of the earliest published records of the observation of red water in the sea is probably that of Charles Darwin made during the voyage of H.M.S. Beagle in 1832. He writes (1860), "On the coast of Chile, a few leagues off Conception, the Beagle one day passed through great bands of muddy water exactly like that of a swollen river...Some of the water placed in a glass was of a pale reddish tint and, examined under a microscope, was seen to swarm with minute animalculae darting about and often exploding. Their shape is oval and contracted in the middle by a ring of vibrating curved ciliae." Judging by this description, the microorganism observed by Darwin was probably one of the naked dinoflagellates, which frequently swarm on the surface of the sea.

The appearance of a red discoloration in fresh water lakes was recorded, however, long before Darwin made his observation. According to Morren (1841) old historical records indicate that in 208 B.C., the water of the Lake Bolsena (also known as Vulsiniensis or Vulsinus) near Rome turned red; a similar phenomenon was recorded in 586 A.D., near Venice, Italy, (Graesse, 1909).

Carter (1858) attributed the various hues of sea water around the island of Bombay to a dinoflagellate, <u>Peridinium sanguineum</u>, and Collingwood (1868) summarized a large number of observations on the occurrence of microscopic algae which cause discoloration of the sea in various parts of the world.

A red discoloration of water is also caused by purple sulfur bacteria. The first detailed accounts of it are given by Warming (1875, 1876) who studied the red water along the Danish Coast. Recent investigations by Utermohl (1925, 1931) disclosed that red water of the swamps along the coast of Denmark was due to the sulfur bacteria (Chromatium, Thiopedia, and Thiocystes) which were present in concentrations varying from 6,000 to 7,000 bacteria per cubic centimeter. Purple bacteria are frequently associated with the decomposing plankton and require the presence of H₂S and sunlight for their maximum development (Gietzen, 1931). In Sicily, Forti (1933) found that the reduction of sulfates provided the source of H2S for the prolific growth of Thiopolycoccus ruber, Thiopedia rosea and other forms responsible for the Sicilian "lake of blood," The distribution of sulfer bacteria is widespread (Ellis, 1932). Their occurrence in the Black Sea (Egunov, 1895, 1901; Knipovitsch, 1926; Issatchenko and Egorova, 1939) and in the Caspian Sea (Knipovitsch, 1938, Brujewicz, 1937, p. 77) is of particular interest because of the role these microorganisms play in the oxidation of H2S which accumulates in deep water or in certain portions of these seas.

The red color of sea water is sometimes caused by the abundance of a blue-green alga, <u>Trichodesmium erythraeum</u>. As a matter of fact, the Red Sea and the Vermilion Sea (Gulf of California) were so named because of the frequent coloration of their waters caused by the preponderance of this species in the plankton (Sverdrup, et al, 1942).

The rapid growth of marine diatoms resulting in a brownish discoloration of sea water rarely attracts public attention, but periodic outbursts of rapid propagation of a diatom <u>Aulacodiscus</u> <u>kettoni</u> along the shore of Copalis Beach in the State of Washington are of particular interest to marine biologists and petroleum geologists (Becking, et al, 1927). Under a certain combination of meteorological conditions the development of this diatom is so rapid that thick brown patches of almost a pure culture of this species float on the surface and within a few hours extend over several hundred feet in area. Eventually they are deposited by surf on the beach in layers several inches thick.

Outbursts of sudden propagation of various species of dinoflagellates frequently occur along the coast of California. The earliest records made in 1746 by a missionary, Gonzag, describes a discoloration of water along the coast of California extending about one-half mile offshore (quoted from Issatchenko, 1914, p.243). In 1902, Torrey described the deep discoloration of coastal water over an area extending from Santa Barbara to San Diego. Allen devoted a number of papers to this subject (1921, 1922, 1928, 1933, 1935). Discoloration of water near La Jolla, California, caused by Prorocentrum micans, was reported by him to have occurred in the years 1906, 1907, 1917, 1924, 1933 and 1935. In 1933 the yellow color persisted from May 17 to May 31, when a marked reduction in its intensity was noticed. The zone of discoloration, about onehalf mile wide, extended for about three miles along the coast. Besides Prorocentrum micans, the plankton contained <u>Ceratium tripos</u>, the ratio between the two forms being 50:1. Of special interest is the mass occurrence in water of <u>Gymnodinium</u> <u>splendens</u>, <u>G. sanguineum</u>, <u>Gonyaulax catenella</u>, and other species of <u>dinoflagellates</u> which have been considered responsible for the mortality of pearl oysters in Japan (Miyajima, 1934) or for the toxicity of sea mussels on the California coast (Sommer, et al, 1937). According to Japanese sources, outbreaks of "red water" in the Gulf of Konsa and in Gokasho and Ago Bays, the principal locations of pearl oyster farms, occurred in 1911, 1922, and 1933, each time causing widespread mortality of pearl oysters.

Outbreaks of red water in the lower part of the Puget Sound area were also attributed to mass reproduction of <u>Gymnodinium</u> <u>splendens</u> and <u>Gonyaulax</u> <u>alaskensis</u>. The concentration of these microorganisms in water (Nightingale, 1936) varied from 37 to 15,800 cells per cubic centimeter. No mass destruction of fish or shellfish associated with red water has ever been reported from this area.

The occurrence of "red water" along the coast of Peru and the ensuing mortality of fishes are of particular interest because of their frequencies and intensity. This condition, which is characterized by discoloration of the surface water, extensive fish mortality, and liberation of H₂S, is known locally as "aquaji." According to Gunther (1936) it may result from a sudden rise in temperature and convergence of the abnormal counter current known as El Nino. The exact cause of the cataclysm which causes fish mortality along the west coast of South America has not been ascertained. Gunther (1.c.) mentions, however, that the orange colored water at Pisco contained large quantities of a flagellate with red pigment.

Similar mortalities and discoloration of water along the Malabar coast were described by Hart (1934) and by Hornell (1917). Hornell (quoted from Gunther, l.c.) states: "All Malabar fishermen agree in saying that every year after the passing of the rainy season and the subsidence of the S. W. monsoon, if there be a continuance of fine weather for a week or ten days, with plenty of sunshine, and a weak coastal current, the water inshore becomes turbid and discolored, brownish or reddish in tint; that this water has such poisonous effect upon fish that large numbers become affected and eventually die. The first effect of poison is to make the fish eluggish, and at this stage, as I have myself seen, boys and men crowd to the shore and make great hauls of the dying fish. Fishermen further state that if favorable conditions continue the

color of this foul water changes and becomes distinctly redder, and emits a stench so strong as to be almost unbearable; when this occurs they state that the poisonous influence increases and fishes of kinds not affected during the first onset of the poison die and are cast ashore. They agree fairly generally that sardines are seldom affected in any quantity. Some stated they saw widespread sardine mortality. In these cases the sea was colored for miles with dead and dying sardines in enormous multitudes." Hornell states that in almost all cases the discoloration was due to swarming of Euglenids to the virtual exclusion of all other organisms.

A serious destruction of oysters and mussels by red water at Port Jackson, Australia, was recorded by Whitelegge (1891); the discoloration was attributed to a dinoflagellate, Glenodinium rubrum. Spectacular destruction of squeteague in Narragansett Bay was described by Sherwood and Edwards (1901). For two weeks or more in September the <u>Peridinium</u> infested the waters of the upper bay in such numbers that the water was almost a blood-red color and, as a result, many young squeteague, together with fish of several other species, perished and were piled in windrows on the shore.

The swarming of dinoflagellates has not always resulted in fish mortalities. The author personally observed their mass appearance along the northern coast of Massachusetts, along the coast of New Jersey (samples collected by Chipman, October 1947, unpublished report of U. S. Fish and Wildlife Service), and in the bays and rivers of the Maryland and Virginia coasts without causing any ill effect on fishes or other aquatic animals. The red water observed in October 1947, off the New Jersey coast contained a large number of Noctiluca. The swarming of dinoflagellates (Amphidinium fusiforme) was recorded by Martin and Nelson (1929) in Delaware Bay and Powers (1932) describes the presence of <u>Mesodinium rubrum</u> in the Gulf of Maine. In neither case was the red water accompanied by mortalities of aquatic animals.

With reference to the recent mortality of fishes along the Florida coast, it is of particular interest to ascertain whether similar destruction was recorded in the past in any part of the Gulf of Mexico. It is well known that mass destruction of fishes caused by freezing weather frequently occurs in shallow waters along the coast of Texas. One of the earliest records can be found in a letter from Brig. Gen. R. B. Marly to Prof. Spender F. Baird, quoted in the Annual Report for 1882 of the U. S. Fish Commission, p. 275. This interesting document states, "you will remember that our troops under General Taylor passed the winter of 1845-46 at Corpus Christi, Texas, and while there we one night were visited by a pretty heavy frost, and much to our astonishment, the beach in the vicinity of our camp the following morning was thickly strewn with fish and green turtles that had floated from the Gulf." Winter mortalities of fish due to a sudden drop of temperature were described by Gunter (1941) and Baughman (1947) of the Texas Game and Fish Commission. It is obvious that the cause of the mortalities is quite different from the destruction of fish by red water.

The earliest incidents of extensive destruction of fish along the west coast of Florida, some of which apparently were associated with red water, occurred in 1814, 1854, and 1880 (Ingersoll, 1882). From a description published on July 20, 1882, in the Sunland Tribune, Tampa, and reprinted in the Bulletin of the U. S. Fish Commission, Vol. II, for 1882, p. 104, we learn that Capt. William Jackson of the Steamer "Lizzie Henderson," on his trip from Cedar Key, encountered "a streak of poisonous water, covered with all varieties of dead fish, of more than a mile extent, off Indian Pass, between Clearwater and Egmont Light. The Captain says that a very offensive smell arose from it, and that a good many bottom fish, such as eels, were floating dead on the surface."

Two years later the Report of the U. S. Commission of Fish and Fisheries (for 1884, p. XLII) states again: "The occurrence of extended mortality among the fish, both of the fresh waters and of the sea, has been a subject of much interest, and the attention of the Commission has been specially attracted to the determination at least of the causes even though they be so general in their action as to be apparently incapable of cure."

"Several accounts have been given of the occurrence, at short intervals of fish pestilences in the Gulf of Mexico, where for weeks at a time, in particular regions, the surface will be found covered with dead or dying fish of all kinds that inhabit the waters. Thousands of tons are estimated to be thus destroyed. Nothing satisfactory has yet been indicated as to the origin of this difficulty. The fish, themselves, do not appear to be diseased in any way. A correspondent of the Commission, however, has suggested that, owing to some unusual condition, the cold waters of the deeper parts of the Gulf are brought near to the surface, where they affect these fish, the sudden chill producing such a shock as to cause either death or temporary disturbance of health. The waters in which these occurrences take place are said sometimes to be discolored as if by the presence of microscopic forms of either animals or plants; and it is not impossible that a careful search, prosecuted by an expert on the spot, may solve the problem."

