Project No. 88-09 April 1989

POTENTIAL FOR LONG-TERM PERSISTENCE

OF THE RED TIDE DINOFLAGELLATE

Ptychodiscus brevis

IN NORTH CAROLINA COASTAL WATERS

FINAL REPORT

Prepared for

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ACKNOWLEDGEMENTS

We would like to thank the following people for their advice and aid in this research effort: Drs. Robert Holman and Doug Rader, North Carolina APES Program; Patricia Tester of Beaufort NMFS; Patricia Fowler of North Carolina Shellfish Sanitation Department; Dr. Bud Cross, Director NMFS, Beaufort; Willie Burton and Kim Durrell, Versar, Inc.; Dr. Gregory Gaines, University of Maryland; and Dr. Carmello Thomas, State of Florida.

FOREWORD

This report was prepared by Versar, Inc., for Dr. Robert Holman, Director of the Albemarle-Pamlico Estuarine Studies (APES) Program sponsored by the Department of Natural Resources and Community Development under contract number C-1669 of the State of North Carolina. The three month project involved the collection of water samples in Beaufort Inlet and Bogue and Core Sounds and the analysis of the physical and biological components of those samples. The purpose is to determine the potential persistance of red tides in North Carolina. This document describes the field and laboratory effort which went into addressing this problem.

ABSTRACT

In October of 1987, the toxic red tide dinoflagellate *Ptychodiscus brevis* which normally resides along the Florida coast was introduced into North Carolina waters via the intrusion of a Gulf Stream eddy. The transported population resulted in the closure of shellfish beds in North Carolina due to the presence of brevetoxin in the shellfish. A major field effort was undertaken in February 1988 to determine the extent of the organism distribution. Benthic samples were collected as well as water column samples and were incubated in the laboratory for a one month period to determine the presence of cysts. Incubation of the sediment samples yielded no motile population which suggests that *Ptychodiscus* did not form a resting stage in North Carolina waters. Without further study, however, one cannot rule out the possibility that *Ptychodiscus* formed a resting stage, but its obligate period of dormancy was not complete during the short one month incubation. Lack of reoccurrence of blooms of this red tide organisms in North Carolina waters in late 1988 give credence to the view that the original 1987 inoculum did not encyst.

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I. INTRODUCTION

Seasonal and sporadic occurrences of high concentrations of phytoplankton, sufficiently dense to color surface waters, are world-wide phenomenon (Ryther 1955; Rousenfell Nelson 1966). The distribution and accumulation of large concentrations of some marine dinoflagellates known as "red tides" have received considerable attention since the early 1800's as massive fish kills often result from the rapid multiplication of certain toxic, single cell planktonic organisms. "Red water" conditions were originally classified by Allen in 1946 as accumulations greater than 10^6 organisms per liter and concentrations up to 10^9 cells per liter have been reported in estuarine waters. Federal regulations require shellfish beds be closed to harvesting if concentraitons in the water exceed 5,000 cells per liter. Rytner (1954) established a number of prerequisites for an outbreak of a red tide including: the existence of a seed population in the area and favorable conditions for the growth of the organism or the existence of a hydrographic concentrating mechanism.

One of the best studied of the toxic dinoflagellates is the organism responsible for the red tide off the Florida Gulf Coast. Ptychodiscus brevis, originally described by Davis in 1948 as Gymnodinium brevis, is a small, obovate, unarmored dinoflagellate approximately 20-30 μm in diameter. This photosynthetic plant is motile and can alter its vertical distribution in the water column using its flagella. Ptychodiscus brevis contains toxins known as brevetoxins which are neurotoxins to humans. These toxics accumulate in shellfish and cause severe nervous system disorders when ingested by humans including convulsions and temporary paralysis. Because P. brevis is easily lysed, toxins may be released into the water column where they cause massive mortalities of fish. The toxin may also be present in aerosols when a bloom forms in the nearshore region and may cause respiratory difficulties.

Many red tide organisms form resting stages during adverse conditions in which they sink to the sediment. They regain their swimming form when conditions become more favorable. This cyst is most often associated with a life cycle changed in which the cell commences sexual reproduction, including gamete production and fusion, planozygote, and hypnozygote (cyst) formation. The existence of a resting form for Ptychodiscus brevis is currently under debate. It has been speculated that cyst populations of this organism may exist in the bottom less than 100 miles off the Florida west coast and under certain conditions are transported in the Gulf Loop Current where they impact upon the Florida shelf and upwell into surface waters. However, no cyst form has been confirmed.

