The Florida Gulf Coast Red Tide

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FOREWORD

The work detailed in this bulletin represents an attempt to evaluate the effects of *Gymnodinium brevis*, its behavior and its distribution; some investigation of factors which may cause its phenomenal growth and of factors which may help in its control. Our laboratory has not attempted to investigate phases of the problem which are under attack at the University of Miami or at the laboratory of the U. S. Fish and Wildlife Service. The problem is so great that duplication of effort should be avoided as much as possible. Fortunately, conferences at which workers from the three laboratories have discussed their work and findings, and have agreed upon allocating certain fields of endeavor, have helped to eliminate repetition. Thus little or no mention is made herein of hydrographic factors responsible for focal concentrations and of meteorological factors. These, as well as other aspects of the problem, have been receiving careful attention from the University of Miami workers and those of the U. S. Fish and Wildlife Service.

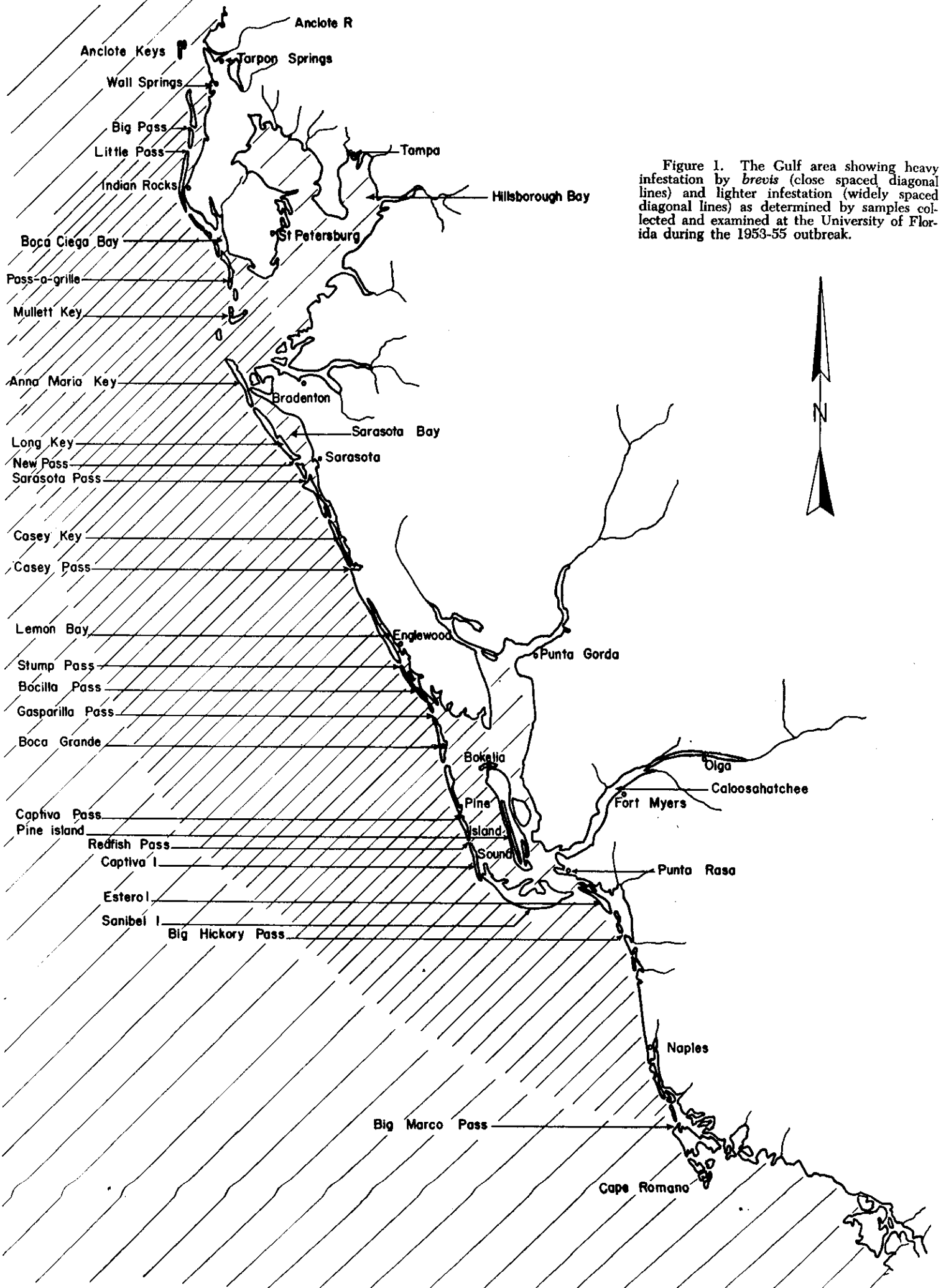

Figure 1. The Gulf area showing heavy infestation by *brevis* (close spaced diagonal lines) and lighter infestation (widely spaced diagonal lines) as determined by samples collected and examined at the University of Florida during the 1953-55 outbreak.

The Florida Gulf Coast Red Tide

INTRODUCTION

The term "Red Tide" has come into widespread use within the past seven years. It was coined to use for the slight to intense discoloration of Gulf water, largely in the Naples-Clearwater area, which occurred whenever there was a substantial fish kill.

Actually it is applied to a phenomenon of "blooming" which occurs frequently in fresh and salt water, records of which antedate history. A "bloom" or a "red tide" is simply an aggregation or a sudden increase in numbers of some microscopic organism to a point where the water in which it is found becomes discolored. There are literally hundreds of organisms which cause blooms, and most of these are harmless. A few cause damage, such as tastes and odors in water supplies, or the death of cattle from drinking water containing intense blooms, or the poisoning of mussels along the California coast, or the killing of fish and other organisms along the Florida Gulf Coast.

Many kinds of blooms occur in Florida coastal waters. Some are widespread, some local. In those cases where the discoloration is brown or red, and deep enough to catch the eye, they are now probably called Red Tides. Of all these blooms, only the Red Tide due to *Gymnodinium brevis* is really damaging. In fact the original connotation of the term applied to a bloom of *Gymnodinium brevis* Davis (Figure 2). This organism is a small dinoflagellate, having some plantlike and some animal-like characteristics. Dinoflagellates are common in fresh and salt water, as are blooms caused by them. They have been incriminated by association with fish kills many times, for example in Japan (1) and along the coast of India (2). *Gymnodinium brevis* Davis was named only as recently as 1947 by a worker at the University of Miami (3), and to date has never been reported from any other location in the world.

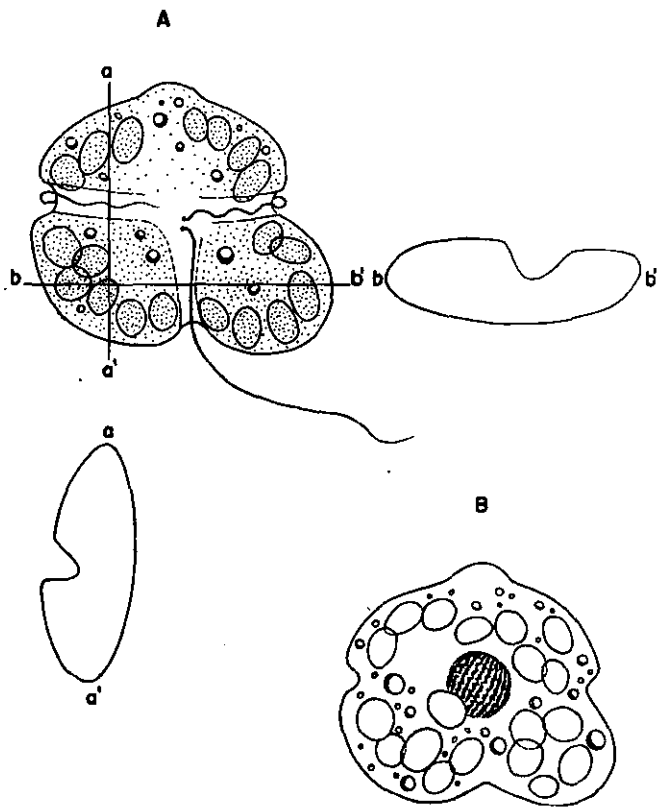

Figure 2. A. Habit sketch of *Gymnodinium brevis*, showing shape, girdle, sulcus, round inclusions, 19 chromatophores, encircling flagellum, swimming flagellum. At right and below are shown cross sections through the regions indicated by the lines b - b' and a - a'. B. A killed and fixed specimen showing the large characteristic nucleus.

DESCRIPTION OF THE ORGANISM

The organism is somewhat discoid in outline, having a transverse groove or girdle, and an indentation or sulcus from this girdle to the posterior body edge, so the body is divided roughly into four quadrants on its ventral surface. At the upper end is a small protuberance or nipplelike projection. The sulcus actually extends to the apical end of this nipple, but this can usually be determined only with the oil immersion objective, at about 930x. There is no shell or armor as in some other dinoflagellates. The width is about 30 microns, and the length about 25. There are approximately 22 discoid chromatophores (chlorophyll-containing structures) which appear pale yellow-brown in transmitted light. Dense swarms of the organisms appear reddish-brown in reflected light. The nucleus is typically large and, as in all dinoflagellate nuclei, it shows closely packed threads in a spiral or parallel arrangement. This is so characteristic, and dinoflagellate nuclei are so resistant to disintegration, as to greatly facilitate identification. In the catenate (chain-forming) *Cochlodinium,* formalin used for preservation disrupts the cells, but the nuclei still stand out and remain connected by amorphous remains of the cells. Sometimes a few *brevis* are similarly disrupted, but the persistent nuclei and chromatophores are diagnostic. The cell contents are clear and finely granular, except that several round refractive bodies may be present. No ingested food has ever been seen, and the organism probably lives holophytically, i.e., like a green plant, although it is believed able to assimilate some dissolved organic matter.

The only observed method of reproduction is by binary fission. Under optimum conditions several divisions may occur in 24 hours, so that blooms are easily accounted for. In general, the behavior of this species corresponds to that of dinoflagellates as a whole, but its effects have made it infamous.