The cause of the poisonous water in the Gulf of Mexico was discussed again in 1886 by John G. Webb (1887) who attributed the mortality of fish to "noxious and poisonous gases which permeate from portions of the Gulf and its bays and which are derived from underground streams of water that flow into the sea." Unfortunately,

no samples of water were collected at this time for analysis and no other observations were made to substantiate the explanation suggested by Webb. The reference to the discoloration of water appears, however, significant and suggests that the mortality of fish in 1882 was associated with the unusual reproduction of some planktonic microorganisms.

Reference to poisonous water along the Florida coast was also found in connection with the descriptions of mortality of sponges which was often ascribed to the drainage of "black" or "poisonous water" from the Everglades. In 1881 in a letter to Prof. Spencer F. Baird, the first U. S. Commissioner of Fisheries, Ingersoll gave the following vivid description of this occurrence (quoted from Collins, 1885).

"This discolored water appeared in long patches or streaks sometimes 100 yards wide, drifting lengthwise with the flow of the tide. The earliest indication of it was the floating up of vast quantities of dead sponges, chiefly loggerheads. All those seen by Mr. Brady were less than 40 miles north of Key West in what is known as 'The Bay' nor has anything of the sort been seen at any time outside (i.e., southward or eastward) of the Florida Reefs."

Several theories were advanced to account for the disaster. According to one, the appearance of the black water was due to the overflow of swamp waters from the mainland. Another theory attributed the origin of black water to the subterranean disturbances which gave rise to poisonous gases which ascended and polluted the sea water, causing general destruction of marine life in the affected area.

In 1908 Moore (1910) reported that the sponge beds in the Jewfish Bush Lakes and to the eastward were destroyed by "poison water." He considered that this destruction was due to fresh water from the Everglades, impounded by the railroad embankments of the Key West extension.

Severe mortality of fish occurred again in October and November 1916, appearing progressively southward from Boca Grande to Marco, and killing all local species of fishes, but not affecting fresh water forms. This mortality was studied by Taylor (1917) who, however, failed to arrive at any conclusion regarding its cause. No red water was observed by Taylor, although the discoloration of the sea was reported by others. With regard to dinoflagellates he writes: "Peridini appear to furnish an exceedingly unlikely explanation but--the evidences contrary to such an explanation are not altogether convincing." During the 30-year period following the mass destruction of fish in 1916, the conditions along the west coast of Florida remained normal; at least no mortality of fish was noticed or reported. In the winter of 1938 an unknown disease inflicted great damage upon the commercial sponges of the Bahama Islands, spread rapidly throughout the West Indies, and by the spring of 1939 reached the northwestern coast of Florida. Here its ravages gradually diminished but not before a large number of sponges had perished. Only the horny sponges were affected by the disease, which wiped out from 60 to 90 percent of the adult populations of velvet and wool sponges, (<u>Hippiospongia gossypina</u>, and <u>H. lachne</u>), of the Bahama Islands. Noncommercial species were not affected, and no signs of unusual mortalities were noticed among other invertebrates and fishes. No discoloration of water was observed in the entire affected area and water conditions remained normal.

Investigations conducted in the Bahama Islands (Galtsoff, et al., 1939), and later on in Florida waters (Galtsoff, 1940) showed that the mortality was associated with a spread of a fungal infection, affecting only certain species of horny sponges.

THE MORTALITY OF FISH AND THE RED TIDE

First Outbreak of Red Tide: November 1946 - April 1947.

The beginning and the spread of fish mortality along the west coast of Florida can be reconstructed from verbal or written reports and statements made by local fishermen and residents of the shore properties. Unfortunately, at the first signs of mortality, in the latter part of November 1946, no Federal or State biologists were engaged in any field studies in this section of the coast. Competent observers arrived at Fort Myers only about the middle of January 1947 and began to make observations and take records of dead and dying fish when the event had already passed its peak (G. Gunter, F. G. Walton Smith, and R. H. Williams, 1947).

The following picture can be reconstructed, however, from an appraisal of widely scattered and sometimes not too critical reports of fishermen and laymen. Dead and dying fishes and turtles were first noticed in red or brownish water on or about November 20 by mackerel fishermen fourteen miles offshore from Naples. The "red tide" spread northward, reaching in December and January the inshore waters around Samibel and Gaptiva Islands, as far north as Boca Grande Pass. At the same time fish continued to die and millions of dead carcasses were floating in the water or were cast ashore. All the beaches in the Fort Myers area became littered with dead bodies which were reported to accumulate at the rate of more than 100 pounds per linear foot of shore line. In February 1947, dead fish were washed ashore on Englewood Beach, marking the northernmost extent of mortality. It is quite probable that these fish perished in the Boca Grande region and were carried northward with the currents.

According to Gunter and Smith (unpublished report in files of the Service), no mass mortality occurred north of Pine Island Sound, however, some "sick" cobia, <u>Rachycentron canadus</u>, were caught by the fishermen 10 miles off Pass a Grille Beach, off the mouth of Tampa Bay. In the opposite direction, Cape Romano marks the southernmost limit of mortality.

According to the observations of Fort Myers fishermen and some of the residents of the Captiva Island who assisted the Service in obtaining water samples for analysis, the strip of discolored water extended from 5 to 8 miles offshore. Beyond that zone the sea water was clear and of normal dark blue hue.

Inasmuch as no air survey was made at this time the exact extent of red water in which fish were dying in large numbers cannot be accurately determined. In general, it may be concluded that the affected area comprised several hundred square miles along the shore from Cape Romano to Boca Grande Pass.

From the accounts of fishermen and residents of the shore properties, as well as from more critical observations made by Gunter and Smith in January, and by the author in March 1947, the conclusion can be drawn that mortality was quite common. All kinds of animals perished in the red water, including a small number of turtles and porpoises. Windrows of dead fishes piled on beaches comprised a great variety of common commercial and noncommercial varieties (Fig. 1). Likewise, the pelagic and bottom invertebrates succumbed to the unknown poison. Large numbers of shrimp were seen dead, as well as common blue crabs, fiddler and mud crabs, barnacles, oysters and coquinas. Observations made by the author in March 1947, around the Fort Myers area, disclosed that about 80 percent of the oysters, Ostrea virginica, grown on piles, were dead. Clean inner surfaces of their shells, free from any fouling organisms, indicated that death had occurred only recently. The surviving oysters were in good condition in spite of the general infestation of their tissues by the spores of a gregarine -- Nematopsis -- a very common parasite of oysters in southern waters.

No mortality was observed among the hard shell clams, Venus mercenaria, and no reports were received of the destruction of ducks, gulls, and other birds inhabiting the inshore waters. Later during the summer information was received from residents of Largo, Florida, that "thousands of sea gulls and pelicans died from eating the fish poisoned by the red tide." The correctness of this observation has not been verified by the author.

After the end of January a few scattered outbreaks of red water and fish mortality were reported in April from Key West and Marathon (Florida Bay), and from Cape Sable, but the damage was insignificant and apparently of a very short duration.

Fish Mortality: June 1947

Late in June 1947, reports were received by the Service of the recurrence of fish mortality in the Fort Myers area. The case was investigated by Wm. W. Anderson of the Service who interviewed a number of persons including Mr. S. W. Smith, President of the Caloosahatchee Conservation Club, commercial fishermen and guide boat operators, and made personal observations. According to this information the first incidence of the death of fish was noticed on June 21. The mortality increased up to June 28 and, as on previous occasions, was rather indiscriminate, affecting a great variety of fishes. Examination made on June 30 of the decaying remains of fish disclosed a preponderance of catfish, which accounted probably for 50 percent of the observed specimens. More accurate quantitative estimate was not made, however, since the beach had been already partially cleaned. Other dead fish comprised pinfish, porgies, white and spotted trout, cowfish, spiny box fish, moonfish, spot, mullet, eel, sand-bream, whiting, thread herring, hogchoker, tongue fish, yellowtail, tripletail, redfish, and drum. Very noticeable among the decaying remains were the carcasses of horseshoe crabs. In spite of a large variety of dead fishes, the June mortality was very light in comparison to that of the preceding winter.

The first fish kill in June occurred from 5 to 7 days after excessive rains, which resulted in the run-off of fresh water in the coastal bays and out through the passes along the beach area. On June 30, the fresh water was still flowing into the bays. On July 1, when a boat trip was made by Anderson over the affected area, the flow of fresh water abated and the bays were clearing.

Reconnaissance survey made by air on June 30 disclosed no red or yellow patches of water. The only discolored water was noticed off the passes where it was brownish, due to the fresh water.

Only a few scattered dead fish were observed in the bays and for several miles off the beaches. In one offshore section there was a large number of dead fish in an advanced state of decomposition. Upon examination they were found to consist primarily of thread herring (90 percent) and the remaining 10 percent of toadfish and eels.

No difference in the color of the water could be detected between the areas where dead fish were floating and those in which there were none. Throughout the entire area the water was a greenish color, typical for the littoral section of the Gulf.

From those observations and from the analyses of water samples discussed below, it appears that this local mortality of fish was not due to red water and probably was associated with heavy rains and sudden discharge of fresh water into the bays.

Second Outbreak of Red Tide: July - September 1947.

Early in July 1947, the red water reappeared along the west coast of Florida, off Venice. Mr. Wm. W. Anderson was again detailed to make the necessary observations and to collect samples of water and plankton for chemical analysis and microscopic examination. He reports as follows: "From conversations with numerous fishermen, guide boat operators, and other interested persons, it was learned that the red water first appeared off Venice and areas to the south about July 6. This colored water was first observed in streaks about 3 to 6 feet wide and a hundred yards long from just off the beaches to approximately 10 miles offshore (Fig. 2). It rapidly spread and in about one week was continuous from the beach out to approximately 15 miles offshore.

"On July 21 observations were made by airplane. Evidence of red tide was found from a point about one-half way up Longboat Key (between Sarasota entrance and Tampa Bay entrance) south to Captiva Island (a short way above Fort Myers entrance). Several runs were made offshore and the infected water at points extended as far as 18 to 20 miles off the beaches.

"From observations of aviators and fishermen it appears that the infected waters were slowly moving northward and at the time of my departure from Sarasota on July 30, amber water was reported to be as far north as Anna Maria Key almost to Tampa Bay. According to an Associated Press release of August 5 from Clearwater, Florida, the red water had by that date reached approximately 15 miles north of Clearwater and was still moving northward." Observations made by the Service on August 15 by low-flying plane over the section of the coast between Captiva Island and approximately 20 miles north of Tarpon Springs and 20 miles offshore disclosed the presence of red water approximately 5 miles north of Egmont Key (entrance to Tampa Bay) to a point almost directly off Tarpon Springs. The affected area extended generally from just off the beaches to an irregular line from 10 to 15 miles offshore. Numerous dead fish were observed within the red area (Fig. 3).

North of Egmont Key and south of Captiva Island the water appeared on August 15 clear and blue. According to the report of fishermen the water in that section cleared rather suddenly on August 7 or 8 and since that time has remained blue.

At Tarpon Springs the red water was noticed by sponge fishermen about August 8 and since that time has been fluctuating about this point. No dead fish were, however, found in the harbor at Tarpon Springs.