In September of 1987, a major red-tide of *Ptychodiscus brevis* was reported in the Gulf off of Naples Florida on the southwest coast. It is possible that a portion of this bloom became entrapped in the Gulf Stream and was physically transported over a thousand kilometers northward. In October of 1987, red water appeared off Beaufort, North Carolina. Dead fish were observed floating on the water, scallops were found dead and oysters and clams that were ingested produced neurotoxic symptoms in those who ate them. Federal and state agencies acted quickly to close shellfish beds and the causative organism, *Ptychodiscus brevis*, was identified. Examination of satellite data from NOAA weather service in Miami, Florida gave evidence that a warm mass of Gulf Stream water moved into the Cape Lookout, North Carolina area toward the end of October, apparently the result of an onshore eddy. This appears to be the mechanism for inoculation with the North Carolina area (P. Tester, personal communication).

The immediate question to be addressed was "What is the potential for the long term persistance of this organism in North Carolina waters"? One of the two requirements established by Ryther for bloom occurrence, that of the inoculum, was met. It remained to be determined whether conditions in North Carolina were favorable for its continued presence. Because the organism is suspected to have a cyst form, the question of survival becomes more complex. The optimal temperature range for growth of *Ptychodiscus brevis* is over 20 °C and it is normally considered a tropical species. The cold North Carolina winters would certainly cause the demise of any *Ptychodiscus* population. However, the possibility that under stress conditions, the motile populations may form a cyst and sink out of the overlying watercolumn into North Carolina sediments only to germinate annually when the weather became warm posed a real potential threat and warrented investigation. This research attempts to address that question by collecting water column as well as benthic sediments in the North Carolina bloom area and incubating them at increased temperatures to determined whether cells would germinate and grow during simulated summer conditions.

II. RESEARCH AND DISCUSSION

A. SAMPLING METHODS AND DESCRIPTION OF THE FIELD PROGRAM

A field sampling program was conducted the 9th through 11th of February 1988 aboard the North Carolina Division of Marine Fisheries R/V Carolina Coast. Sampling areas (Fig. 1) included seven locations in the straits, three locations in Bogue Sound (Fig. 2), nine locations in Morehead and Beaufort Harbors (Fig. 3), and seven locations in the Beaufort Inlet area of the Atlantic Ocean (Fig. 3). At each location, the water column was vertically sampled and profiles of salinity, temperature pH, and dissolved oxygen were obtained with Versar's Hydrolab. At each station, a minimum of 3 and maximum of 5 water samples were taken at discrete depths with a Van Dorn sampling bottle. Duplicate samples were taken at select stations for quality assurance. These samples were subdivided into two 500 ml aliquots and one preserved with lugols iodine for phytoplankton identification and the second kept cold and dark for laboratory experimentation. In addition, samples of sediment were taken at each location. Surface sediment was obtained by lowering the intake of a peristaltic pump into the sediment and pumping the slurry into 1 liter sample bottles. These sediment samples were also subdivided and one sample preserved with lugols for enumerating cysts while the other kept cold and dark for laboratory incubation experiments.

Salinities in the sampling area ranged from 25.6% in the straits to 33.4% in the inlet. High winds kept the system relatively well mixed with the only notable stratification in Beaufort Inlet with a $3^{o}/_{oo}$ top to bottom salinity gradient. Temperatures in the system ranged from 5.1°C in the straits to 8.4°C near Shackleford Point. A small temperature gradient of approximately 0.5°C was noted at Morehead City Port. The pH was relatively constant ranging from 7.8 to 8.4. Dissolved oxygen of 9.7 to 12.4 ppm indicated a well mixed, oxygenated column to depth (see Tables 1 through 4).