DAMAGE

Damage caused by this particular organism has been described as running into millions of dollars. Its immediate effect is a wholesale killing of fish, both sports and commercial varieties. In addition, fingerlings (baby fish) and many other animals such as crabs, shrimp, scallops, marine worms, minute copepods which serve as fish food, and even porpoises die. This killing affects the activities of both charter boats and commercial fishing boats. It also stops skiff rental and the sale of bait, fishing gear, and fuel. Dead fish accumulate in windrows on the beaches, and the cost of burying or removing them must be met. They also accumulate in coves and bays, and produce a nauseating stench. This stops bathing, so the whole tourist trade is adversely affected. Some sort of gas or windborne spray is produced at times which, while odorless, is a mild bronchial irritant, and is feared when it accumulates sufficiently to be noticeable. Woodcock (4) studied this substance in the 1946-47 outbreak and showed that the irritating factor was confined to droplets of windborne spray. Since the irritant is present only in droplets originating from water containing *brevis* or water in which *brevis* has lived, it appears likely that it may be identical with the toxin. While its effects on human respiratory tracts are slight and transient, the necessity for isolating and studying it is strong. It must be emphasized, however, that this has not been termed a health hazard.

There have also been rumors of enteric troubles due to eating shellfish from a Red Tide infected area. This point of human illness is emphasized by La Cossitt (5) in a popular journal, but it should be stressed that there has been no definite tracing of any human illness to *brevis,* and there has been no widespread illness when the Tide was present. The county health officers of the affected area have reported no health troubles from it. Dr. Wright, of Sarasota County, personally obtained clams dug in a heavily infested area, and bacteriological examination of them by a trained shellfish bacteriologist showed nothing unusual. It should be pointed out that Hornell *(loc. cit.)* says the people along the Indian Malabar coast harvest and eat the fish killed by their Red Tide. To toxic effects like those due to *Gonyaulax* on the California coast have been demonstrated. Quite respectable strings of sheepshead and other fish have been caught in waters containing up to 100,000 *brevis* per liter. These were eaten with no untoward effects whatever. Nevertheless, publicity in newspapers and nationally circulated magazines concerning actual damage plus rumored effects has tended to magnify conditions and probably has further contributed to financial loss in the area.

Such accounts should be viewed against a long period of time to be correctly appraised. Acute conditions rarely last more than a very few days. A strong offshore wind or a spell of heavy weather is sufficient to disperse a tide-borne invasion of dead fish or to break up concentrations of *G. brevis.*

Some ideas as to the quantities of fish killed may be gathered from pictures which have appeared in a wide range of publications. Table I gives some numbers of fish washed up on beaches or enumerated

floating at various places and times. When flying over an area or cruising through it, it may frequently be noted that dead fish are distributed "15 feet apart" or some similar estimate. This is at best a poor method of estimation and does not take account of fish which are not floating, the size of the area, and when and where the fish were killed. Usually during a fish kill the small bottom fish appear first, inshore, then the larger ones. The kill of baby fish is especially bad because it slows replacement, and because it seriously affects the food chain for adult fish which move in after the Tide has passed. Since other elements of the food chain—shrimp, copepods, shellfish, worms—are killed, the whole ecologic picture is a dark one. In addition, even the bait may be wiped out, which further hurts the fishing.

The only redeeming feature is the fact that replacement is either rapid or, despite the numbers of dead fish observed, the percentage of kill is low. There is some indication that both of these conditions occur. Schools of fingerlings have been observed in the very shallowest inshore waters where there was a heavy kill a few hundred yards away. The upper part of Tampa and Hillsborough bays was not invaded by the Tide in 1953-54, and at least some large fish were present there. It was also observed that while a bad outbreak with attendant fish mortality might be in progress at one point, a few miles away one would find sports fishing and bathing going on as usual, and no scarcity of mullet for cut bait. At the time of writing, the last localized outbreaks of the 1953-54 Red Tide are only a few weeks past, yet sports fishing (grouper, speckled trout, mackerel, and redfish) has been generally good throughout the entire area to judge by newspaper accounts. The whole picture of other than locally acute conditions seems to demand more intensive collection of data and better evaluation with less careless talk.

Galtsoff (6) has shown that outbreaks apparently have occurred in this area since 1844, and that they usually occur many years apart. We have known of *G. brevis* as such only since 1946, and while there have been three outbreaks since that time (1946-47; 1952; 1953-54), previous outbreaks such as the "green tide" mentioned by Canova (7) in 1885 might have been fully as intense, but less irritating to the much smaller tourist industry of earlier years and so less publicized. This infrequent occurrence along our Gulf Coast might well be contrasted with the yearly fish kill noted above along the Malabar Coast of India.

Nevertheless, the Red Tide is responsible for heavy damage, and whether frequent or infrequent deserves careful study aiming at its prevention and/or cure.

TABLE I

COUNTS OF DEAD FISH

On Land

LONGBOAT KEY

September 18, 1953

Fish Counted in Quarter Mile

Yellow Tails	298	Mullet	11
Whiting	11	Speckled Trout	4
Jack Crevalle	2	Puffer	1
Redfish	3	Angel Fish	1
Pompano	1	Porcupine Fish	8
Eel	21	Red Snapper	1
Ladyfish	3	Shiner	2
Catfish	11	Horseshoe Crabs	2
Toadfish	6	Unidentified Fish	33
Triggerfish	2		421

SIESTA KEY

September 19, 1953

Fish Counted in 125 Paces (yds.)

Grouper	2	Catfish	4
Whiting	14	Flounder	1
Eel	3	Sheepshead	1
Porcupine Fish	8	Angel Fish	1
Mullet	14	Yellow Tails	200
Ladyfish	1		256
Toadfish	7		

VENICE JETTIES

September 19, 1953

Fish Counted in 100 Paces (yds.)

Mullet	418	Other Species	15

MIDNIGHT PASS

December 18, 1953

Fish Counted in Quarter Mile

About 1000 Sheepshead — About 8000 Other Species

At Sea

SOUTHWEST OF PASS-A-GRILLE, 10 MILES

September 18, 1953

Huge windrow here, but for several miles an average of 1 dead fish per 10 feet.

OFF SANIBEL LIGHT, 6 MILES

March 18, 1954

Dead fish averaged 1 per 15 feet for an area of several square miles—perhaps 5 x 5 miles.

As mentioned previously *G. brevis* at present is known only from the Florida Gulf Coast area (Figure 1). Its actual occurrence has been studied most extensively in the 1953-54 outbreak. Much of the information in prior outbreaks has been inferential, based on the presence of dead fish and other organisms. Since there are many reasons for widespread fish kills, evidence other than microscopic identification of *G. brevis* must be regarded askance in speaking of it as a cause of fish kills in this area. Therefore, extensive studies of the distribution of the dinoflagellate are a necessity and are under way.

DISTRIBUTION

Methods of Sampling and Analysis of Samples

For purposes of future study, water samples containing *G. brevis* are preserved by adding 5 ml. of formalin per 100 ml. of sample. *G. brevis* then rounds up and becomes a flattened oval or circular disc, usually losing its two flagella. Color is ordinarily lost in a few days. Its chromatophores frequently assume a median bandlike position, and the girdle and sulcus become indistinct. The anterior nipple often can be distinguished. With very little practice the species is totally identifiable, even after the color has wholly disappeared from the chromatophores.

In the field, grab samples are taken in shallow water simply by lowering a container from a bridge, dock, or boat, and getting an approximate surface sample. For vertical sampling Foerst bottles are used, and the depth calculated roughly from meter markings on the line and the angle of the line. This is further correlated with the depth for the station as indicated by a Coast and Geodetic Survey map.

Preserved samples are sedimented in the dark for two weeks or more, when the supernatant may be siphoned off, and the catch concentrated by centrifuging the final 50 or 100 ml. for five minutes at about 2200 rpm in pointed-end 50-ml. tubes.

Samples to be examined alive are protected from the sun and from any sudden increase in temperature. Such living samples should be concentrated and studied within eight or ten hours, also by centrifuging. No tendency to disintegrate has been observed at the speeds used (up to 2300 rpm.), but living organisms will cytolyze within 15 or 20 minutes under a cover glass.

Counting is done by a drop method. The catch is concentrated in ratios such that 6 drops of catch = 100 ml. of raw water. In dense blooms this ratio may be doubled or quadrupled, i.e., 12 or 24 drops = 100 ml. of raw water. One drop, therefore, equals 16, 8 or 4 ml. of raw water, approximately. A 25 mm. square No. 1 cover glass is used, and with the usual 10x oculars and 10x or 43x objectives in the microscope there are 16 or 64 paths—not circular fields, but paths—across such a cover; that is, in one drop of catch. If a drop of catch = 16 ml. of raw water, obviously one path at 100x (10x oculars and 10x objective) equals one ml. of raw water and at 430x one path equals ¼ ml. Using the mechanical stage, one counts two paths across each drop on a slide, bisecting the cover and at right angles to each other. This usually compensates for inequality of distribution beneath the cover, and often enables one to identify much smaller organisms than can be identified in a counting chamber. The method is fast and easy, and the amount of error is small. The number of paths counted depends to some extent on the accuracy desired. Common practice has been to count eight paths (two paths for each of four drops), which at 430x usually equals two mls. of raw water. *G. brevis* is not always certainly identified at 100x, but is unmistakable at 430x.

Area Covered in Sampling

Using the techniques described above, workers of the College of Engineering Red Tide Project have examined hundreds of samples, both living and preserved, since December, 1952. Most of these were collected from inshore waters in the Big Marco Pass-Tarpon Springs area. Samples were taken at the mouths of creeks such as Gottfried, Palm River and others; at the mouths of such rivers as the Alafia, Caloosahatchee and Peace; from bays such as Lemon and Big Sarasota; in the various passes; and a few from the Gulf, 30 to 100 miles out. Large numbers were also sent in from such places as Cedar Keys, Alligator Harbor, Santa Rosa Sound, the Gulf Stream off Miami, and more recently a large number have been taken out in the Gulf through the courtesy of the U. S. Coast and Geodetic Survey. In addition, for purposes of comparison, several hundred samples from the North temperate Atlantic (Woods Hole, Georges Bank, the Delaware and Chesapeake Bays) and from the equatorial Atlantic have been examined. A very large number of samples from inshore waters around Long Island were carefully examined with reference to pollution effects on their biota, while the offshore samples were examined relative to productivity. While many of these antedate the present Red Tide work, *G. brevis* would certainly have been recognized, if present. None have been found. Since *G. brevis* has been found in all months of the year in its known habitat, it seems from all these examinations that at present the waters around and offshore from the Tarpon Springs-Big Marco Pass area must be regarded as its endemic habitat.