While an accurate estimate of the quantity of fish killed is hardly possible, the total will no doubt run into millions of pounds. To the thousands of tons that were deposited on the beaches (Fig. 4), must be added a great quantity that never reached the coast, but floated and disintegrated in the offshore areas. On one occasion, after several days of winds from off the land, a mass of dead fish was located approximately 10 miles offshore from Venice. These fish were packed tightly together in a band some 100 to 200 feet wide and extended for miles (Fig. 5). Besides these areas of densely packed dead fish, one was seldom out of sight of dead fish bobbing on the surface.

No attempt was made to count dead fish by species, for virtually all varieties were equally affected. An exception was the pelagic species such as mackerel, bonito, et cetera, which were not found on beaches. In the opinion of mackerel fishermen these fish would not float when dead. Sponge divers working last winter off Marco, Florida, reported that the bottom was littered with dead mackerel, although rarely were these fish found on the beaches.

Although few dead crabs were observed by Anderson, this may be due to the fact that these animals tend to sink upon death and would, therefore, be less noticeable. On the other hand, he frequently observed small species of crabs swimming in the infected waters in apparently good condition. On several occasions crabs were observed feeding on dead fish at the surface. He thinks that it is entirely possible that these crustaceans were less affected by the red water than were the fish. Horseshoe crabs (<u>Limulus</u> polyphemus), however, suffered a heavy mortality and thousands were washed onto the beaches and, in addition, quantities were observed floating in the Gulf.

While there are no extensive oyster beds in the vicinity of Venice, observations on "coon oysters" growing in clumps on the rocks just inside Venice Inlet, showed evidence that numbers of these oysters had been recently killed. At the same time numerous hermit crabs were found alive among these same rocks.

From field observations made in July and August, Anderson draws the conclusion that great numbers of fish of the inshore areas have been killed wherever tides carried the red water into the bays. In this respect, Clearwater suffered probably more than any other community. On August 22 fish were still dying in Clearwater Bay, while dead carcasses from the outside waters were driven by the onshore wind through two wide passes and cast almost right onto the streets of the city. The same situation was observed in a number of other places (Fig. 6).

Inquiries made among the Tarpon Springs sponge-boat captains, divers, and other persons engaged in the sponge industry, disclosed no evidence of any damage to sponge grounds by red water.

CHARACTERISTICS AND EFFECTS OF RED WATER

Color

The coloration of the water in which the fish died was described as green, greenish yellow, yellow, amber, brown, reddish, and red. The sequence of changes from clear, blue water of the sea to slightly turbid and yellowish was very gradual as the boat approached the affected area. Undoubtedly, the exact color depends on a number of conditions, such as the abundance of pigmentbearing microorganisms, presence of suspended nonliving material, and the angle of the reflected light. In the minds of local residents and fishermen, the deep amber color was definitely associated with the mortality of fish. Some of the samples of concentrated plankton collected in August in the Fort Myers area and delivered to the Woods Hole laboratory for examination had a deep reddish-brown color. Both the plankton and the water, to which small amounts of chloroform or formalin were added as preservatives, were of the same color. Spectrographic analysis was made at Woods Hole by Dr. Eric G. Ball of the Harvard Medical School. The light absorption curve of the sample of red water collected on July 24, 1947, three miles off Musketeers Pass, is presented in Figure 7.

In the wake of the ship the water had an oily appearance. When dipped up and allowed to stand for 5 to 10 minutes, it became thick, sometimes almost of a consistency of Karo syrup, and slimy to the touch. Strands of mucus were easily discernible in the preserved sample. These characteristics were much more pronounced in the deeply colored water and apparently were associated with the abundance of pigment-bearing organisms.

Differences in the surface tension in the red and normal water were recorded by Woodcock in his microscopic examination of the droplets collected on slides treated with lyophobic substance. These results of his work are presented in a separate section of this summary.

Within the area of the sea affected by the red tide, the intensity of the color was not constant. As a matter of fact, all the observers agree that the color was variable and occurred in patches, which may range from a few square feet to areas about 200 feet wide and several hundred feet long. The analysis of plankton samples shows that the intensity of coloration was proportional to the abundance of plankton.

Plankton

Plankton samples were collected by towing a number 20 net through the discolored water and were preserved in formalin. The portion of the samples sent to the Service's College Park laboratory in January 1947 and examined by the author contained both animals and plants represented by a number of species commonly found in littoral waters. There was a considerable amount of unidentifiable masses of jelly-like mucus with small granules probably derived from the cells of dinoflagellates damaged by preservatives. Some of the dinoflagellates were sufficiently well preserved to recognize them as a species of the genus Gymnodinium. The colonies of the blue-green alga, Trichodesmium sp., were equally abundant. Common pelagic diatoms of the genera Chaetoceros, Coscinodiscus, Rhizolenia and Navicula were also present, but much less abundant than Trichodesmium and Gymnodinium. Zooplankton was very abundant. the predominating forms being annelid larvae, which were very numerous, Rotatoria, and larvae of gastropod and lamellibranch mollusks. Of the latter group the clam larvae (probably Venus) were quite conspicuous in the sample taken at Boca Grande Pass. The remainder of the zooplankton comprised copepods and their larvae, mostly of the copepod stage. The yellow color of water may have been caused by Gymnodinium which contains brown or reddish pigment granules, or by bundles of Trichodesmium sp. containing blue-green and yellowish pigment. The slime or mucus observed in the samples of water may have derived from either form.

In the sample obtained by towing of a number 20 plankton net, the numerical relationship among the various members of plankton does not represent their actual relationship in the sea. Because of the small size of Gymnodinium, measuring about 30 microns. this dinoflagellate easily passes through the meshes of plankton cloth and escapes capture. Thus, in a sample collected by means of a plankton net, the number of Gymnodinium would be much smaller than that of the larger forms -- as for instance, Trichodesmium, Chaetoceros and all zooplankton, which are caught in relatively greater proportion. For a correct quantitative sampling, other methods, such as centrifuging or filtering through collodion membranes, should be used.

On July 1, 1947, seven samples of plankton were collected by W. W. Anderson in the Fort Myers area by using a foot net of number 20 silk towed on the surface of water. At this time the water was not discolored and samples of plankton preserved in formalin had the ordinary appearance. The salinity of water computed from specific gravity reading, made with hydrometers, is shown in Table 1. The results indicate normal conditions and apparent dissipation of the effects of the run-off of fresh water.

TABLE I.- Salinity of water near Fort Myers, July 1, 1947

San No	ple Location	Sp. gr. 17.5°C	Salini %	ty Remarks
נייי וויי וויי	9 miles west of Hickory Pass	1.0267	34.83	Large number of dead fish, mostly thread herring; also toad-fish and eels
2	12 miles west of Clam Pass	1.0274	35.83	Same as at Station 1
3	6 l/2 miles off Wiggins Pass	1.0272	35.64	Some dead fish, mostly thread herring, toad- fish and eels
4	2 1/2 miles off Wiggins Pass	1.0270	35,35	Scattered dead fish, thread herring, toad-fish, eels.
5	At Big Hickory Pass	1.0244	32.01	No dead fish observed
7	Estero Bay, 1/2 miles north of mouth of Estero River			No dead fish observed
8	l mile north of Ketchel Key, mouth of Caloosahatachee River	1.0093	12,18	Two dead catfish

A summary of plankton examination is presented in Table 2. As can be seen from the latter table, with the exception of Station 1, plankton collected at the offshore stations is a typical marine plankton which may be encountered during the summer in the coastal water of the Gulf of Mexico. In a contrast to the plankton collected in January, <u>Gymnodinium</u> and <u>Trichodesmium</u> were absent.

Plankton taken at Station 1 (9 miles off Hickory Pass) was unique in the predominance of a species of Radiolaria tentatively identified as <u>Acanthochiasma</u>. This Radiolarian with its long siliceous spicules formed large flocculent masses engulfing the copepods, marine Cladocera, and diatoms. The amount of plankton in this sample far exceeded that in other samples. This probably can be considered as an index of a greater abundance of plankton in the water, for it is reasonable to assume that approximately the same time was used in towing.

Plankton at Station 2 (12 miles west of Clam Pass) was similar to that of Station 1 but its volume was less and the <u>Acanthochiasma</u> sp. was relatively less abundant.

TABLE 2---Plankton of the samples collected near Fort Myers on July 1, 1947

(Symbols used to designate abundance: 5 crosses--Extraordinarily abundant; 4--very abundant; 3--common; 2--scarce; 1--one or two specimens in the sample.)

Stations							
	1	3	<u> </u>	5	77	8	
Blue-green algae						x	
Rhizosolenia	XX	n	X	X	•	·. ••	
Chaetoceros, sp.	X						
Melosira	. 1				XXXX	XX	
Pleurosigma	•						
Cocconeis		-	· .			X	
Peridinium					XX	X	
Ceratium		XX	XX				
Acanthochiasma	XXXXX	XXXX	XX				
Ctenophores				X	XXXX		
Hydromedusae				XXX			
Oyster, larvae		1. A. A.	XXXX	XX	XXX		
Clam, larvae	X		XXX	XX	XXX		
Other Pelecypod larvae							
Gastropod Larvae			с." С	XX			
Oithona	XX	XXX	X	XXX		X	
Centropages	XX	XXX	X	XXXX	÷	XXX	
Nauplii		XXX		XXX		X	
Metanauplii				XXX			
Evadne	XX		XX	XX		· X	
Crab zoeae	1. 			XXXX .		XX	
Sagitta	X	XX					
Dikopleura	XX	XX				x	
Detritus			•			XXX	

Plankton of the brackish water (Station 8) contained large amounts of detritus, small pieces of decaying wood and blue-green algae.

Normal conditions prevailing at this time in the sea may be concluded from the fact that the copepods were reproducing very actively, many of them carrying their spermathecae. Lamellibranch larvae, including oyster larvae, were very abundant. Judging by the sizes of oyster larvae, the spawning of these mollusks took place in June.

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In spite of great mortality of oysters observed by the author in March in the Fort Myers area, the surviving stock was capable of producing a very large population of larvae.

There was nothing in the plankton of these samples which would suggest its toxicity. It is quite possible that the localized mortality of fish in June was due to a sudden drop in the salinity of water but the scarcity of observations and a delay in obtaining samples of water for analysis do not permit the drawing of definite conclusions regarding its cause.

Plankton obtained in July and August in the deeply discolored water 3 1/2 miles off Musketeers Pass presents an entirely different picture. The material in these samples, preserved with chloroform, consists primarily of a very large number of Gymnodinium, Counts made by Woodcock and Anderson in the field show that the number of Gymnodinium in the surface layer of red water varied at this time from 13,000,000 to 56,000,000 per liter. Samples collected by plankton net contained also large numbers of Evadne. The intestines of this cladoceran were stained deep red by ingested Gymnodinia. The relative abundance of Evadne in samples collected by plankton nets may be, however, misleading because the majority of Gymnodinia pass through the net and are lost, whereas most of the Cladocera are caught. There was a small number of copepods and diatoms in these samples. It was clear that Gymnodinium was the predominant organism of the red water.

The relative abundance of various forms in the samples collected on July 24, 3 miles off Musketeers Pass, is presented in Table 3.

Several samples for chemical and toxicological analyses were collected in this area and were forwarded to the Woods Hole Ocea-Midneybt por nographic Institution and the U. S. Food and Drug Administration. At the time of collecting, the color of the water in this section of the Gulf varied from "amber," to "deep amber," and "red."