At the time of our field sampling, motile populations in the watercolumn had declined rapidly from the previous month's numbers, most probably due to low water temperatures and the retreat of Gulf Stream waters offshore. Counts of <u>P. brevis</u> by North Carolina Division of Health Services during the week of 7 February 1988 in Beaufort Inlet reached 0 (or less than 1 cell/liter). Many clamming areas were reopened within the next month as toxicity declined. Of the 26 locations sampled, only station within the inlet showed any planktonic population of <u>Ptychodiscus</u> (Fig. 4). At I-3 and I-6 populations of

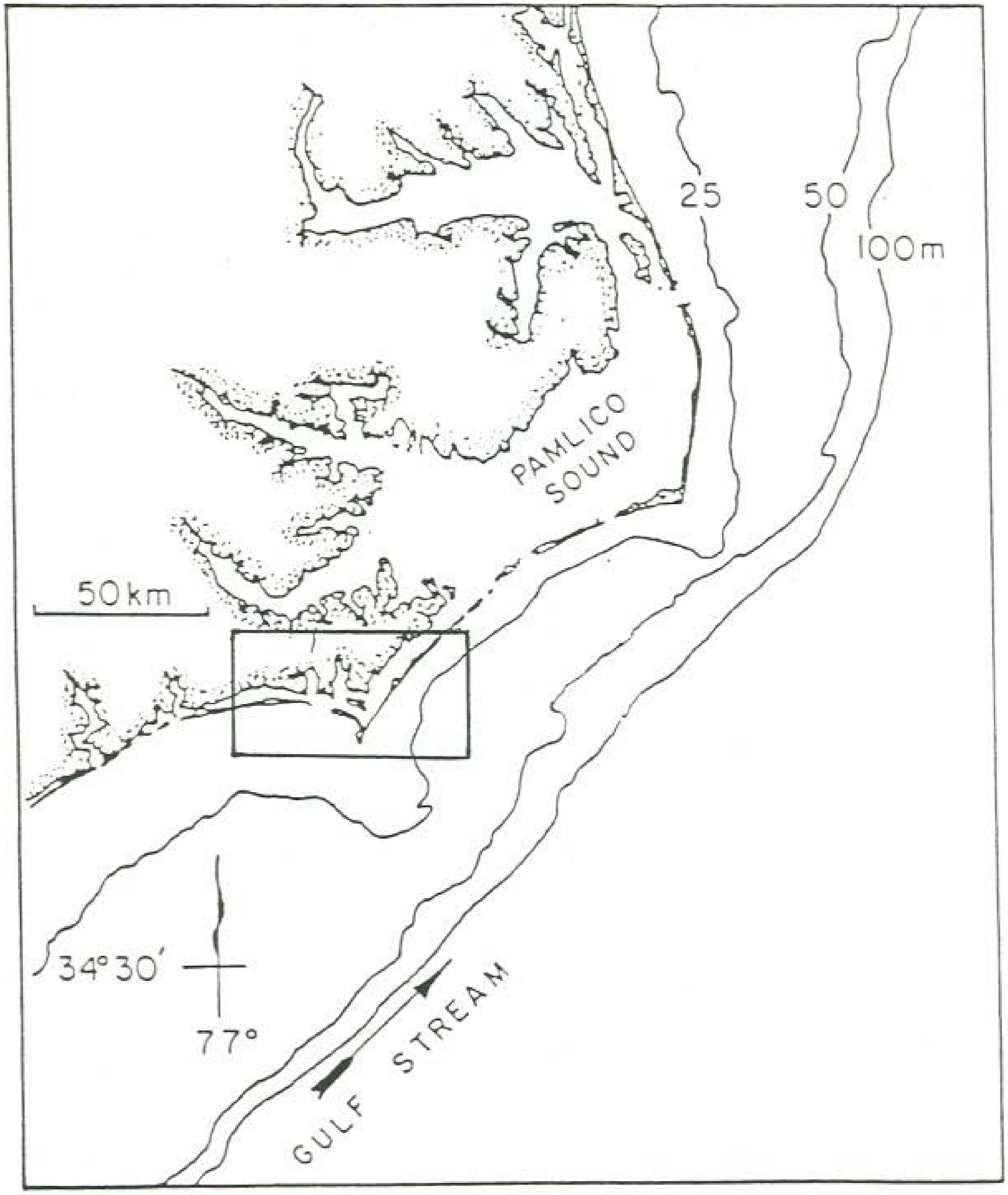

Figure 1. Sampling area of Ptychodiscus brevis, February 1988 (hatched area)