Routine sampling has been very inadequate, except for inshore sampling from the head of Hillsborough Bay to Big Marco Pass. In this area, for all of 1953, health department sanitarians of Manatee, Sarasota, Charlotte, and Lee counties took samples once each two weeks from 24 stations, which coverage is deemed adequate for that period. Much nonroutine sampling has been done during the 1953-54 outbreak, both for the University of Florida, and for the University of Miami and the U. S. Fish and Wildlife

Service, but sampling well offshore and vertical sampling have been very sparse. We still cannot claim to know how far westward, nor to what depths, the endemic habitat of *G. brevis* extends.

Organisms Other Than Gymnodinium brevis *In the Areas Surveyed*

In all of the Gulf samples, analysis was primarily a search for *G. brevis*, but qualitative and quantitative counts and identification were made for all other microscopic organisms above bacteria. In some fish holocausts, bacteria have been reported (2) as being the only biologic agents present. Actually, unless vertical sampling proved the contrary, there may have been deep stratification of other organisms. According to Zobell (8) the bacteria of the Gulf are poorly known. However, some investigations of bacteria from Red Tide waters have been made, and Bein (9) credits some of the chromogenic types with toxic qualities. Our laboratory has about 14 strains of marine bacteria, some of them chromogenic and presumably pure, mostly isolated from the tanks at Marineland, Florida. Extracts from mass cultures of these should shortly be available for testing.

It would be exceptional if bacteria (saprophytic types, not killers of fish) were not abundant in areas where there were many dead fish. But the possibility of some symbiotic relationship between *brevis* and bacterial species needs investigation, at least as a part of the ecologic picture, if for no other reason. On the whole, our knowledge of bacteria in the areas in question is very scant.

There is available, however, a great deal of biologic data for Gulf samples generally, and a full year's study (for the Hillsborough Bay-Big Marco Pass area) of protista (algae and protozoa) of these waters, since counts which were begun in January, 1953, were continued well into 1954 after *G. brevis* appeared in late August, 1953.

This plankton is a rich one; exclusive of local variations, there is an abundance of diatoms, dinoflagellates, minute flagellates of several categories, and ciliates, and lesser numbers of blue-green algae and rhizopods. It is a plankton such as might be expected in nutrient-rich waters, and it showed frequent local blooms; for example, a bloom of the diatom Coscinodiscus in the area around the mouth of the Myakka river in 1953 colored that already brown water a deeper brown. Table II shows the most common species or genera counted in the Big Marco-Tarpon Springs area since work began in 1953. It should be noted that in many instances only a genus is given; it is often impossible to make species identification and count at the same time. The species list in Table II is probably far from complete for the area. It indicates that there is an abundance of food for plankton feeders, and that the great majority of species found here are not unusual, but that this list is directly comparable to the species lists for other locations such as Long Island Sound or Chesapeake Bay. There are some unusual species, of course, and some which occur in numbers not elsewhere encountered. The most important indication is that the area is not markedly different in its microscopic biota from other areas, except perhaps in some one aspect which might account for the *brevis* blooms.

Predominant are the diatoms. In fact they are so abundant that after the first year (1953) counting of them was discontinued, and only the species or genera present were noted. Next in importance, if not in abundance, are various dinoflagellates. The small colorless and colored flagellates of other taxonomic groups are very abundant also, and at certain times or places, other particular groups abound. Thus the upper part of Hillsborough Bay tends to have exceptionally large numbers of ciliates. This is evidence of the large bacterial populations in the area, and this in turn, of some degree of pollution of the area by organic wastes.

The surveys have also shown that many other organisms live and even bloom along with *G. brevis*. Table III shows a comparison of this sort for three samples collected January 6, 1954. It indicates that other organisms outnumbered *G. brevis* and that the same group was not predominant in each of the three samples. It is well shown by the total group of analyses that the inshore waters are rich in fish food, or in the supply of protista at the base of the fish food chain. Analyses of the type shown in Table III are evidence that there is no break in this food chain at its lowest level. There is no evidence here that the toxins of *G. brevis* are effective against other protozoa and algae. If there is a break in the food chain, it must be at a higher level, a point which is currently under investigation.

The analyses show that most of the genera and species of protozoa and algae found elsewhere, likewise occur in the *brevis* area. *G. brevis* is limited in its distribution, and is one of the very few of its group that is. Of course, it may yet be found to be widespread, but so far it is one of a very few organisms which seem peculiar to a given habitat. There are some other dinoflagellates which behave in similar fashion—Noctiluca, for example. This organism is abundant where there are records of its occurrence, but it too has been totally lacking in the samples thus far analyzed.

TABLE II

Organisms occurring commonly in water where *Gymnodinium brevis* has been found, or simultaneously with it. Virtually all of these organisms are suspended, not bottom-dwelling forms.

PROTOZOA

Rhizopoda

Amoeba spp.
Biomyxa sp.
Chaos spp.
Difflugia sp.
Microgromia parva
Pelomyxa sp.

Flagellata

Bicoeca mediterranea
Bodo globosus
Bodo spp.
Monas spp.
Monosiga ovata
Phyllomitus amylophagus
Pleuromonas jaculans

Ciliata

Amphorella brandti
Amphorellopsis sp.
Askenasia volvox
Aspidisca costata
Codonella cratera
lusitanica
Coleps hirtus
Coxliella ampla
Craterella obscura
Cyclidium spp.
Cyclotrichium gigas
meuneri
Cymatocylis sp.
Didinium nasutum
Dysteria navicula
Euplotes spp.
Favella campanula
composita
panamensis
Halteria grandinella
Helicostomella kiliensis
subulata
Holophrya sp.
Lacrymaria sp.
Leprotintinnus nereticus
Lionotus fasciola
Mesodinium acarus
pulex
Metacylis jorgensii
mereschkowskii
Metacylis vitreoides
Onychaspis sp.
Parafavella sp.
Placus socialis
Pleuronema chrysalis
Rhabdonella torta
valdestriata
Stenosemella nivalis
Strobilidium sp.
Strombidium cornucopiae
spp.
Tiarina fusus
Tintinnidium primitivum
Tintinnopsis beroidea
butschlii
cylindrata
dadayi
lata
minuta
mortensi
platensis
spp.
Tintinnus angustatus
apertus
parvula
pectinis
tubulosus
Trochilia marina
Vaginicola sp.
Vorticella spp.

ALGAE

Myxophyceae

Anabaena sp.
Calothrix spp.
Gomphosphaeria sponina
Lyngbya spp.
Merismopedia sp.
Microcystis incerta
Nodularia sp.
Oscillatoria spp.
Richelia intercellularis
Spirulina sp.
Skujaella sp.

Chlorophyceae

Carteria spp.
Chlorella spp.
Pyramidomonas montana
Pyramidomonas spp.
Pedinomonas sp.

Euglenophyceae

Anisonema ovale
Eutreptia lanowii
viridis
Petalomonas mediocanellata
Tropidoscyphus octacostatus

Cryptophyceae

Chilomonas sp.
Chroomonas sp.
Cryptomonas spp.
Cyathomonas truncata
Rhodomonas spp.

Xanthophyceae

Chloramoeba marina
Heterochloris mutabilis
Olisthodiscus luteus

Ebriaceae

Ebria tripartita
Hermesinum adriaticum

Silicoflagellata

Dictyocha fibula
Dictyocha speculum

Coccolithophoridae

Acanthosolenia mediterranea
Pontosphaera sp.
Syracosphaera carterae
Syracosphaera mediterranea
sp.

Chrysophyceae

Chromulina ovalis
Chrysamoeba radians
Chrysococcus cingulum
sp.
Dinobryon balticum
Prymnesium saltans
Synura uvella

Dinoflagellata

Amphidinium fusiformis
herdmani
spp.
Ceratium furca
fusus
longipes
tripos
spp.
Cochlodinium achromaticum
archimedes
Dinophysis tripos
Diplopeltopsis minor
sp.
Diplopsalis lenticula
Exuviaella lima
marina
Goniodoma sp.
Gonyaulax digitale
monilata
polygonatum
scrippsae
triacantha
spp.
Gymnodinium aeruginosum
brevis
fusca
hyalinum
minor
nelsoni
pellucidum
simplex
Gymnodinium splendens
variable
spp.
Gyrodinium lachryma
sp.
Massartia sp.
Minuscula bipes
Oxytoxum gladiolus
Peridiniopsis asymmetrica
Peridinium cerasus
depressum
divergens
mite
pentagonum
punctulatum
quadridens
roseum
tabulatum
trochoideum
umbonatum
spp.
Phalacroma rotundatum
sp.
Podolampas bipes
palmipes
Polykrikos labourae
schwartzii
Prorocentrum gracile
micans
triangulatum

Bacillarieae

Actinoptychus spp.
Amphiprora spp.
Amphora ovalis
Asterionella formosa
japonica
Bacteriastrum delicatula
hyalina
Biddulphia spp.
Campylosira spp.
Cerataulina bergonii
Chaetoceras gracilis
spp.
Climacodium frauenfeldianum
Cocconeis placentula
Corethron hystrix
Coscinodiscus spp.
Denticula sp.
Ditylum brightwelli
Eucampia zoodiacus
Eunotia sp.
Fragilaria sp.
Grammatophora marina
Guinardia flaccida
Gyrosigma spp.
Hemiaulus hauckii
sinensis
Lauderia borealis
Leptocylindrus danicus
Licmophora abbreviata
Lithodesmium sp.
Melosira abbreviata
moniliformis
nummuloides
Melosira sulcata
Navicula spp.
Nitzschia closterium
longissimum
paradoxa
seriata
Planktoniella sol
Pleurosigma spp.
Plagiogramma vanheurcki
Rhabdonema sp.
Rhizosolenia alata
calcar avis
fragillisima
robusta
setigera
styliformis
stolterforthi
spp.
Rhoicosphenia sp.
Skeletonema costatum
Stephanopyxis turris
Streptotheca sp.
Striatella unipuncta
Surirella spp.
Synedra spp.
Thalassionema nitzschoides
Thalassiosira spp.
Thalassiothrix delicatula
longissima
sp.
Triceratium sp.
Tropidoneis sp.