TABLE 3--Red Water Plankton, July 24, 1947, 3 miles off Musketeers Pass

(arranged in order of abundance in the samples collected by plankton net; designation of abundance the same as in Table 2.

Evadne sp. Gymnodinium Ceratium . Rhizosolenia Oscillatoria sp. Centropages	XXXXX XXX XXX XX XX XX XX XX XX	Nauplii Lamellibranch larvae* Hydromedusae Ostrea virginica larvae Gastropod larvae Fish eggs	XX XX X X X X X X X
Centropages	XX	rish eggs	X

*Exclusive of oyster larvae

Phosphorus Content of Red Water

During the flowering of Gymnodinium in the water along the west coast of Florida in July 1947, three 5-gallon samples were collected at three different stations and shipped to the Woods Hole Oceanographic Institution for analysis. No preservatives were added to the samples. Dr. Bostwick H. Ketchum, microbiologist of the Institution, was kind enough to analyze the samples for the total phosphorus content. During the winter bloom of Gymnodinium, analyses for inorganic phosphorus in the surface samples were made by the investigators of Marine Laboratory of the University of Miami. Using the colormetric method of Denige's modified by Florentjin. they were unable to detect the presence of any phosphates. The results are not at all surprising, for it is to be expected that a large phytoplankton population had utilized the available nutrient salts. The analyses of the total phosphorus content in the July samples made by Ketchum present, however, an entirely different picture and are of great interest (Table μ).

TABLE 4--Total phosphorus content of sea water samplescollected in the areas of intense growth ofGymnodinium off the west coast of Florida.No preservative added. All samples acidifiedat Woods Hole Oceanographic Institution.

	Sample	Date	Inorganic P mg A/liter	Total P mg A/liter
A.	3 1/2 miles off Sarasota Pt. Deep amber color	July 22	5.0	9 •9 *
B₊	1 1/2 miles above Pt.O'Rocks and 1 1/2 from beach off Sarasota Key. Deep amber color	July 22	7•4	19•5 17•7
C.	3 miles off Musketeers Pass	July 24	4.5	10.6 10.2

*Duplicate analyses of total P were made for each sample.

Dr. Ketchum makes the following comments on the phosphorus analysis made at the Woods Hole Oceanographic Institution, "The analyses were made by the method described by Redfield. Smith and Ketchum, Biological Bulletin, 73: 421-443, 1937. It was necessary to adopt a procedure which insured that all particulate matter in the water was included in the sample. Most of the water from the carboy was poured into a fresh clean carboy. The remainder was acidified with concentrated sulphuric acid and the carboy was vigorously shaken after rubbing the walls with a rubber policeman. This was then added to the water sample, the whole shaken vigorously, and two 50 cc portions were removed for analysis. Samples were also taken for the determination of inorganic phosphorus in the water. The results of the inorganic analyses are. of course, considerably greater than those reported by F. G. Walton Smith. This is to be expected since the water was stored for a month before our samples were taken and considerable decomposition of organic matter had taken place.

"The total phosphorus found was between 9.75 and 19.5 microgram atoms per liter. These values are from 5 to 10 times as high as those ever encountered in uncontaminated oceanic water. Two ways by which this degree of concentration could be obtained may be postulated. If the <u>Gymnodinium</u> cells could absorb all of the phosphorus from the entire water column, and then swarm at the surface where these samples were collected, a concentration factor of about 10 might be expected. Since only surface samples were collected, it is impossible to tell whether or not this is the case. Since no water from areas free of the <u>Gymnodinium</u> bloom was provided, the 'normal' phosphorus content of these waters is unknown*, and the concentration to be expected by a tenfold increase cannot be estimated.

"If, on the other hand, the samples analyzed are characteristic of the entire water column, considerable fertilization or contamination must have taken place. This increase in phosphorus could not be attributed to peculiar hydrographic conditions such as upwelling of nutrient-rich deep water, since these concentrations are greater than would be obtained in such water. The water column in the area from which these samples were taken is on the average, 10 meters deep. The amount of phosphorus found would, therefore, correspond to a total of 3 to 6 grams per square meter of sea surface. The addition of three grams of phosphorus per square meter would require almost 8000 kg. (17,000 lbs.) P per square mile.

* Although analyses of inorganic phosphorus are available, none have been made for total phosphorus.

"These results suggest several problems which should be undertaken in studies of the phenomenon when additional outbreaks occur.

"1. Samples of water should be taken at several stations extending throughout the area of plankton flowering to determine the total quantity of phosphorus added to the water mass. It will be necessary to take samples at various depths to determine whether the flowering is concentrating the phosphorus in the surface and to permit the total contamination to be computed properly.

"2. The distribution and source of such a large supply of phosphorus should be the principal object of further studies of this phenomenon. If sufficient hydrographic and chemical data are available, the source could be localized quickly. If it appears that nitrogen is also present in excess over its normal content in sea water, a similar survey for the source of nitrogen fertilizer should be made.

Sufficient water containing large masses of Gymnodinium **"**3. should be filtered to obtain adequate samples for determination of the carbon: hitrogen: phosphorus ratios. The plants in the sea normally contain these elements in the proportions of 100:17.5:2.44 grams. Under culture conditions it has been found that relative deficiencies of either phosphorus or nitrogen can be developed. However, if this population contains only the nitrogen normally present in sea water, combined with the tremendously increased phosphorus found in our analysis, the relative nitrogen deficiency would be greater than anything measured so far. The alternatives are that a source of nitrogen has also been added to the sea water, or that the Gymnodinium is capable of fixing atmospheric nitrogen. There are no experimental data which would substantiate this latter view. Culture experiments can be designed to investigate this problem."

Additional samples of water collected by the U. S. Fish and Wildlife Service on July 22 and August 22, 1947, in the area between Venice Inlet and Sarasota in water of deep amber color and in clear blue water were turned over to Dr. Ketchum for analysis. He reports (Ketchum and Keen, 1948) that within the discolored area the total phosphorus content varied from 4.9 to 20.4 microgram atoms per liter while normal phosphorus concentrations varying from 0.62 to 1.22 microgram atoms per liter were found in the same places in August. The authors think it is unlikely that water-borne contamination has caused these results and suggest that future studies of intense plankton blooms include total phosphorus determinations at various depths.

Poisonous "Gas"

The winter outbreak of the Red Tide was accompanied by a strange phenomenon which greatly added to the discomfort and distress of the residents of the beaches and islands of the west coast of Florida. With the onshore wind and breaking of the surf an odorless but highly irritating "gas" emanated from the water. It caused spasmic coughing, a burning sensation in the throat and nostrils, and irritation of the eyes. Local residents, experimenting with the red water dipped from the sea, observed that strong coughing was produced also by inhaling vapor from this water heated over the kitchen range. For several days when onshore winds persisted, life on Captiva Island was very uncomfortable. Virtually the entire population was sneezing and coughing and suffering from other symptoms resembling those of a heavy cold or hay fever.

Unfortunately, the samples collected through the effort of Mr. J. N. Darling of Captiva Island were taken too late, when the conditions in the sea were apparently returning to normal. Boiling tests made at the Woods Hole Oceanographic Institution gave negative results. Likewise, chemical analysis of the water disclosed no abnormal conditions. The pH of the two samples, measured by glass electrode, was 8.1 and 8.28 at 15° C., and 7.98 and 8.18 at 23° C. These changes are perfectly in accord with the effect of temperature changes on the pH of sea water.

The buffer capacity of sea water, which indicates the quantity of total carbonates and borates, was found by Dr. Ketchum to be within the usual limits. If any large amount of free acid had been added to these waters, the buffer capacity would be low and would account for the low pH. In normal sea water the ratio of buffer capacity to chlorinity is 0.125. Excessive photosynthesis (removal of CO₂) lowers the value for this ratio. Pollution or excessive decomposition processes raise its value. The buffer capacity and its ratio to the chlorinity of the sea water of the two samples are shown in Table 5. Sample 1, the Gulf water, shows a perfectly normal buffer capacity: chlorinity ratio. Sample 3, the Bay water, has a high value for this ratio but no higher than would be expected for waters receiving a moderate amount of land drainage.

The oxygen content of the water was found to be 5.23 ml. per liter for sample 1 and 4.90 ml. per liter for sample 3. These values are well within the limits expected for normal sea water. The total gas was extracted from these samples after acidifying to pH 1.0 with sulphuric acid. The gas extraction, however, was not complete as determined by its oxygen content and, since all previous tests had indicated negative results, no further analyses of the gas were made.

The negative results were possibly due to the fact that the sea water was already returning to normal before the samples were collected.

TABLE 5--Chlorinity, pH oxygen content and buffer capacity of samples of Florida water, February 1947. Analyses made by Dr. Ketchum, Woods Hole Oceanographic Institution.

Sample #1 Gulf Water	Sample #3 Bay Water
19.29	19,16
34.85	34,61
8,10	8,28
7.98	8,18
2.42	2.74
0.125	0,143
0	+,018
5.23	4.90
	Gulf Water 19.29 34.85 8.10 7.98 2.42 0.125 0

The strange effect of red water in causing coughing and sneezing lead to the general belief among the local residents that the water of the Gulf was poisoned by the dumping of mustard gas and other poisonous gases and ammunition. This belief was somewhat strengthened by a report issued by one of the commercial chemical laboratories in Florida that the flesh of the dead fish contained large amounts of arsenic. Samples of dead or dying fish were collected on August 17 and 18 inside Clearwater Bay, Florida, and sent to the College Park

*Method of Thompson & Bonner, 1931, Ind. Eng. Chem. Anal. Ed. 3, 393.

Technological Laboratory of the Service where they were analyzed by Charles F. Lee. Four samples of water from the area affected by red tide were forwarded to Dr. N. W. Rakestraw at the Scripps Institution of Oceanography. These samples were taken on July 22-24, 1947, at the following points: 3 1/2 miles off Sarasota Point; 1 1/2 miles above Point O'Rocks; 1 1/2 miles from beach off Sarasota Key; and 3 miles off Musketeers Pass (2 samples). Two samples of Woods Hole water were added as control.

Samples of fish on arrival at the College Park laboratory were toughened by formalin. The whole bodies were ground and 30 grams of the mixture were digested with sulphuric and nitric acid, according to the procedure of the A.O.A.C. method for determination of arsenic in organic material. The results show very low arsenic content, varying from 2.7 to 0.7 parts of As₂O₃ per million. These values, being within the expected content of arsenic in the body of fish, do not substantiate the results reported by the local commercial laboratory. Likewise the analyses of water made by Rakestraw gave negative results. He writes: "We were unable to find any abnormal amount of arsenic present in any of the samples submitted, either those from Woods Hole or Florida. The method used should have detected an amount in excess of 30 micrograms per liter (that is, parts per billion). Such a value is the upper normal limit in sea water."