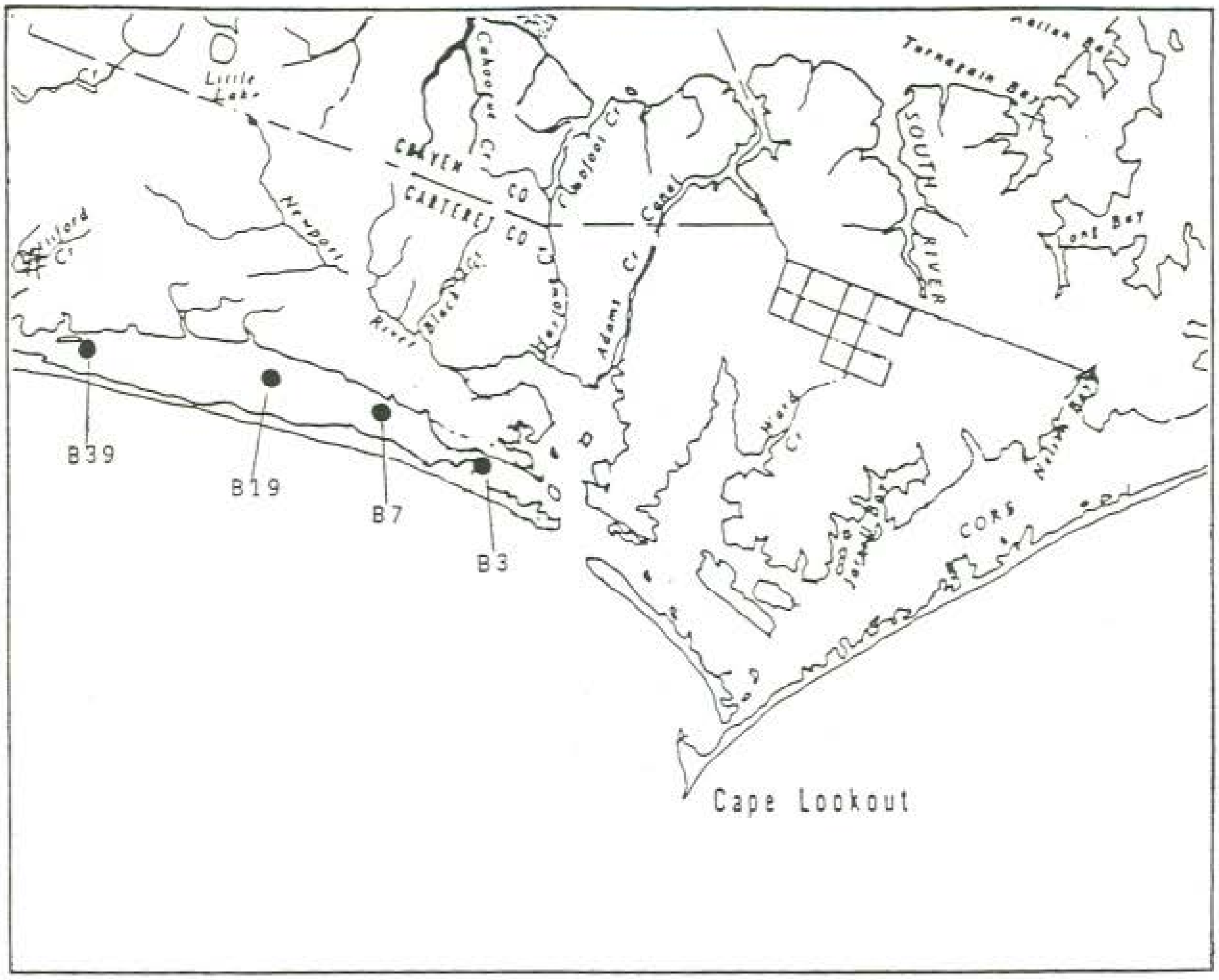

Figure 2. Sampling areas in Bogue Sound

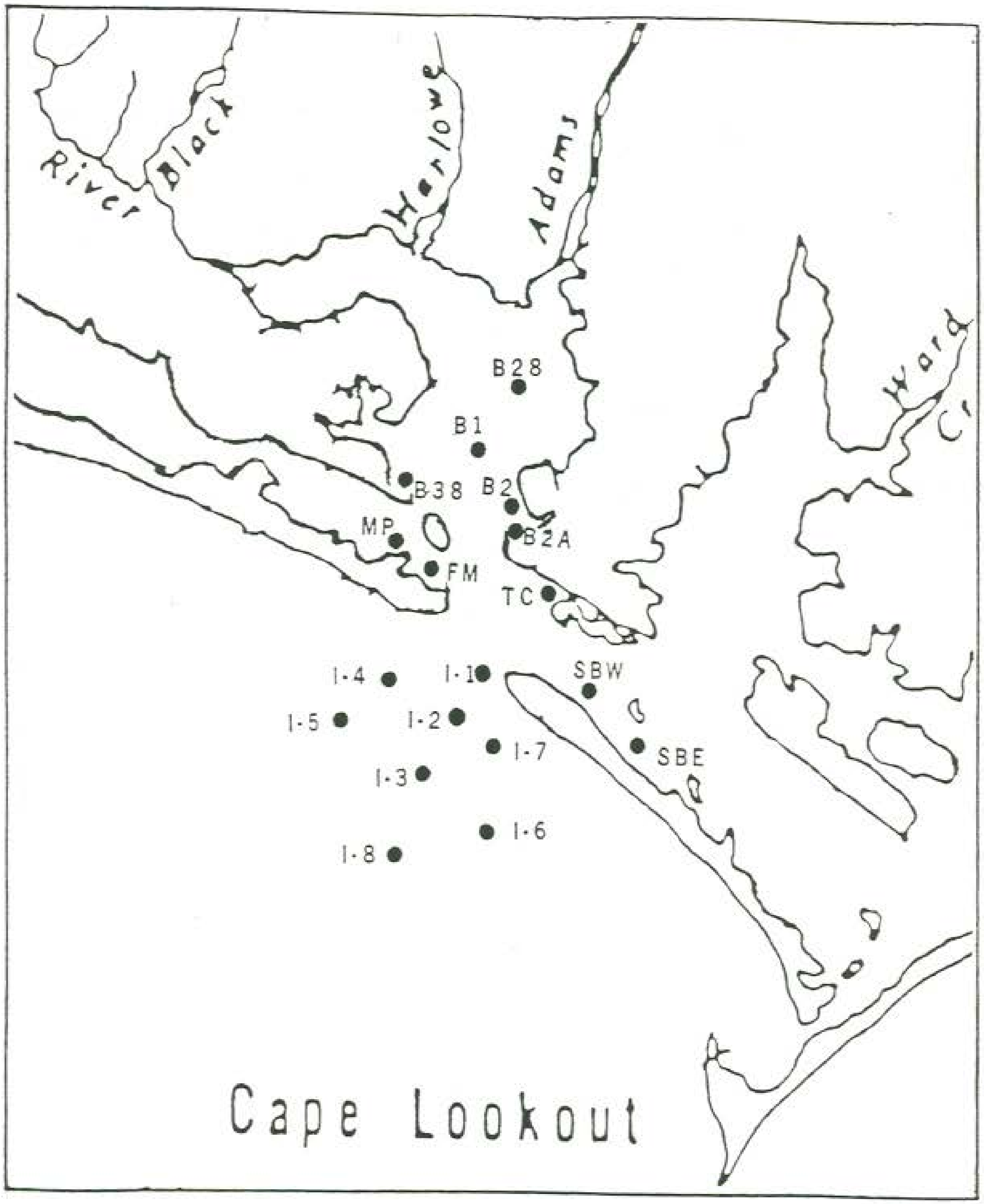

Figure 3. Sampling locations in Beaufort Inlet and near Beaufort and Morehead City