Gymnodinium brevis *Distribution As to Area and Time*

The areas in which the 1946-47 Red Tide was active and identified are set forth by Gunter *et al* (10). There are no figures which show its exact time of beginning or end and the total area in which it was active. The 1952 outbreak was apparently rather limited in the area affected, which seemed to center around Sanibel Island. There is little published information on this outbreak, at least at the present time. A very few cells of *G. brevis* were present in some samples taken in this area in November, 1952, but by January 9, 1953, no *brevis* were found in samples taken from Sarasota to Big Marco Pass. At this time the sampling stations referred to above were set up. Thereafter they were visited once in two weeks, and at intervals of about six weeks the accumulated preserved samples were collected and returned to the Gainesville laboratory for analysis. On these six-weeks' collecting trips live samples were taken and examined in transit, to aid in recognizing organisms which preserved poorly.

The first *brevis* to appear in 1953 were found in samples taken in Lemon Bay from the bridge on August 19, and in Sarasota Bay at the Cortez bridge, August 26. The first of these was a preserved sample, containing 116 *brevis* per ml. and the second a living sample, showing 178 per ml. On the latter date, August 26, there were no brevis at Naples or at Piney Point. On September 1 the organism appeared at the Placida Ferry wharf, and on September 3 there were 1,732 per ml. in Big Sarasota Bay, about three miles north of the bridge, and 600 per ml. in the surf at Golden Beach on Longboat Key. They were also present as far out as 22 miles off Anna Maria, and on this date dead fish were appearing. It is significant that no dead fish were seen, or at least reported, until after the populations of *brevis* had attained considerable numbers.

Such was the beginning of the 1953-54 Red Tide. From the very first it was marked by local concentrations rather than a widespread uniform distribution. The area covered was very large, and *brevis* was found in samples taken as far north as Tarpon Springs and as far south as Big Marco Pass, a distance of 175 miles, and from landlocked waters such as Lemon Bay to 45 miles offshore. A sample network for an area this size was not possible, despite the efforts of the three research organizations at work and those of the Gulf Coast Coordinating Committee. In the event of another outbreak, some such set-up to assess the magnitude of the invasion seems almost a necessity if distribution is to be ascertained.

At the north and south ends of the infested area there was a rather sharp tapering off of *brevis* populations. No fish kills were reported from Tarpon Springs or Big Marco Pass, although dead fish were reported as abundant off Anclote Key on at least two occasions. These may well have been carried there by the northbound current of the eddy reported by Hela (11). Out in the Gulf, the most westerly reported occurrences of *brevis* were about 140 miles southwest of Fort Myers in samples taken by the U. S. Coast and Geodetic Survey ship "Hydrographer." At the time of this writing some of the offshore Gulf samples are still awaiting analysis. However, the diminution in numbers along the western edge of the shaded area (Figure 1) indicates that the area of intense growth is fairly well delimited.

Clearwater had a single small fish kill along its beaches, with some attendant discomfort; Naples had a rather larger one. The beaches between these two

TABLE III

Groups, Number of Species and Number of Organisms per Liter Present with *G. brevis*, January 6, 1954.

Organisms or Groups	Opening of Tampa Bay at Anna Maria		One Mile Off Venice Jetties		Big Sarasota Bay at Sarasota	
(Single Sample)	No. Species	No. Organisms	No. Species	No. Organisms	No. Species	No. Organisms
Gymnodinium brevis	1	240,000	1	125,000	1	14,000
All other dinoflagellates	4	31,000	5	1,106,000	5	65,500
Diatoms	18	132,250	21	90,250	8	165,500
Blue-green algae, filaments	1	2,000	1	1,000	1	2,000
Chrysomonads	2	107,000	1	4,000	2	4,000
Cryptomonads			1	24,000		
Ciliates	2	750				
Excess of other protista over *G. brevis*	26	32,750	28	1,100,250	15	219,000

points, from Fort Myers Beach to the Pinellas county beaches, all had one or more very heavy fish kills, and the inhabitants were subject to a great deal of discomfort. Probably the most continuously affected area was from Casey Key to Sanibel, and the heaviest continuous infestation was in the Boca Grande area.

Table IV gives an idea of the numbers of *brevis* which we have encountered in given areas of dense growth during the present Tide. The total for sample 1011, of 4,814,000 per liter, is the highest natural concentration found in any sample. This is far below the 15,000,000 per liter reported by Lasker and Smith (12) for the 1946-47 Tide. It is also below the density which sometimes occurs when a large sample is brought into the laboratory and allowed to stand. Such an aggregation is shown in Figure 3, in which the dark cloud represents perhaps 99 per cent of all the *brevis* in a five gallon carboy. In laboratory cultures, Wilson (13) reports the same phenomenon, which in his cultures is manifestly a response to light.

Such dense aggregations under field conditions are usually at or near a focal point for a swarm. This is well shown by the Lemon Bay samples of Table V. Nevertheless, widespread concentrations of up to 500,000 per liter were encountered with some frequency in 1953-54 when well over 150 samples from widely scattered localities showed concentrations of over 100,000 per liter. To the layman these may seem to be prodigious numbers, as indeed they are.

TABLE IV

Numbers of *Gymnodinium brevis* per ml. of Raw Water in Selected Areas of Dense Growth During the 1953-54 Red Tide Outbreak.

Date	Station	Depth	No. per ml.
8-26-53	Cortez bridge	Surface	178
9- 3-53	Entrance, Sarasota Bay	Surface	780
9-18-53	Gulf, surf at Golden Beach Longboat Key	Surface	2,140
10- 6-53	Gulf, off Clearwater Beach	Surface	140
11-24-53	Venice, at jetties	Surface	392
12-10-53	Gulf, 5 mi. off Siesta Key	Surface	580
1- 6-54	Anna Maria Key, N. end	Surface	240
2-10-54	Stickney Point Bridge	Surface	504
3-18-54	Pine Island Sound, off York Island	Surface	234
3- 6-54	Off Sanibel, 3.5 mi. S.	4 meters	684
4-30-54	Off Stump Pass, 1 mi. W.	8 meters	1,042
6-18-54	Lemon Bay, center, off Englewood	Surface	4,814
8-12-54	Off Sarasota, 20 mi. S. W.	15 meters	29
8-12-54	Off Sarasota, 20 mi. S. W.	23 meters	19

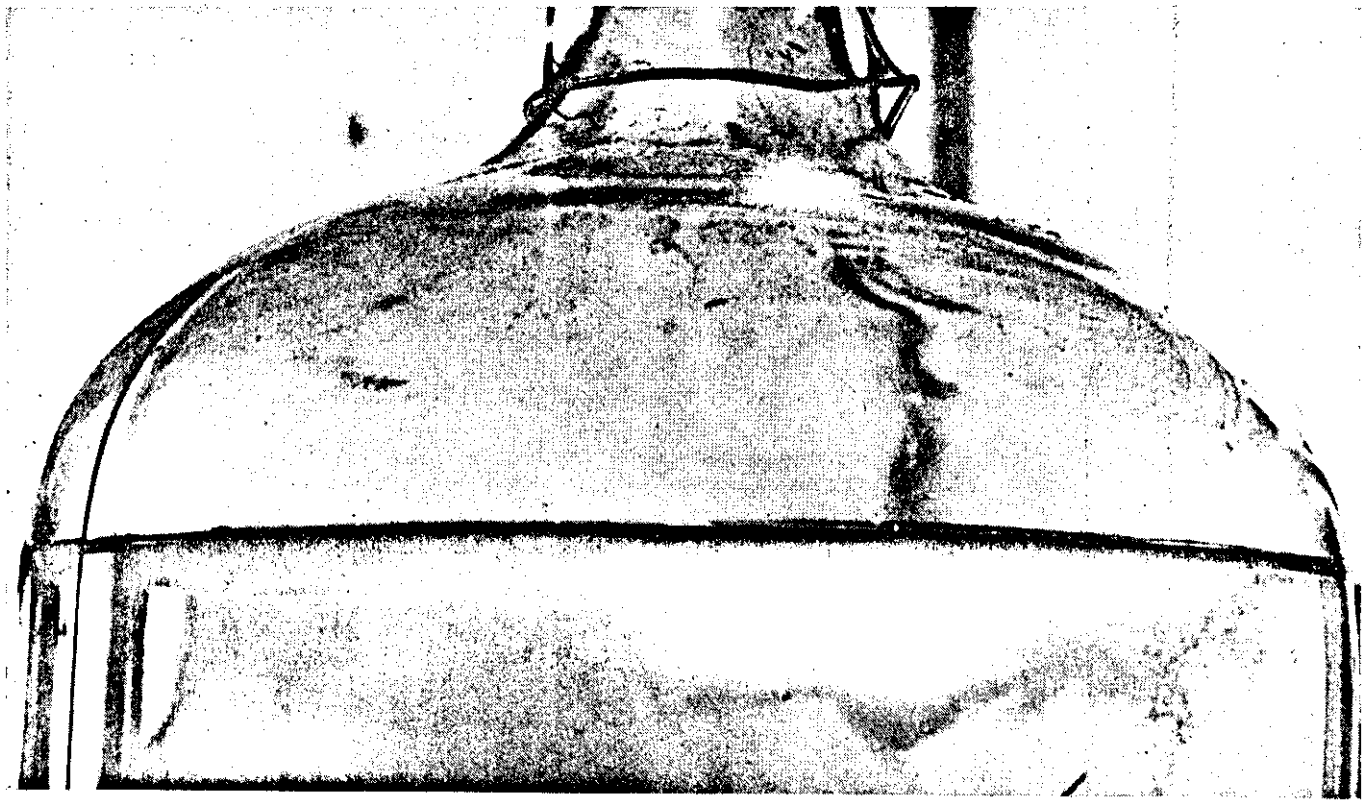

Figure 3. Illustration of the peculiar aggregating or clumping effect exhibited by *Gymnodinium brevis*. The dark cloud hanging below the surface of the water in the approximate center represents a collection of virtually all *brevis* in a five gallon carboy. When the carboy was filled, the organisms were uniformly distributed in the water.

Nevertheless, one cannot detect a discoloration of the water at the surface until the number of *brevis* exceeds 250,000 or more per liter, and apparently there is no quick fish kill until the number approaches or exceeds 450,000 per liter. Actually at Fort Myers Beach on March 18, 1954, a non-working member of an investigating crew had a nice catch of sheepshead and other fish from water containing about 20,000 *brevis* per liter, while many samples in the vicinity showed between 100,000 and 300,000 per liter. Evidence both from fish kills and from field samples indicates that dense aggregates are rare, and that even during a heavy infestation, the number taken in any random sample will tend to be low.