The occurrence of an irritating substance in discolored sea water is not entirely new. It was observed in the same area during the fish mortality of 1916. Harden F. Taylor (1917), who investigated the mortality of fishes on the west coast of Florida in October-November 1916, quotes from the letter he received from the deputy collector of customs at Boca Grande. "While on the beach I felt a slight tendency to sneeze and cough; shortly afterwards my attention was called to the action of the dog which was sneezing violently and seemed to be in acute distress, choking and showing every symptom of asphyxiation. I carried him back, and the same thing happened again. I then noticed that my lungs were feeling sore and that my breathing was labored in much the same manner as when I board ships after fumigation, except that I noticed no odor For the past few days the beach has been lined with tarpon, jewfish, grouper, and many varieties of top fish which seemed to escape the first attack The gas was very violent this time and many people telephoned for medical assistance for 'cold in the head,' 'sore throats,' 'cold in the chest, etc.... I myself have suffered quite acutely for the past five days, but the worst of the gas seems to be going now."

It appears highly improbable that any ammunition or poisonous gases could have been dumped into the Gulf water in 1916 when the menace of the World War I threatened the country. The symptoms described in the above quoted letter are identical with those observed during the last outburst of red water.

Another record of the presence of "poisonous gas" in water during a mortality of fishes in Texas is found in the report of Lund (1934).

A. H. Woodcock of the Woods Hole Oceanographic Institution suggested that the irritant may not be gaseous but particulate and that it may be transported with the drops of sea water found in the air. These drops are presumed to be projected into the air by bursting bubbles associated with breaking waves. The irritating organic compounds, or perhaps the organism producing these irritants, may be carried directly from the sea to the nose and throat of persons near the shore. This suggestion was made by Woodcock on the basis of his measurements of air-borne drops taken from an elevation of 6 feet on December 7 and 8, 1946, at Daytona Beach.

In July 1947, Dr. Woodcock, at the request of the Service, made observations at the Fort Myers area and, later on, conducted laboratory studies with the samples of red water delivered to him at Woods Hole. A complete report of Woodcock's observations will appear in one of the technical periodicals. For the purpose of this report it suffices to summarize his principal conclusions.

Upon arrival at Venice, on the west coast of Florida, he found that the shift of wind from onshore to offshore resulted in a cessation of respiratory irritation among people living along the shore. Experiments conducted with the assistance of W. W. Anderson of the U. S. Fish and Wildlife Service consisted in spraying into the nose and throat a small amount of sea water containing from 15 million to 56 million <u>Gynmodinium</u> per liter. The tests invariably resulted in coughing and in a burning sensation in the nose and throat. Spraying of water filtered through a Yena fritted glass bacteria filter produced the same irritation although no particulate matter could be seen in this filtrate using a magnification of 500 times.

Involuntary coughing and a burning sensation resulted also from inhaling the vapor produced by warming the samples of red water to temperatures varying from 80° to 90° C. At these temperatures clouds of fine bubbles rose to the surface and burst. When the heat source was removed, the bubbling ceased and the irritating effects also ceased.

Regarding the formation of drops, their size and shape, Woodcock reports as follows: "Small glass slides, covered with a hydrophobic silicone film, were exposed for 10 seconds at a height of 2 cm. above vessels containing clear sea water and red water under various conditions of temperature and effervescence. Drops of liquid on these slides were later examined microscopically while they were in a sealed container and in vapor pressure equilibrium with a relatively large volume of sea water of known salinity. Table VI shows the size range of these drops and the conditions under which drops were or were not deposited on the glass slides.

"Drops caught over heated effervescing 'red water' had very different contact angles when compared with drops caught over heated effervescing clear sea water. This difference in contact angle suggests a marked lowering of the surface tension of the drops, which may be due to some surface-active agent. Since the small drops originate at the surface and are probably composed largely of surface film water, there is reason to suppose that the mechanism producing the drops might bring about a concentration within these drops of surface-active materials."

Woodcock found that a pad of absorbent cotton 2 cm. thick, held over the mouth and nose is sufficient to prevent irritation. According to his opinion this indicates that "the irritant, as it exists in the air-borne form, is filterable and that it is probably present as a liquid or solid."

The irritant is quite stable, as is shown by the fact that samples of red water collected in July and stored at room temperature for four weeks were still effective in producing a positive coughing reaction in humans.

The author had the privilege of examining Mr. Woodcock's "drop" preparations of normal and red water. Some of the droplets caught on silicone film contained small, slightly greenish granules, in shape and coloration similar to the granules found in <u>Gymnodinium</u> preserved in formalin. The granules inside the droplets were about 1 or 2 microns in diameter. The mechanism by which they were carried into the air is apparently similar to that described by Zobell (1942) for marine bacteria. According to this author marine bacteria are carried considerable distances inland. The amount of water transported through the atmosphere of our globe as water droplets which may contain bacteria is surprisingly large. According to Zobell it averages 12.7 cubic miles per year. The present observation shows the mechanism by which the irritating substance of red water may be transported through air. It does not prove, however, that the granules observed in the droplets produce irritation, although the suggestion appears to be plausible.

The effect of red water on human beings does not seem to be confined to a group of allergic persons. So far every person who consented to subject himself to a "sniff test" reacted positively. The total number of about 25 tests is too small, however, to make a positive statement and the possibility of a widespread allergy to red water requires further study.

TABLE 6---Effervescence, drop size and irritating effect of red water and clear sea water. (Observation by A. H. Woodcock)

		RI	RED WATER				CLEAR SEA WATER		
ю с.	26°	85°-95	o 260	85°-95°	25°	85°-95°	25°	85°-95°	
icrons	15-80	15-80		no drops	15-80	15-80	no drops	no drops	
rritating Effect	Yes	Yes	No	No	No	No	No	No	

The question arises whether the irritating substance is definitely associated with the red water or whether it may emanate from any highly concentrated plankton sample. To test this possibility two experiments were performed by the author. Concentrated samples of plankton were collected by towing a No. 20 foot net for 20 minutes in the Woods Hole Harbor and near the entrance at the "Hole." The samples were greenish in appearance and consisted primarily of <u>Chactoceros</u>, <u>Rhizošolenia</u>, <u>Peridinium</u>, and a relatively small number of copepods and other zooplankton. They were heated to 85° -95° C. in a shallow aluminum pan. Five persons working in the laboratory were asked to sniff the vapor. In all the cases the results were negative. The samples had a distinct grassy or hay odor but the irritating effect was entirely absent.

Toxic Effects on Fish

Many vivid descriptions of the manner in which the fish die upon encountering red water were given by fishermen and local residents. Critical observations and experimental data are, however, few and incomplete.

In describing the death of mullet, several fishermen interviewed in the course of these studies, stated that death comes rather suddenly. As the fish enters "red water" it begins to act rather strangely, "coming to the surface, whirling around, then turning on side or lying stomach up, and then sinking to the bottom." Spadefish acted apparently in the same way. Some of the untrained observers described a gulping of air by fish, which would suggest the lack of oxygen. Dead and dying specimens, mostly thread herrings, which were sent to the College Park laboratory were dissected and carefully examined by the author. The internal organs appeared to be normal and the gills were not clogged by plankton or other material. If death was due to asphyxiation, it was not caused by the occlusion of the gill filaments.

Field observations suggest that the cause of death may be attributed to some strong poison produced by or associated with red plankton. This conclusion is confirmed by several experiments conducted by the author at the Woods Hole Laboratory and by the investigators of the Miami Biological Laboratory.

Toxic Effect of Alcoholic Extracts of Red Plankton

Concentrated samples of red water plankton collected on July 24, 1947, near the Musketeers Pass were preserved by addition of small amounts of chloroform. Both the supernatant liquid and precipitate were of deep brownish-red color. A considerable portion of the plankton was cytolysed but large numbers of <u>Gymnodinium</u> and <u>Evadne</u> were easily recognizable.

The material was poured into tall glass cylinders. After 24 hours of standing, the transparent supernatant liquid was siphoned out and the material settled on the bottom measured and transferred to a 1-liter glass-stoppered bottle. To a 200 ml. portion of this material were added 600 ml. of 95 percent ethyl alcohol and 10 ml. of strong HCL. The bottle was stoppered and placed for 24 hours on a shaking machine. The construction of this apparatus, the only one available at Woods Hole, was such that no vigorous shaking was possible but the bottle was subjected to a gentle horizontal movement. After 24 hours the bottle was set aside for 72 hours. During this period it was occasionally and vigorously shaken by hand. The extract, of amber-red color, was filtered through No. 40 Whatman paper and evaporated over a hot bath (75-85°) to dryness. The yield of the crude extract was from 0.73 to 0.97 grams. The dried extract consisted of a greasy, dark substance with a noticeable amount of crystallized mineral salts.

In one instance, when through an oversight the extract was overheated, both the irritating effect and the odor were lost. Subsequent tests showed that the overheated sample had also lost its toxicity. All other extracts were toxic to fish.

A number of tests were made with the killifish, <u>Fundulus</u> <u>hetroclytus</u>. The first test was made in the concentration of 1 part of dried extract in 1000 ml. of sea water. Both the test fish and the control were kept in 500 ml. of water which was not aerated. The salinity was 31,54 and the temperature varied from 21.8° C. at the beginning of the test to 22.1° at the end. The results of the test are summarized in Table 7.

TABLE 7-Effect of the concentration of 1:1000 of red water extract on Fundulus, August 29, 1947.

Time	Behavior of Fish	······································	
	Experiment	Control	
2:36 p.m.	Start	Start	
3:30 p.m.	Fish in distress, lying on side	normal	
	Occasionally gulping for air, lying on side	đo	
4:07 p.m.	Unable to swim even when pushed, respiratory	5 . E	
• • • •	movements slow down.	do	
5:30 p.m.	Floats on side, no respiratory movement	do	
6:00 p.m.	Appears to be dead, placed in running sea		
Ferre	water	đo	
7:30 p.m.	Respiratory motion starts, floats on side	do	
8:30 p.m.	Begins to swim	do	
8:30 a.m.	Normal	đo	

At 4 p.m., when signs of distress were pronounced and the fish was unable to swim, the pH of the water was 6.84 in the control and 6.80 in the experimental tank. The <u>Fundulus</u> appeared to be dead after about 2 1/2 hours of exposure, but completely recovered after its removal to running sea water. On August 30, 1947, the same extract as used in Exp. 1 was tested in the concentration of 1:2000. The volume of water in which the fish was placed was 1000 ml. <u>Fundulus</u> lived both in the control and in the red water extract for 6 days. There were no signs of distress in either fish.

An experiment with the concentration of 1:250 (Table VIII) resulted in death of the fish within $1 \frac{1}{2}$ hours. Removed from the solution and placed in running sea water in which it was kept for 24 hours, the fish failed to recover.

Three other experiments gave similar results, namely, a concentration of 1:1000 or greater was highly toxic to fish while no ill effect was observed in weaker solutions.

TABLE 8--Effect of the concentration of 1:250 of red water extract on Fundulus, Fish kept in 500 ml. of water, salinity 31.48, temperature 22° C.