TABLE V

Red Tide Organisms in Lemon Bay
June 18, 1954.

Sample No.	Location	No. per Liter
1001	Off Stump Pass	211,000
1003	Between Stump Pass and Punta Gorda Beach	9,500
1005	Mouth of Ainzes Creek	94,000
1007	Between Forked Creek and Godfrey Creek	290,000
1009	Bay, offshore at Englewood	276,000
1011	Center of bay, off Englewood	4,814,000
1013	Off Lemon Key, opp. Englewood	3,235,000
1015	Center of bay, opp. Godfrey Creek	212,000
1017	Center of bay, opp. Punta Gorda Beach	82,500

Red Tide Organisms in Sarasota Bay
June 19, 1954.

Sample No.	Location	No. per Liter
1019	Middle Sarasota Pass No. 9	500
1021	Below Cortez-Bradenton Beach Bridge	33,000
1023	Inside Longboat Pass	317,000
1025	Off Longboat Key, in Sarasota Bay Channel	186,000
1027	Off Longboat Key, in Sarasota Bay Channel	193,000
1029	Middle Sarasota Bay, off Stevens Point	45,000
1031	Middle Sarasota Bay, off Whittaker Bayou	580,000
1033	In New Pass	300,000
1035	In Sarasota Bay, opp. Tank Stack	316,000
1037	In Sarasota Bay, opp. Cedar Point	241,000

Frequently, samples taken a few miles from a rather dense local swarm will show no *brevis* at all. Examples of this are easy to cite. On September 18, 1953, samples taken just off Pass-a-Grille had 2,444,000 per liter, while there were none at John's Pass or in Sarasota Bay at Cortez. Samples at the latter station contained 83,200 per liter three days later. The whole picture for the area shown in Figure 2 is one of usually low or absent *brevis* populations during all of 1953-54, but with sudden erratic, scattered flare-ups to enormous numbers. In short, no discernible pattern can be detected for these flare-ups, and perhaps when one can be found, the greatest single step in understanding and possibly controlling the Red Tide will have been achieved.

The time at which an outbreak occurs might be important, because there is a fair correlation between time and water temperature, especially in the open Gulf. However, seasonal fluctuations in temperature are less pronounced in the Red Tide area than in the Woods Hole area for example. Considering the fact that most of the unicellular algae and protozoa live throughout the year in the active state, and that they attain their population maxima at various times of the year, it seems normal to find *brevis* present around the calendar. There is not even a "bloom season" for it, unless the organism can bloom in a rather wide temperature range. Thus concentrations approaching bloom proportions have been encountered at temperatures ranging from 18° C. to 30° C., and during this past outbreak, in every month from August, 1953, to December, 1954. It is concluded, therefore, that temperature, within the Gulf area where *brevis* has been found, is not a limiting factor.

Vertical Dispersion

The preceding discussion has largely applied to lateral distribution. Thus far there has been too little attention paid to the dispersion of *brevis* in waters deeper than perhaps ten feet.

Much attention has been directed toward recognition of red water from boats and planes. This is entirely proper, and it is frequently possible to spot patches of bloom in this way. In Peconic Bay it has been possible to spot blood-red concentrations of a related dinoflagellate, Cochlodinium, as far as a half-mile away from a small cabin cruiser. However, no such vivid discolorations have been noted in hunting for *brevis* either by boat or by air, and red water has frequently been all but impossible to locate, or has been a slightly brown discoloration rather than red. Obviously any water located in such a manner must be examined microscopically, especially since there are many other organisms which bloom in patches.

The difficulty is further augmented because in deep water bottom growths are deceiving, especially when viewed from a plane. They are then easily mistaken for patches of discolored water. In addition to this uncertainty about such apparent discoloration, the surface pattern produced by clouds is also a cause of trouble, and when there is a combination of this

pattern, plus one due to a change from sand to silt, plus patterns produced by bottom growths, actual sampling and microscopic identification of *brevis* become a necessity. Concentrations in deeper water would almost certainly be missed in an aerial or boat survey, unless depth sampling apparatus was used.

Table VI shows the concentration of *brevis* in ten samples taken March 6, 1954, when both surface and depth samples were obtained. This area is subject to considerable tidal action and to wind action, and probably the water is well mixed, top to bottom at about 12 feet. Such mixing is suggested or verified by the counts of *brevis*. It thus appears that in shallow areas, so long as there is wind and tidal action, concentration will be uniform in the upper few feet of water. In lakes it is not uncommon to find a concentration of microorganisms in the upper few inches. However, one has difficulty picturing such a condition in the Gulf, at least for an organism like *brevis* whose rate of movement is slow.

Vertical migration of dinoflagellates in response to light is well known (14), but is neither a constant effort to remain at the top, nor is the same behavior common to all species. Sampling shows that this species at least is governed by other factors as to the level at which it tends to concentrate. Thus there were no *brevis* at a station close to Sarasota Point, unless they were between the surface and 24 feet. A few miles away they were equally abundant at the surface and at 42 feet, with no indication of how many were between the two levels. On the basis of samples taken at the 10- and 15-mile distances, one might expect that a depth of about 50 feet was their lowest level of choice, and that they tended to inhabit somewhat higher levels. But twenty miles out they are shown in some abundance as deep as about 70 feet. Certainly no generalization can be made for a distribution pattern such as is shown by this data. The following figures also tend to support the idea that it would be unwise to assume a pattern at this time.

TABLE VI

Distribution of *Gymnodinium brevis* Vertically at Certain Points, March 6, 1954. Numbers per Liter.

Off Punta Rassa	Surface	123,000
Off Punta Rassa	8 ft. bottom	118,000
Off Punta Rassa	In channel, surf	466,000
Off Punta Rassa	In channel, 9 ft.	456,000
3.5 Miles South of Sanibel	Surface	612,000
3.5 Miles South of Sanibel	9 feet	744,000
3.5 Miles South of Sanibel	12 feet	684,000
6 Miles South of Sanibel	Surface	480,000
6 Miles South of Sanibel	9 feet	256,000
6 Miles South of Sanibel	12 feet	336,000

4-30-54	3.5 Mi. W. of Little Gasparilla	Surface	104,000/1
4-30-54	3.5 Mi. W. of Little Gasparilla	30 feet	432,000/1
4-30-54	3.5 Mi. W. of Stump Pass	Surface	88,000/1
4-30-54	3.5 Mi. W. of Stump Pass	24 feet	1,042,000/1

Most of these samples were taken in clear or partly cloudy weather, and sufficiently late in the day so that if there is a diurnal migration upward in response to light, it should have been evident. An apparent failure to respond positively to light, at least to the point of accumulating at the surface, is shown in Figure 4. This series of six samples was taken 35 miles west of Fort Myers Beach, Jan. 11, 1955, about 1:00 p.m. on a sunny day. Considering the tendency of phytoplankton to accumulate in the topmost few

TABLE VII

Analyses of 18 Samples of Red Tide Water for Nitrogen, Phosphorus, and Salinity.

Sample No.	Location	Nitrogen ppm	Phosphorus ppm	Salinity
1010-11	Center of Lemon Bay opposite Englewood, 6-18-54	0.507	0.	34.4
1022-23	Inside Longboat Pass, Sarasota Bay, 6-19-54	0.133	0.	35.1
1018-19	Halfway between Anna Maria Key and Perico Island, Sarasota Bay, 6-19-54	0.417	0.0081	29.5
1028-29	Halfway between Stevens Point and Mangrove Point, Sarasota Bay, 6-19-54	0.134	0.	34.2
1026-27	Off Longboat Key, Sarasota Bay, Fl R "26", 6-19-54	0.490	0.0019	33.6
1024-25	Off Longboat Key, Sarasota Bay, Fl G "21", 6-19-54	0.247	0.	35.1
1032-33	New Pass, Sarasota Bay, 6-19-54	0.138	0.0019	35.5
1034-35	Off Tank stack, Sarasota Bay, 6-19-54	0.484	0.	35.3
1030-31	Opposite Whittaker Bayou, Sarasota Bay, 6-19-54	0.217	0.011	35.3
1006-07	Between Forked Creek and Godfrey Creek, Lemon Bay, 6-18-54	0.242	0.	33.7
1014-15	Center of Lemon Bay across from Godfrey Creek, 6-18-54	0.188	0.011	34.6
1012-13	Off Lemon Key, across from Englewood, Lemon Bay, 6-18-54	0.541	0.	33.8
1008-09	Near shore, off Englewood, Lemon Bay, 6-18-54	0.259	0.	34.4
1016-17	Center of Lemon Bay, opposite Punta Gorda Beach, 6-18-54	0.178	0.	34.5
1000-01	Stump Pass, 6-18-54	0.160	0.	34.6
1002-03	Between Stump Pass and Punta Gorda Beach, 6-18-54	0.149	0.021	34.6
1020-21	Between Cortez and Bradenton Beach, Sarasota Bay, 6-18-54	0.221	0.0047	31.9
1004-05	Mouth of Ainzes Creek, 6-18-54	——	0.	35.3

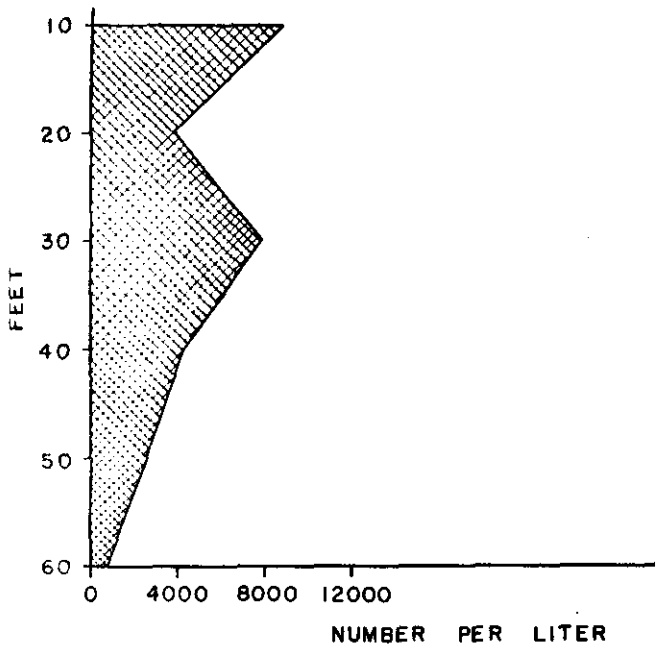

Figure 4. Distribution of *brevis* from the surface to the bottom in 60 feet of water, 35 miles west of Fort Myers Beach at 1:00 p.m., January 11, 1955. Note the two maxima, the second being at the 30 foot depth.

inches in lakes, ponds and even streams as a direct response to light, it is evident that *brevis* presents no such clear-cut response. Not nearly enough vertical sampling has been done at this time, but at least a trend to accumulate in deep water is sometimes evident and will be more closely investigated in future work.

Manifestly, heavy concentrations in bottom waters are not going to be seen by aerial or boat observers. Actually the depth to which they may be noted will depend to some extent on the amount of suspended silt, as well as other organisms present, in the water. Since much of our idea of the distribution of *brevis* has been based until now either on surface samples or (perhaps) on dead fish, these observations suggest that currently accepted distribution patterns, or areas of infestation, be accepted tentatively, for depths greater than ten feet.

These findings on vertical distribution must also be taken into account in any methods of foretelling future outbreaks, and specially in methods of control. This is especially true of control by killing agents. It is one thing to control an acre of water four or five feet in depth, but quite another for an acre of water 70 feet or more in depth. Generally the first indication of an outbreak has been the appearance of dead fish. Usually these have been small bottom fish and found close inshore. But if there is an initial build up of *brevis* populations well offshore and in deep water, then the whole matter of its biology may need reconsideration. Clearly there are too many unanswered questions so long as there is insufficient knowledge of vertical distribution.

ERADICATION OR CONTROL OF BREVIS

The Necessity for Laboratory Study of Living Organisms

One of the prime requisites for studying life cycle, behavior and handling of any microorganism, is laboratory cultivation. In the case of *brevis* and some of its close relatives, we have been handicapped until recently because they could not be cultivated in the laboratory. By agreement the U. S. Fish and Wildlife Service undertook to grow *brevis*, and our laboratory undertook the growth of related species. This phase of the work has been successful, and the U. S. Fish and Wildlife laboratory supplied us with cultures and information on the growth of *brevis*. Our laboratory, meanwhile, had successfully grown two related organisms so that now there are several species available for experimental work. Figure 5 shows the microscopic work of isolating and transferring such organisms, and Figure 6 shows culture tubes and bottles in the specially designed culture room where light, temperature and other factors can be carefully controlled.

By growing these organisms in the laboratory, it is possible to determine facts which cannot be determined in the field. The growth rate (number of divisions in 24 hours) is very important in accounting for bloom production. The effects of particular substances in accelerating growth can be easily determined in the laboratory on a quantitative basis. One can investigate the toxicity of many substances on known populations and over definite periods of time. And with pure cultures (those free of other organisms) it is possible to harvest the culture, make extracts and then test the effects of these extracts on fish or other organisms.

These are some of the reasons for maintenance and study of laboratory cultures. Ultimately laboratory findings are applied in the field, and in this fashion progress is made.

Extent of Control Measures

The Protista are supposedly cosmopolitan in distribution, although there are a number of species which have been recognized by only one observer, or have been recorded from only one area. Usually such areas are quite small, i.e., a bay or a single coastal station. In the case of *brevis*, they have never been reported from any other part of the world than

the Gulf of Mexico area outlined above. That area is quite large, and there is the possibility that *brevis* will yet be found in other parts of the world. Certainly it is difficult to imagine any single factor such as the presence of some chemical component in unusual quantity or ratio throughout the entire known *brevis* area which would account for their presence only in this area. It is even difficult to imagine a build-up of nutrient matter throughout this large area sufficient to account for the presence throughout the area of any single unique species. This would be an easier postulate, if all species, or at least many, increased in numbers at the time *brevis* increased or appeared. But there is little or no evidence that such is the case. Despite the fact that an increase in nitrogen and phosphorus is generally sufficient to increase the numbers of many photosynthetic species which use them, the *brevis* blooms represent by far the most noticeable increases in phytoplankton in the general area. However, they do not seem to represent one of a succession of blooms by a succession of species, such as frequently occur seasonally, but rather an intermittent invasion by one species which repeatedly reaches bloom proportions in small isolated parts of its area.

Figure 5. Method of picking *brevis* for inoculating into test tubes containing culture media. The microscope is a stereoscopic binocular. The organisms are picked out of the hollow ground slide on the stage of the scope. The glass tube in the right hand of the operator has its lower end drawn out into a capillary tube which can pick up a single organism at a time.

As shown above, there is less difficulty in postulating a local build-up of nutrients here and there which could account for a local bloom. Since we have a poor picture of the *brevis* distribution pattern in epidemic years, the postulate of local richness of some food combination, or combination of other factors with attendant heavy local blooms is a safe one, and with precedents easy to cite. Some attention should be given to the possibility of controlling blooms by controlling such accumulations of bloom factors *if the conditions can be discovered.*

The eradication of *brevis* from its area is quite another matter, a most difficult one. There simply is no feasible way of eradicating an organism from an ocean area up to two hundred miles long, perhaps equally broad, and in water up to 100 feet deep. Such elimination has not been possible for many larger land dwelling creatures such as the yellow fever mosquito, *Aedes aegypti,* which is very selective in habitat.

Control of this mosquito has been accomplished, however, which keeps its density below the danger point. Therein lies a promising approach to the *Gymnodinium brevis* problem. There may be some method of controlling the density of the population. While few figures are available, it is believed that a considerable density of *brevis* (about 450,000 per l.) is necessary to produce killing effects, and such densities usually cover relatively small areas, so that some method of control could be obtained. Such control is usually based on some knowledge of when to expect an increase. Lackey and Sawyer (15) have shown that phytoplankton generally blooms when nitrate and phosphate values approach or exceed 0.2 and 0.015 p.p.m. respectively in fresh water. These values are too high for most Gulf waters as shown in Tables VII and VIII. But a knowledge of the nutrient threshold for rapid reproduction, or of the ratio of certain nutrients to each other, or of the amount of necessary organic matter seems necessary in order to know when to expect an increase in *brevis* production. Regardless of other factors which might cause an increase, unlimited growth—from a few cells to millions in a unit quantity of water—is simply not possible without the necessary nutrient substrate.

It is also necessary that a seeding population of *brevis* be present. Once the nutrient requirements are known, the next step is a periodic check to deter-

TABLE VIII

Analyses of 18 Samples of Red Tide Water for Nitrogen, Phosphorus, and Salinity.

Sample No.	Location	Nitrogen ppm	Phosphorus ppm	Salinity
1022-23	Gulf side Siesta Key, Columbus Ave., 8-11-54	0.101	0.0062	35.0
1018-24	Point of Rocks (dead fish, seaweed), 8-11-54	0.111	0.0094	30.3
1004-56	Gulf side Longboat Key, South of Rocky's Restaurant, 8-11-54	0.149	0.020	35.0
1062-1	Boat slip, Bay side Longboat Key, South of Rocky's Restaurant, 8-11-54	17.618	0.010	—
1016-35	New Pass Bridge, 8-11-54	0.255	0.010	35.1
1019-31	23.52 Mi. West Sarasota Point, Surface, 8-12-54	0.194	0.0031	36.3
1020-00	23.52 Mi. West Sarasota Point, 69 ft, 8-12-54	0.413	0.0062	36.3
1059-601	23.52 Mi. West Sarasota Point, 45 ft., 8-12-54	0.133	0.0112	37.3
1037-50	17.83 Mi. West Sarasota Point, Surface, 8-12-54	0.112	0.0062	35.9
1008-64	17.83 Mi. West Sarasota Point, 54 ft., 8-12-54	0.183	0.015	36.0
1014-02	17.83 Mi. West Sarasota Point, 36 ft., 8-12-54	0.159	0.0081	36.3
1067-12	11.21 Mi. West Sarasota Point, Surface, 8-12-54	0.176	0.010	36.0
1013-33	11.21 Mi. West Sarasota Point, 48 ft., 8-12-54	0.183	0.0306	36.3
1034-17	11.21 Mi. West Sarasota Point, 33 ft., 8-12-54	0.175	0.0044	36.0
1030-63	6.79 Mi. West Sarasota Point, Surface, 8-12-54	—	0.0075	35.5
1060-81	6.79 Mi. West Sarasota Point, 42 ft., 8-12-54	0.228	0.0106	36.7
1041-80	0.95 Mi. West Sarasota Point, Surface, 8-12-54	0.169	0.	35.3
1015-32	Venice jetty, 8-12-54	0.083	0.0025	35.4

mine: (a) when these are present in optional quantities, and (b) when the seeding population is present.

Control by Heavy Metal Ions

There are several possible controls which are either under investigation, or should be under investigation. The U. S. Fish and Wildlife Service is currently investigating the use of copper compounds as a control measure. There is no doubt of the effectiveness of copper. For example, a copper compound is used at Marineland to control bacteria and other microorganisms in the tanks. For a year now, monthly counts have been made of bacteria and other microorganisms in the ocean water just off the Marineland intake and in the aquarium water. There is a great reduction of the incoming organisms by the filters. Due to the

Figure 6. Interior of the culture room in the Sanitary Engineering Laboratory. In this room the temperature is kept constant. Constant uniform lighting is provided by batteries of neon tubes. Cultures grow in the test tubes, some of which have screw tops. Most of those with such tops contain living *brevis*.

copper compounds there is almost no growth in the tanks—one filamentous green alga, a few ciliates and naviculoid diatoms grow well, but little else, despite ample seeding after filtration.

There are, however, two factors which have to be taken into account for any killing agent—cost and effects on other life. Since the major *brevis* areas to be controlled are probably shallow inshore areas, cost is fairly easy to estimate. Effects on other living things is an experimental matter, probably largely unknown for copper in sea water, and necessary to evaluate, since indications are that it is quite toxic. Use over wide areas might have undesirable effects on the food chain of fish, on fingerling fish, or even on adults. While copper is the usual ion selected for algal control, there are some others which deserve consideration for marine waters. Arsenic is one, although its use is outlawed in Florida waters.