Time	Behavior of Fish		
	Experiment	Control	
1:30 p.m.	Start	Start	
1:40 p.m.	Normal, no signs of distress	Normal	
2:00 p.m.	Lying on side, unsuccessful attempt to		
-	right the body	do	
2:40 p.m.	Floating with belly up; almost dead	do	
2:55 p.m.	Dead	dó	

The toxicity of red water was also ascertained in an experiment performed by the investigators of the Miami Laboratory (Gunter, et al, 1947, manuscript). For this test Florida Bay water containing live Gymnodinium brevis was brought to the laboratory and poured in a glass aquarium. An identical tank with equal amount of Biscayne Bay water and placed side by side with the first one served as control. Both aquaria were strongly aerated. Eight sheepshead minnows, <u>Cyprincdon variegatus</u>, two puffers, <u>Sphoeroides</u> testudineus, and two majarras, <u>Eucinostomus gula</u>, were placed in each aquarium. In Florida Bay water the minnows died in 22 to 46 hours; both puffers died in 48 hours; and the majarras succumbed one in 88 hours and another 118 hours.

Two crabs, Portunnus sayii, survived for a week when the experiment was discontinued. In the control tank all fishes and crabs survived with the exception of one puffer which jumped out during the third night and was found dead on the floor.

One week after the last fish died in the tank containing Florida Bay water, the control fishes were placed in it. They all survived for three weeks and the test was discontinued. From these observations the authors conclude that "the original 'poison' had been absorbed or taken up by the fishes it killed or the initial concentration and any which was subsequently produced had been broken down or undergone chemical change rendering it innocuous."

The preliminary experiments here reported are inadequate for the understanding of the toxicology of the red water, the manner in which it acts upon fish, or for determining the resistence of various species to this poison. Much more experimental and quantitative information is obviously needed for the solution of this difficult problem.

Effect on the Rate of Cleavage of Arbacia Eggs

Interesting tests were performed at Woods Hole by Dr. Ivor Cornman of the Sloan-Kettering Institute of New York City. Using the unpreserved samples of red water and preserved and concentrated samples of red plankton collected 3 1/2 miles off Musketeers Pass on July 22, Dr. Cornman studied their effect on the rate of cleavage of eggs of the sea urchin, <u>Arbacia</u>. Since the samples contained noticeable amounts of H₂S, this gas was removed with a vacuum pump. After the odor of H₂S was gone, the retardation in cleavage was 9 percent in 1:10 dilution and 27% in 1:5 dilution. Cytolisis resulted from exposure to 1:2 dilution. This inhibitory potency is equal to that of crude filtrate from some Pennicillium cultures.

According to Cornman's report (1947) this sample of red water killed <u>Fundulus</u> in 2 hours in 1:2 dilution and in 5 1/2 hours in 1:10 dilution. Cornman thinks that there appears to be some parallel between the toxicity to i sh and the retardation of cleavage of <u>Arbacia ers</u>, "but whether the same portion acts upon both, and whether decomposition plays an important role remains to be determined. Studies conducted near the site with fresh samples of sea water and dinoflagellates should prove more helpful if uncontaminated test organisms are available."

Toxicity of Clams from Fort Myers Area

During the winter of 1947 reports were received by the Service of the toxicity of clams and fish in the area affected by the red water. A barrel containing a quantity of reputedly poisonous hard shell clams and fish was received on March 5, 1947, by the College Park Technological Laboratory of the Service. Extracts for the toxicity tests were prepared both by the method of Sommer and the method of Gibbard. The fish, inspected organoleptically, showed a strong odor of iodoform in the gills, but the toxicity tests for clams (Sommer and Gibbard method) and for fish (Gibbard method) were negative.

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CONTROL MEASURES

Observations on the occurrence, spread, and toxicity of red water provide impressive but nevertheless indirect evidence that the mortality of fish is caused by the outburst or "blooming" of certain dinoflagellates, particularly of the genus <u>Gymnodinium</u>. Final proof that the toxic effect of red water is caused directly by <u>Gymnodinium</u> requires experimentation with pure cultures kept under controlled laboratory conditions. In the absence of definite information regarding the nature and origin of the poison, the present evidence cannot be entirely conclusive. That such an investigation is highly desirable and that it should be conducted on a large and comprehensive scale is obvious and requires no additional arguments. The direct and indirect economic losses and the public health aspects of the problem fully justify the expenditure of public funds for the initiation and conduct of such a research project.

The question naturally arises, whether the information gained from these studies may be employed for the prevention or control of the red tide. Control of any natural phenomenon such as weather, tides, floods, etc., presents a difficult, but not entirely impossible task. The history of science teaches that the solution of problems which is considered impossible at present may become only "difficult" in the future, and that the understanding of natural phenomena and the ability to foresee their occurrence is the first step toward their eventual control.

With reference to the red tide, attempts at controlling the growth of the populations of dinoflagellates have already been made and reported by American and Japanese biologists. Dinoflagellates, like other Protozoa, are very sensitive to copper sulphate and hypochlorite. Kofoid and Swezey (1921) state that copper sulphate in a concentration of 1 part per million killed all Ceratium hirundinella. This method was used by the Japanese biologists in their attempts to control the red water in the Gokasho Bay and in the Gulf of Konsa. Miyajima (1934) states that all dinoflagellates are instantly killed by a copper sulphate solution in the concentration of 2 parts per million. The concentration of 1 part per million kills them within a few minutes. In practice the copper sulphate was applied by attaching bags filled with this salt to the sides of motor launches which were run back and forth in the bay. After the treatment large numbers of destroyed dinoflagellates were found floating in the water.

To prevent the growth of bacteria which may develop after the destruction of dinoflagellates, the Japanese biologists suggest the use of 10 percent solution of calcium hypochlorite or the addition of liquid chlorine. Both solutions can be used simultaneously and their effective concentrations, according to Kominarui (quoted from Miyajima) should be adjusted to the salinity and temperature of the water. He states that a 13.6° C. ordinary bleaching powder containing from 34 to 35 percent of free chlorine is effective in killing dinoflagellates at the concentration of 1:500,000. At 10° C, the concentration should be increased to 1:400,000. In combination with copper sulphate the concentration of hypochlorite can be reduced to 1 part per million. It is interesting to note that after the red water was destroyed by chemical treatment the Japanese biologists noticed the appearance of another dinoflagellate of the genus <u>Polykrikos</u> which apparently was harmless to pearl oysters.

In view of this experience it appears promising to try the spraying of red water from aeroplanes or from boats with a solution of copper sulphate or dusting it with powdered calcium hypochlorite. Other chemicals, harmless to fish and shellfish, may be tried. The use of powdered calcium oxide (unslackened lime) suggests itself, for its addition to sea water will raise the pH to a level which is beyond the tolerance of the dinoflagellate and, at the same time, it is unlikely that the increased concentration of Ca salts in the water will adversely affect fish or shellfish, for the excess of Ca in sea water will be rapidly precipitated.

Advantage may be taken of the fact that the greatest concentrations of dinoflagellates appear in patches which are, probably, the centers of their more rapid propagation. It is possible that the destruction of the initial populations may stop their further spread.

No definite recommendation for control can be made at present. Various methods are mentioned here only as possibilities which should be tried at the first opportunity.

ECONOMIC LOSSES

Evaulation of the economic losses caused by the recent fish mortality presents great difficulties. Estimates made by the coastal residents of the number of dead fish washed ashore in January-February, 1947, vary from 50,000,000 to 100,000,000 pounds. The figure obviously represents only a fraction of a total number of fish which perished in the sea, for large numbers of dead carcasses were carried away by the currents and tides. Following the winter mortality of 1947 there was a noticeable drop in the quantity of fish landed at Naples, Fort Myers, and other ports of the Florida west coast. Many fishermen left their occupation at least temporarily and sought other employment. The exodus of winter vacationists driven from the beaches by the offensive smell resulted in considerable monetary losses, which are impossible at present to determine. Local wholesale fish dealers declined to give estimates of their losses on the grounds that failure to catch fish would not be considered a "loss." Some idea of the economic significance of the disaster can be obtained, however, from the fact that during December 1946-February 1947, the principal wholesale dealers operating out of Fort Myers and Fort Myers Beach suffered from 70 to 90 percent decrease in the volume of their business.

The evaulation of other indirect losses caused by fish mortality cannot be made at present with any degree of accuracy.

CONCLUSIONS

There is strong but indirect evidence that previous and recent mortalities of fishes in the Gulf of Mexico were associated with the occurrence of red water which was caused by the rapid reproduction of <u>Gymnodinium</u>, a protozoan flagellate, belonging to the subclass of Dinoflagellata. Several members of this group-namely, <u>Gonyaulax</u>, <u>Glenodinium</u>, <u>Mesodinium</u>, <u>Gymnodinium</u>, and <u>Peridinium</u> are known to cause yellowish, brownish, or reddish discoloration of sea water in various parts of the world. In many instances these microorganisms have been considered as agents responsible for the mortality of fish, or for causing the poisoning of shellfish (<u>Gonyaulax</u>). Although direct experimental evidence of the toxicity of these microorganisms is lacking, there are many well established facts which force us to suspect the dinoflagellates of the red water as a primary cause of the mortality.

Observations made during the recent outburst of red water in Florida showed without any doubt that the yellowish or reddish color was associated with the presence of a large number of <u>Gymnodinium</u>. It was evident that the color was more intense in the samples of water containing greater numbers of this dinoflagellate. The number of <u>Gymnodinium</u> in poisonous red water varied from 13,000,000 to 56,000,000 per liter. There is no evidence that the red water killed the fish by clogging their gills. The gill filaments of the fish which died in red water were found to be normal and not covered by an accumulation of plankton. The poison contained in red water and in its plankton can be extracted in acidified 95 percent ethyl alchohol. After evaporation, the residue redissolved in water killed Fundulus in concentrations stronger than 1:1000. The fact that <u>Gym-</u> nodinium is predominant in the red-water plankton and that the mortality of fish ceases with the disappearance of the red discoloration provide sufficient grounds to suspect the <u>Gymnodinium</u> as the principal cause of mortality.

Final proof of the toxicity of this microorganism can be obtained, however, by experimenting with pure cultures of this species and by testing their toxicity under controlled laboratory conditions.

There is no evidence that the mortality of fish was due to the dumping of poisonous gases or ammunition. The abundance of plankton organisms in the red water area speaks against this possibility. Water containing strong poisons would be expected to be almost free from planktonic organisms which, as a rule, are very sensitive to various chemicals and poisons. It would appear crystal clear and transparent.

The red water contained an irritating substance which, under certain conditions such as heating, breaking of waves, strong aeration, became airborne and was transported through the air. When inhaled it caused spasmic coughing and irritation of the mucus membranes of the nose and throat. It is not known whether this substance is identical with that which kills fish or whether two different poisons are associated with the red tide. This important question can be answered only by further research.

Theories, advanced in the past, attributing extensive mortalities of fishes along the west coast of Florida to underwater eruption of poisonous springs released by earthquakes or to leaching of poisonous substances by streams are not supported by evidence and appear to be groundless. The existing evidence gives considerable support to the hypothesis that rapid growth of the <u>Gymnodinium</u> population is the principal cause of both the mortality of fish and of the presence of an irritating air-borne substance. This working hypothesis requires further verification.