Organic Algicides

Lately a whole series of algicides, mostly organic, have been developed by various chemical manufacturers. Some of these are specific as to the organisms they kill. These are to be given trials for *brevis* and other organisms, bearing in mind cost and method of application. Experiments with *Phygon* have already been made, and demonstrate that it is toxic, but not selectively so; also that the amount required is too great for the substance to be economically used. In the light of past experiences with algicides, it seems probable that one of rather high specificity and very high killing power can be developed for *brevis*. While this would serve to control the small local swarms and might even be used economically, there appears little hope of all-out control by such a method. The area and the depths to be penetrated are too great, and there is the problem of *brevis*' developing tolerance, as flies have developed tolerance to DDT. However, the coastal cities use dusting or spraying from planes to fight mosquitoes (Naples, Fla., for example), and control of *brevis* in the shallow inshore areas along the coast by the use of plane-dispersed algicides must not be discounted, until very thoroughly evaluated.

Carbon

Gymnodinium brevis requires small quantities of organic substances in its nutrition. Wilson (13), in his culture medium, includes vitamins and soil extract. These are present to some extent in Gulf water, and Odum, Hynes and Slater (16), have shown that charcoal may effectively remove them by adsorption. Since the organic portion of the substrate is very small, only small quantities of charcoal are needed. This work is in its preliminary stages, and there is no field work at present to show the effectiveness of carbon. Experiments are underway to show how effectively it strips out the growth-promoting matter, how many other organisms are affected, how long its effects last, and to what extent it penetrates (how deep it is effective).

Carbon has three other characteristics which recommend it. It is relatively cheap, and the supply is inexhaustible and readily available. It is not in itself toxic; it will not harm fish. There is no reason why it should affect organisms which have no organic requirements. Finally it has been shown by Block (17) that it will adsorb the waterborne toxin of *brevis*. The effect of this would be to cleanse water already poisoned. Clearly charcoal and activated carbon need a very careful investigation.

Physical Agents in Control Work

Biophysics has demonstrated many ways, principally through wave action, of affecting living microorganisms. Here again the work at the University of Florida is just beginning. It has already been shown, however, that there is no useful killing action in a high-frequency radio field. Cultures of five organisms, including *brevis*, were unaffected in glass containers in such a field. The only effects demonstrable were heating effects on exposures of one minute or more. Still to be investigated are electric currents, especially pulsations, ultrasonic vibrations, and some other phases of wave action. Special consideration will be given to the area of lethality in relation to percentage of kill and cost.

POSSIBLE CAUSES OF THE RED TIDE

Phosphorus

As shown above, phosphorus and nitrogen are always suspected as limiting factors in phytoplankton growth, and since *brevis* is at least partially photosynthetic, these were the first chemical components to be investigated. The rich deposits of phosphorus in Bone Valley and the processing plants along the Peace and Alafia rivers have been spoken of as possible factors in Red Tide production. In 1950, Specht (18) gave some figures showing the phosphate content of these streams to be very high. There is a rapid disappearance of phosphorus as the water moves downstream, but frequently enough reaches the Gulf to show high amounts (as compared to the open ocean), well above the 0.015 ppm which Lackey and Sawyer (15) considered as necessary for a bloom of

phytoplankton. The following are examples of the values which may be found:

		ppm
2-19-53	Peace River, Arcadia, Odum	0.850
2-19-53	Peace River, Punta Gorda, Odum	0.240
3-30-53	Placida (Peace Estuary), Odum	0.028
4- 1-53	El Jobean (Peace Estuary), Odum	0.144
4-17-53	Bokeelia (Peace Estuary), Odum	0.024
3- 7-54	Peace River, Punta Gorda, Odum and Hynes	0.670
3-18-54	St. James Point (Peace Estuary), Odum and Hynes	0.040
4- 3-53	Kissimmee River above L. Okeechobee, Odum	0.018
2-19-53	Lake Okeechobee, Moore Haven, Odum	0.039
2-19-53	Caloosahatchee Estuary, Sanibel, Odum	0.009
2-19-53	Caloosahatchee Estuary, Punta Rassa, Odum	0.060
3-30-53	Caloosahatchee Estuary, Ft. Myers Beach, Odum	0.042
3- 6-54	Caloosahatchee Estuary, Ft. Myers, Odum and Hynes	0.094
3- 6-54	60 miles off Naples, Odum	0.001
3- 6-54	70 miles off Naples, Odum	0.042
3- 6-54	80 miles off Naples, Odum	0.001
3- 6-54	110 miles off Naples, Odum	0.001
4- 1-53	Hillsborough Bay, Odum	0.410
4- 7-53	Piney Point, Tampa Bay, Odum	0.120
4- 7-53	Pinellas Point, Tampa Bay, Odum	0.108
4- 6-53	Ballast Point, Tampa Bay, Odum	0.580
4- 6-53	Gandy Bridge, W. end Tampa Bay, Odum	0.240
3- 7-54	Tampa Bay, Odum and Hynes	0.410

All of these indicate a heavy phosphorus contribution from the land, and in reality the only reason for singling out the Peace River is its volume. Even the smaller streams may contribute large amounts of phosphorus. Thus Phillippi Creek, 7-23-54, was shown by Hynes to contain 0.0294 ppm as well as 0.480 ppm nitrogen.

All of the samples in the list above, except the last, indicate a progressive diminution of phosphorus as the water travels into the Gulf. It is a comparatively easy matter to find samples wholly lacking in detectable amounts as is shown by Tables VII and VIII for June 18 and 19 and for August 12, 1954. These samples were analyzed by Hynes. Twelve out of 36 showed no detectable phosphorus, and one of the deep water, offshore samples exceeded this value of 0.015 ppm. In truth, conflicting records are too easily obtained. Of course some of the differences might arise because of the use of different analytical methods, or a chance of impurity in an analytical reagent; one reason for always repeating a piece of work. Table IX shows the values found by Chew (25) in 12 samples obtained November 14-15-16, 1952, in which 8 samples exceeded the critical value.

Samples taken in July during the 1946-47 outbreak, and reported by Ketchum and Keen (26) contained 0.302 to 0.604 ppm. These seem fantastic values for the open Gulf, five to ten times higher than any previously encountered in uncontaminated oceanic waters. Additional samples were taken between Venice Inlet and Sarasota, July 22, 1947, from red water, and one month later when the water was clear blue.

TABLE IX

Total Phosphorus Concentrations at Various Locations and Depths as Determined by Chew (25).

Date and Location	Depth	Phosphorus ppm
11-14-52		
Off Ft. Myers (Sta. 2) heavy red water	Surface	0.071
Off Ft. Myers (Sta. 2)	6 M	0.009
Off Ft. Myers (Sta. 2)	10 M	0.053
11-16-52		
Off Ft. Myers (Sta. 6a) heavy red water	Surface	0.016
Off Ft. Myers (Sta. 6a)	3.9 M	0.034
Off Ft. Myers (Sta. 6a)	7.9 M	0.019
11-15-52		
Off Ft. Myers (Sta. 5) clear	Surface	0.118
Off Ft. Myers (Sta. 5)	6 M	0.062
Off Ft. Myers (Sta. 5)	12 M	0.0
11-16-52		
Off Ft. Myers (Sta. 10) clear	Surface	0.016
Off Ft. Myers (Sta. 10)	5 M	0.003
Off Ft. Myers (Sta. 10)	10.9 M	0.009

At these times the concentrations were from 0.152 to 0.632 ppm and 0.019 to 0.038 ppm. The July samples were still very high, exceeding virtually all of this laboratory's samples, except those from the rivers, and Chew's samples. Even the lowest values from Ketchum and Keen's normal samples exceed those of most of our samples.

Two possible explanations were given for this high phosphorus content. It was suggested that the *G. brevis* cells may have taken up all of the phosphorus from the entire water column and then have swarmed at the surface where the samples were taken. In water 30 feet deep it would then be possible to account for a ten-fold increase in phosphorus concentration in the upper three feet of depth. But if the analyzed surface samples were representative of the entire water column, large-scale fertilization or contamination must have taken place. If the latter situation were true, about 17,000 pounds of pure phosphorus per square mile would have been required, and areas of several square miles were involved. Salinity data on these samples indicate that there was little or no excess fresh water drainage into the water represented by the July samples, so the possibility of terrigenous contamination appears unlikely. The recent findings concerning the vertical distribution of *G. brevis* would seem to rule out the surface swarming phenomenon, and the high phosphorus concentrations found during the 1947 outbreak are still not explained.

Graham (19) and others found that the mean total phosphorus content over a 16-month period from May, 1949, to August, 1950, in this area was about 0.011 ppm. These figures are considerably below those for

the 1947 samples, as are those found for the present bloom.

Actually *brevis* has been found blooming in water practically lacking in detectable phosphorus (June 18, 1954, Center of Lemon Bay, Tables IV and VII: phosphorus 0.000 ppm, *brevis* 4,814,000 per liter) and in water containing abundant phosphorus. The organism has also been wholly lacking in water such as Hillsborough Bay, and the upper part of Tampa Bay at times when those waters have shown abundant phosphorus. In view of these facts, and in view of the amounts of phosphorus contributed by waters coming from other sources than the Alafia and Peace rivers, it seems premature to indict the phosphate mining industry as causing the Red Tide.

Nitrogen

It certainly appears that phosphorus is not the entire answer to the problem of what initiates a Red Tide bloom. The nitrogen compounds must also be considered. Among those which may be important to dinoflagellate growth are nitrates, nitrites and amino acids. In studying a bloom in Great South Bay off Long Island, Ryther (20) found that the ratio of nitrogen to phosphorus was the factor determining the amount of growth of the organism involved. The principal dinoflagellate which bloomed in Great South Bay was *Prorocentrum triangulatum,* a species whose nutrition has not, until now, been investigated, but which is photosynthetic, and which (Lackey, unpublished data) sometimes reached very high numbers.

Insofar as field observations go, nitrogen has never been lacking when *brevis* was present either in low numbers or in blooms. In this respect it is different from phosphorus and one would be tempted to infer that it is necessary at least in concentrations of 0.1 ppm. Unfortunately there is little correlation between the observed amounts of nitrogen and observed numbers of *brevis.* Figure 7 shows graphically the relationships between *brevis* and total nitrogen in Lemon Bay. Probably the Lemon Bay situation is closest to a normal relationship. There were few other organisms present, and the Bay itself receives few if any sources of large amounts of nitrogen, such as sewer outfalls. Therefore, one concludes that since the largest amounts of nitrogen coincide with the greatest numbers of *brevis,* the source of the nitrogen in the analyses is in the bodies of the plankters. Odum (21) failed to find any nitrate nitrogen in a series of about 15 field samples in *brevis* water, Oct. 6, 1953. Since his standards gave positive tests he states "the suggestion is thus made that *Gymnodinium* [*brevis*] is not a nitrate user." This further strengthens the conclusion advanced above, and indicates that the source of nitrogen for the organism may be an organic one. It likewise helps explain the failure to find *brevis* in Hillsborough Bay, where some of the high nitrogen content might be nitrate from the Tampa Sewage Treatment Plant.

While nitrogen has never been lacking in our field samples, phosphorus has frequently been below detectable levels, and even when present, there is no evidence of a nitrogen phosphorus ratio remotely approaching constancy in *brevis*-containing water. Culture work in the laboratory thus far has not shown what form of nitrogen is utilized by *brevis,* and thus the concepts of Lackey and Sawyer (15) and of Ryther (20) apparently do not hold for this organism.

Laboratory cultures have other nutritional requirements which are necessary. One of these is additional sulfur; another is the complex organic mixture in soil extract. This latter may well be the source, not only of some of the necessary sulfur but of organic nitrogen. It also probably includes some growth-promoting substances such as vitamin B_{12} and others. Continuous refinement of laboratory culturing methods should eventually give a precise answer to requirements and provide the key to the outbursts of blooming.

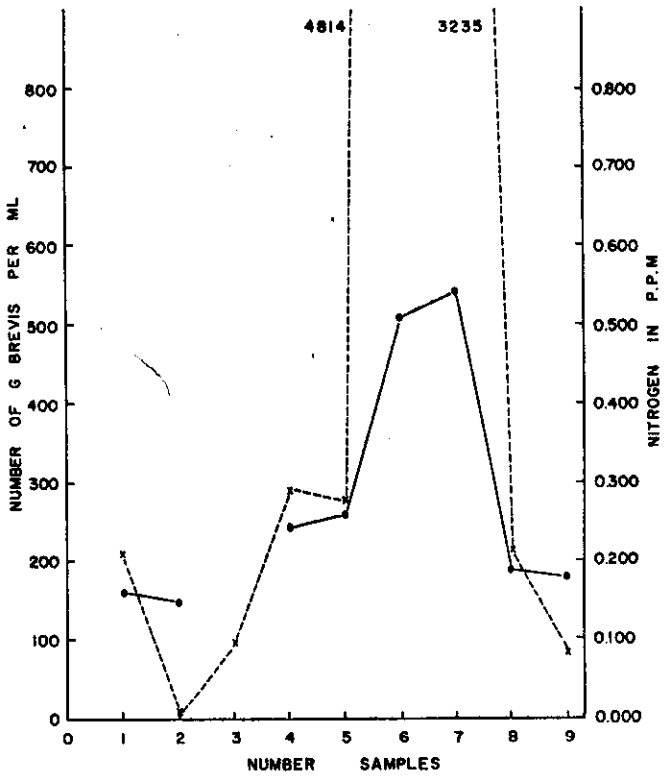

Figure 7. Relationship between *G. brevis* and total nitrogen, in Lemon Bay June 18, 1954. Solid line represents p.p.m. total nitrogen scale at right; dashed line, numbers of *G. brevis* per ml., scale at left.

Then, where is the source of these nutrient materials? Upwellings from nutrient-rich deep waters are fairly well ruled out by the shallowness of the water along Florida's West Coast, where the edge of the continental shelf is about 150 miles offshore. Upwellings in shallow water are infrequent and would bring about little increase of nutrient materials if they did occur.

Rivers passing through phosphate-laden areas inland may carry phosphorus into the Gulf. This same water could pick up nitrogen compounds from the cattle lands, or from the extensive peat deposits, or from sewage treatment plants emptying into them. It has been shown that certain fresh waters contain vitamins. Although the Red Tide blooms apparently are not associated with particular seasons of the year, they do seem to occur more frequently following weather disturbances, especially periods of heavy rainfall. Several workers (Slobodkin 22); Hela, *et al* (11) have used these facts on which to base causal theories for the bloom.

Other Chemical Elements or Compounds

Except for changes in Gulf water due to dilution with land run-off, there seems little else which is peculiar to the *brevis* area. Ground water in the general area is frequently highly charged with H_2S, and Wilson (13) uses some sulfide in his culture medium. However, the ground-water springs in the Gulf flow constantly, and there are perhaps as many on the Atlantic side, where *brevis* has not been found. There is no reason to suspect that the flow is excessive during intermittent *brevis* outbreaks. John H. Davis (23) says exposure of mangrove peat by storms might be a factor. This peat contains H_2S when first exposed, and has been accumulating for at least 5,000 years. Davis says it is four feet thick at Naples, but presumably it is equally thick on the Atlantic coast of Florida.

Organic acids (humic, tannic) are abundant in run-off from the West Coast, and from the air, the brown discoloration as this water comes out of the passes is spectacular. Experimental work on these organic compounds still remains to be done.

Rare metals have been considered. Collier, *et al* (24) showed that titanium and zirconium were significantly present in the *brevis* area, but of themselves they have failed to be a stimulant in laboratory cultures. Thus far there is little or no data on radioactive elements in the area. This question is still under investigation. Failing to find some particular element or compound as a proper stimulant, one is tempted to conclude that rapid reproduction, in this case, is due to some combination of elements and compounds, as yet not recognized.

Salinity Effects

Rivers, when swollen by heavy rainfall, empty larger quantities of water than usual into the Gulf. This nonsaline water, being less dense than sea water, can form a discrete water mass which, under relatively calm weather conditions, should exhibit only a slow rate of mixing at its boundaries (Slobodkin, 22). The low salinity of the mass favors growth of the Red Tide organism, for it has been shown in laboratory culture work that *G. brevis* and many other dinoflagellates grow best at salinities lower than those usually found in open Gulf water. Salinities of samples taken from Red Tide water have also been below those normally found in the Gulf. The water mass must also supply other physiological requirements of the organisms, which it can do by carrying down any or all of the substances mentioned earlier. It seems likely that any such newly isolated water mass should contain the nutritional requirements of at least one *brevis* in the mass, and once these requirements are fulfilled, the only factor limiting a bloom is the rate of diffusion of the mass.

This laboratory has not done extensive work on salinities in the *brevis* area, since data will eventually be available from other sources, notably the Galveston Fish and Wildlife Service Laboratory. It is realized, however, that the function of lower salinity in producing discrete water masses in the Gulf, and in effect segregating certain nutritive factors until complete mixing has occurred, is an important one and must be evaluated as in the above theory.

In direct contrast to this idea is one proposed several years ago by Chew (25). He found, during the Red Tide outbreak in November, 1952, that where Gulf and river water were being mixed, the regions of low phosphorus coincided with those of river water and those of high phosphorus with those of Gulf water at all depths. This suggested that the phosphorus was being diffused from the Gulf water into the river water at the point of mixing and may have been supplied through the leaching of submarine deposits of phosphates some distance offshore. Such deposits might be denuded of overlying sand by a change in currents, or by storms, and thus be exposed to leaching. However, the 1953-54 data of this laboratory do not bear out these ideas. In the water samples analyzed for total phosphorus the highest concentrations have been found in the rivers and their estuaries. These have been much higher than any concentrations found elsewhere in Gulf waters.

It was also shown (Graham, *et al* 19) that a blue-green alga, Trichodesmium, is sometimes concentrated into long bands by winds and tides. It was suggested that the alga could serve as a mechanism for concentrating phosphorus, and that when conditions became unfavorable for its survival and it died, a large quantity of organic material would become available. Decomposition of this, followed by a regeneration of inorganic substances would make the phosphorus, and perhaps other substances, available. If G. *brevis* were present in the area, and if other conditions were satisfactory, a bloom might then occur. Neither the literature nor observations of this laboratory over the past few years have borne out the idea of an extensive algal bloom of blue-greens preceding Red Tide outbreaks.

Other Physical Factors

A number of physical, or environmental factors other than chemical, have received some attention. This laboratory has not investigated meteorological or current effects which are presently under investigation by both the University of Miami and the U. S. Fish and Wildlife Service. The latter factors certainly come into play in distributing the land-contributed nutrients and in dispersing the organisms, and meteorological effects are seen when heavy weather breaks up a heavy *brevis* concentration.

Temperature variations are small and very gradual in the Gulf area, and *brevis* has been found, and has bloomed, throughout the year. Odum has measured the light intensity in varying concentrations of *brevis*, and while at the surface intensities may be very high, the organizisms have been found concentrated at depths where the light intensity was greatly reduced. These and other considerations would seem to rule out light as a single decisive factor.

There is not even anything detectably unusual about the other inhabitants of the area. Inshore, they (the microplankton) are somewhat more abundant than in other situations such as Delaware Bay, but are far from being as abundant as over Georges Banks. They are the cosmopolitan types. There are few if any unusual ones, and the population "pressure" is neither great inshore, nor weak offshore. As a physical effect this is somewhat farfetched, but it is a possible one at times, and seems wholly lacking here.

In review, no single substance, no combination of substances, no ratio of substances to each other, no single source of substances, no critical temperature range, no condition of intense or decreased illumination, nor any combination of chemical and/or physical factors has thus far offered a satisfactory explanation for the behavior of this exceptional organism.

The Red Tide is like a disease—only it is not a disease of the human body, but of the body politic. In human disease we discover the cause; if the cause is a living organism, we study the biology of the organism, learn the cure, aim at prevention. Some diseases are still not conquered. So it is with the Red Tide. We have only recently recognized it as a disease, still later recognized the causative organism. But we are still learning the biology of that organism, and until we understand it, cure and prevention may be a long way off. To the average person, yellow fever is little more than the name of an obscure disease. To others who saw the last exodus of frightened people from New Orleans in 1900, it is a horrible disease, now happily wiped out. To a few workers in the field of public health, it is a dangerous disease, whose control is so important that in the year 1955, a conference of interested workers is being called in Washington, to be on guard against any possible return.

Gymnodinium brevis has a habit of disappearing for periods of years. Responsible men must see to it that the necessary studies are carried to their ultimate end—such a thorough knowledge of the organism that if there is a cure or prevention, it will be learned. Wherever available—in Florida and elsewhere in the nation—qualified scientists and laboratories should coordinate their efforts toward solving this problem. To that end research on the Red Tide at the University of Florida is dedicated.

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