The causes of sudden acceleration in the rate of propagation of dinoflagellates should be studied. Rapid growth of a population of any member of a complex marine community of animals and plants usually takes place when the right combination of temperature, light intensity, chemical composition of water, and availability of nutrient salts and food provide the most favorable conditions for the reproduction and survival of the species. In the case of the red tide, it must be postulated that an additional supply of nutrient salts became available for the rapidly growing population of dinoflagellates. Determination of the total phosphorus in the samples of red water made by . Yetchum indicated that the quantity of P far exceeded the concentration of this element in the sea water ever before observed over the continental shelf. The origin of the phosphorus used by the red water organisms is not known, however. It may have come from the greater depths of the Gulf of Nexico or had its origin in the rich phosphate ore deposits which are extensively mined in Florida. If the latter is the case, the manner in which the phosphate salts reach the sea should be determined and, if possible, controlled.

Phosphates alone are not sufficient to support the growth of the dinoflagellates. Other salts, such as nitrates and nitrites, and salts of heavy metals are also needed. Since lack of any of the nutrient salts may become a factor limiting the growth of the population, it is necessary to make a complete study of the cycles of all the nutrient salts and to trace their origin in the Gulf waters. Furthermore, the nutrient requirements of <u>Gymnodinium</u> should be determined by experimental studies in the laboratory. Thus, a comprehensive research program of the red tide problem should include detailed studies of the seasonal changes in the physical and chemical conditions of the water of the Gulf, its currents, cycles of principal nutrient salts and experimental investigations of the nutrient requirements of <u>Gymnodinium</u> and other dinoflagellates.

A thorough knowledge of the ecological conditions preceding the outbreak of red tide, as well as those existing during and after its maximum development, are necessary for the understanding of the problem.

The chemical nature of the poison and its toxicology must comprise an independent phase of the investigation. Of greatest interest is, of course, the question whether the poison that kills fish is identical with the airborne substance causing coughing and other respiratory difficulties in humans. The public h alth aspects of the red water problem fully justify an extensive investigation along these lines. Prediction and control of the red tide constitute the ultimate goal of the studies which can be attained only after the completion of a well planned and comprehensive program of research.

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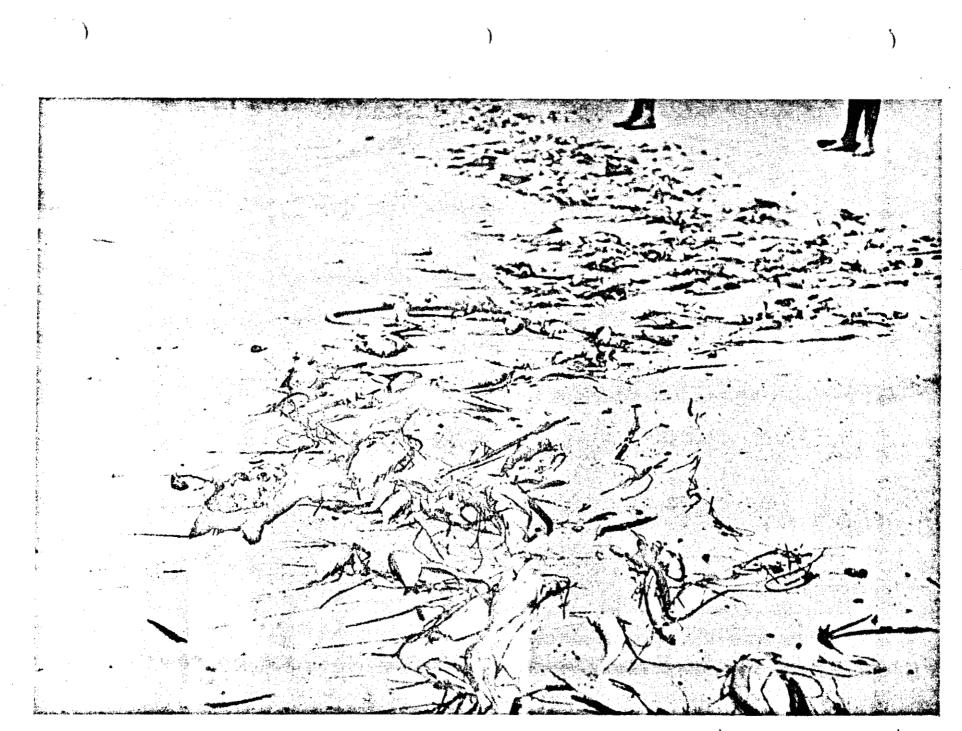


Figure 1.--Dead fish of various kinds and turtles litter Madeira Beach, Fla., at 145th Street, August 1947. (St. Petersburg Evening Independent Staff Photograph)

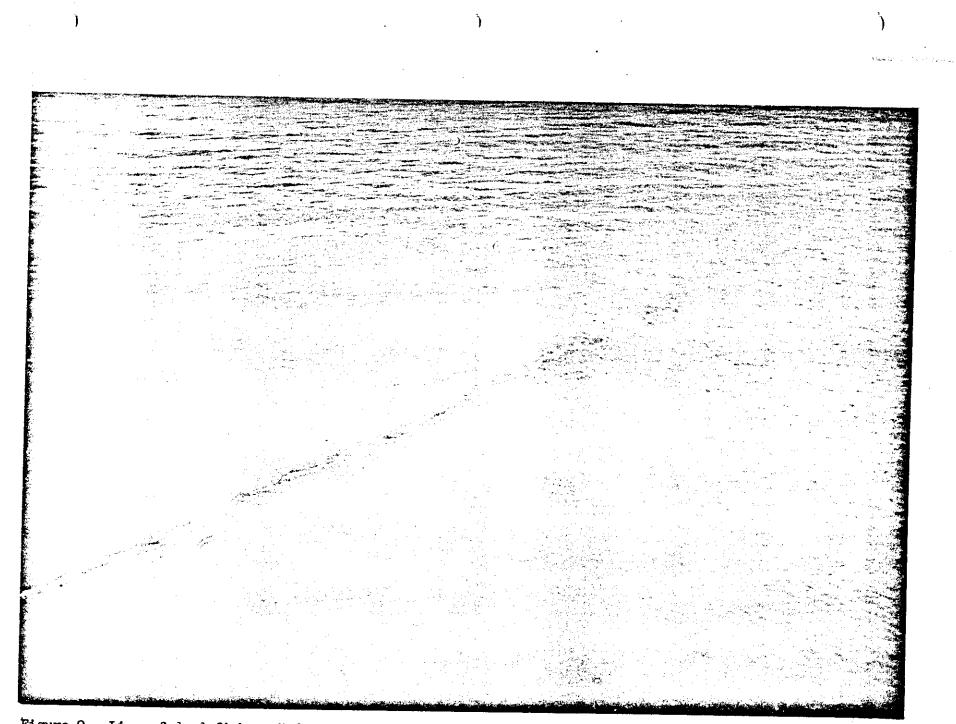
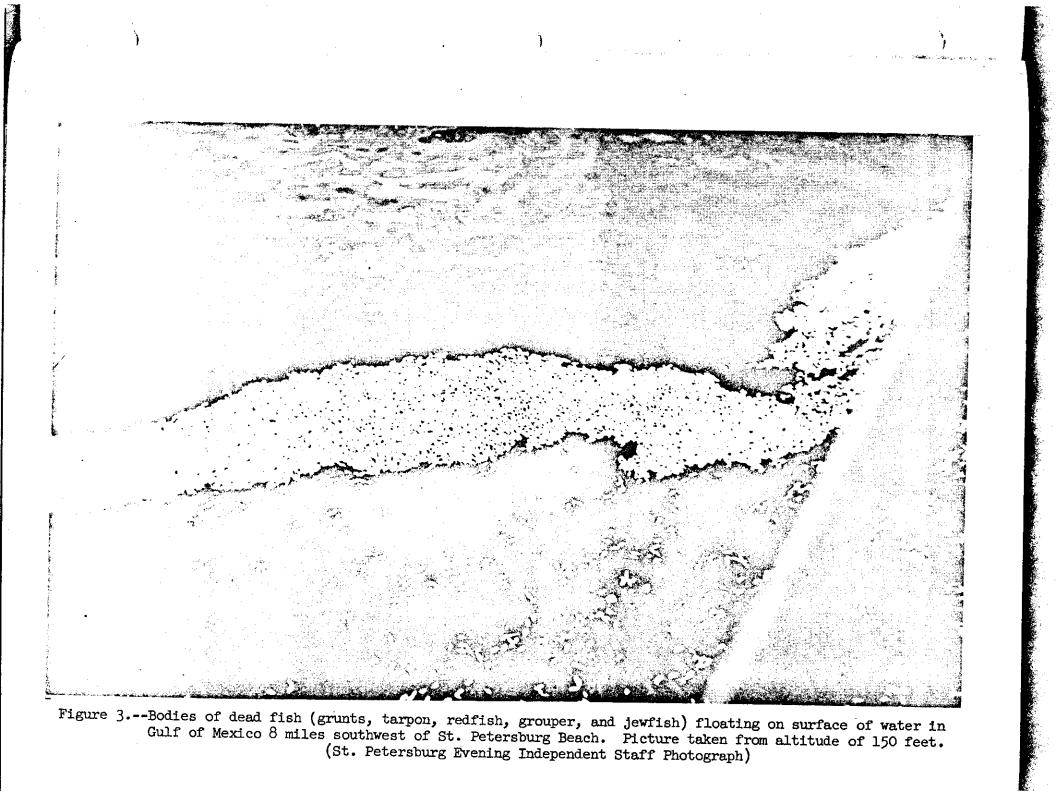


Figure 2.--Line of dead fish as Red Tide invades the inshore waters. Picture taken from altitude of 500 feet. (St. Petersburg Evening Independent Staff Photograph)



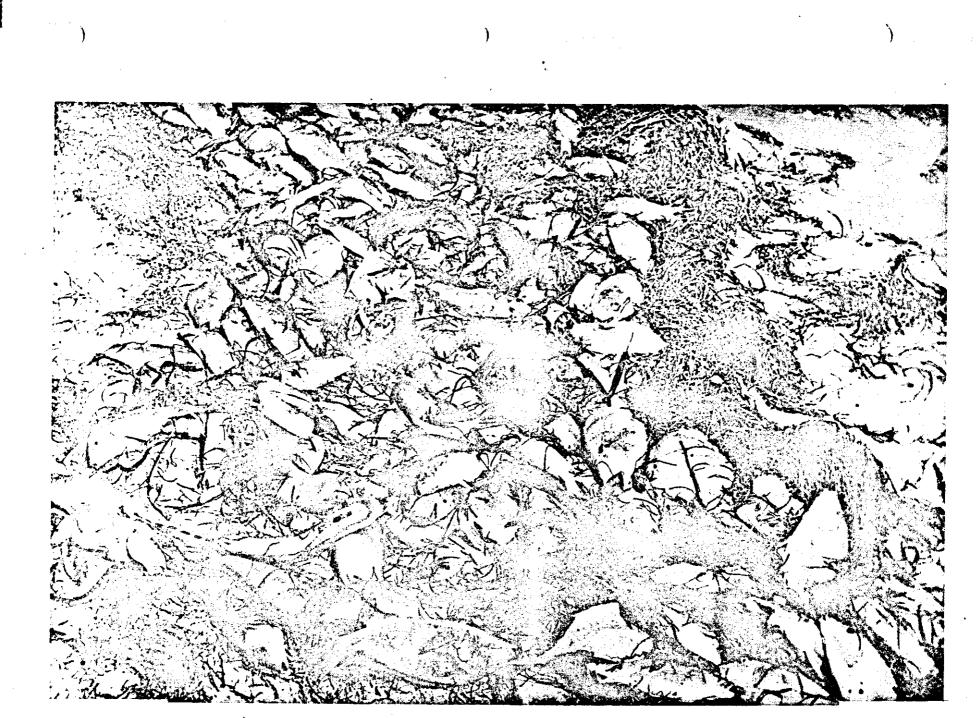


Figure 4.--Mass of dead fish deposited at edge of water at high tide. (Photograph by John Evans, Staff Photographer, St. Petersburg Times)

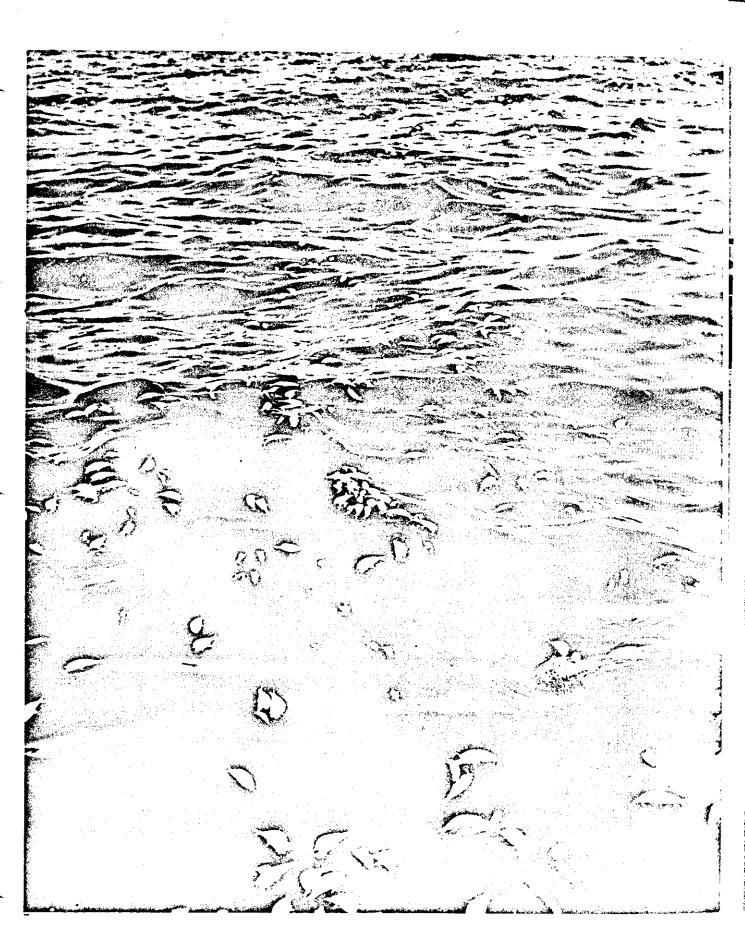


Figure 5.--Typical view of surface of water during mortality of fish in Gulf of Mexico, August 1947. (Photograph by John Evans, Staff Photographer, St. Petersburg Times)

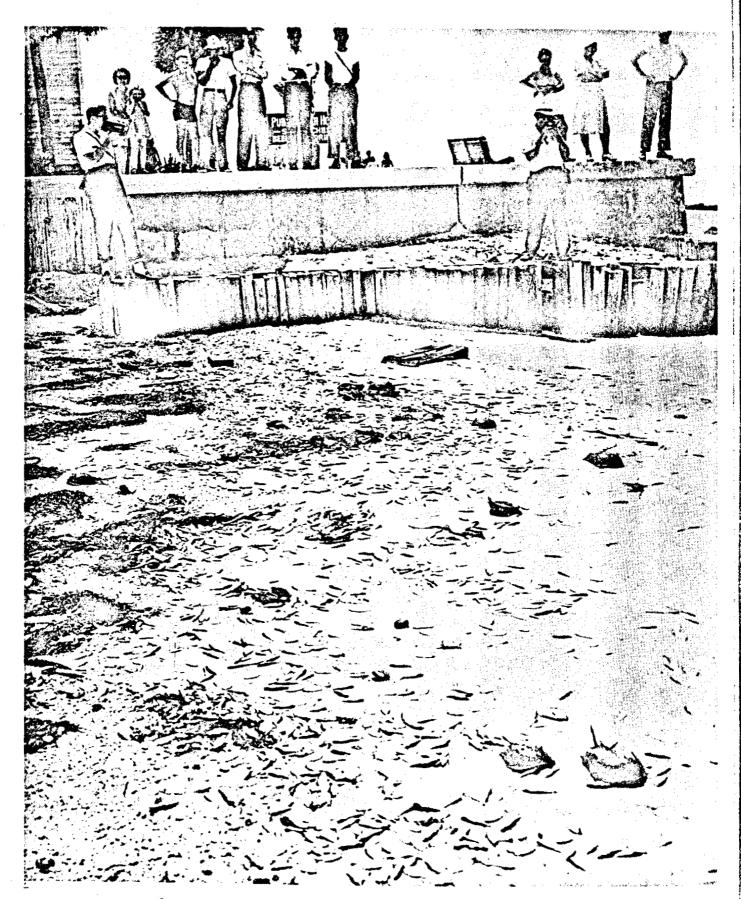


Figure 6.--Dead fish and horseshoe crabs on Pass-a-Grille Beach, Fla. (Photograph by George Trabant, Staff Photographer, St. Petersburg Times)

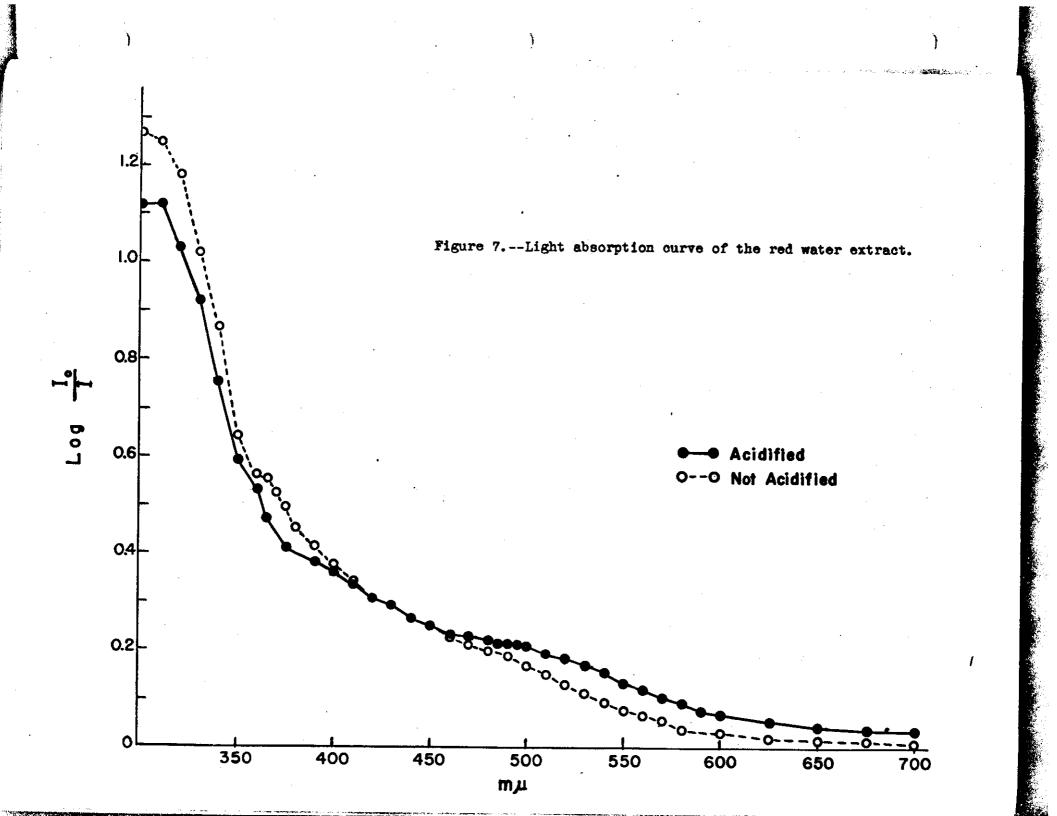




Figure 8.--Spraying dead fish with DDT and deodorants, Madeira Beach, Fla., August 1947. (St. Petersburg Evening Independent Staff Photograph)

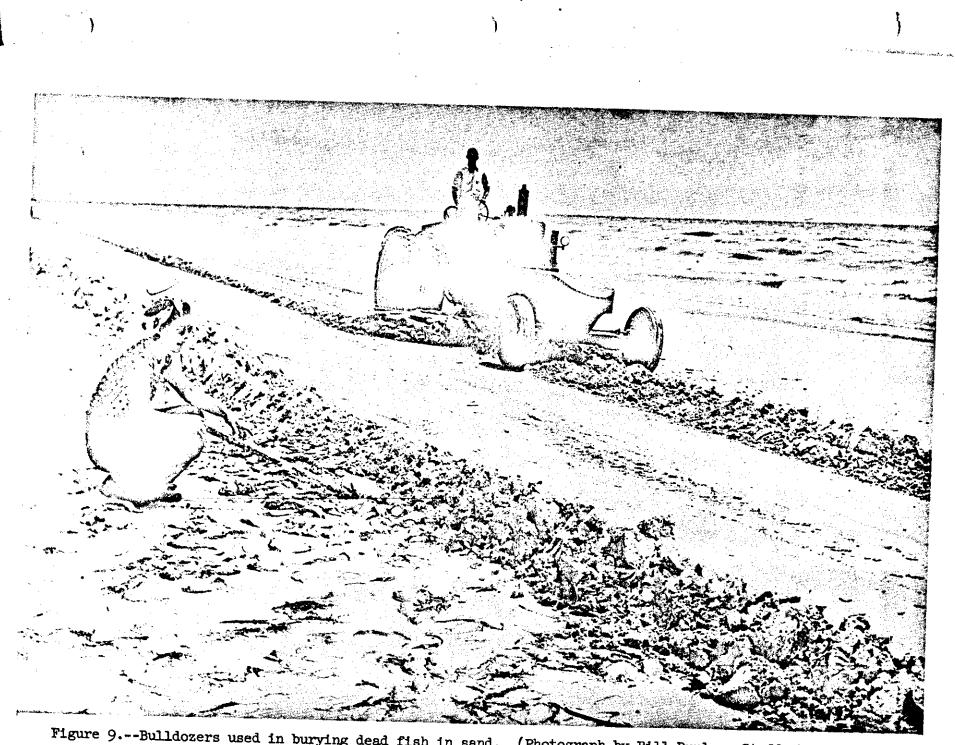


Figure 9.--Bulldozers used in burying dead fish in sand. (Photograph by Bill Dunlap, Staff Photographer, St. Petersburg Evening Independent)