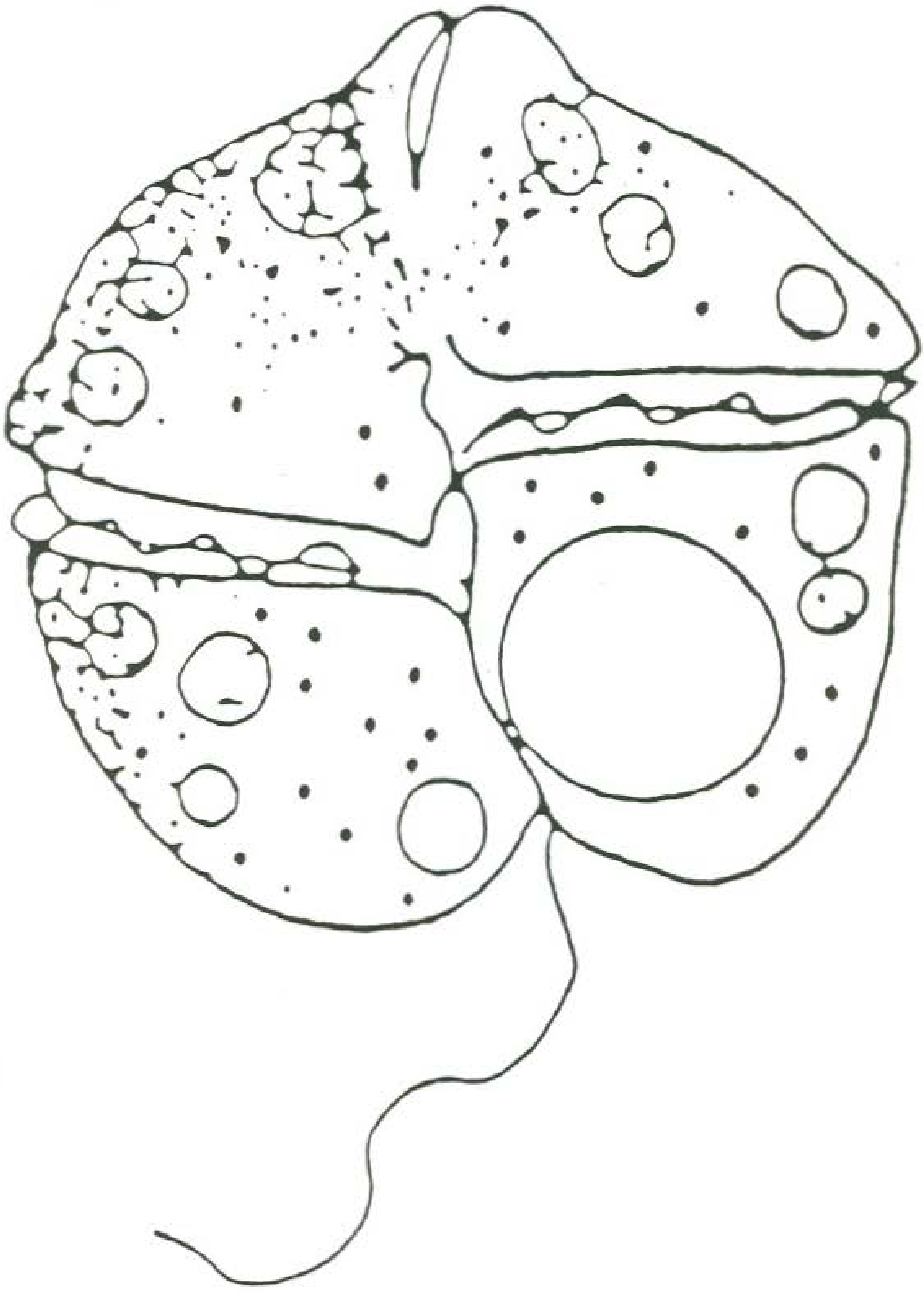

Figure 4. Ptychodiscus brevis -- red tide dinoflagellate

Table 1. The straits

Date	Time	Station	Depth Total	(m)	$S^{o}/_{oo}$	T°C	pH	D.O	Bottle Number	Sediment Type
2/9/88	10:37	B44	8 m	S(.5)	25.6	5.78	8.36	11.3	1	
			24 ft	7.0	26.1	5.7	8.38	10.8	2	
				3.5	26.0	5.16	8.38	10.7	3	
						Sediment			4	Sand
	11:22	B47	29 ft	3.0	25.9	5.7	8.3	11.5	6	
			3.2	1.5	25.8	5.7	8.4	11.1	7	
				0.5	25.8	5.7	8.4	10.9	8	
						Sediment			9	Sand
	11:43	B48	3.6	3.0	25.9	5.7	8.4	10.9	10	
				1.5	26.0	5.7	8.4	10.9	11	
				0.5	25.1	5.8	8.4	10.8	12	
						Sediment			13	Sand
	12:10	B56	4.7	4.0	28.3	6.3	8.4	10.9	14	
				2.0	28.1	6.3	8.4	10.7	15	
				0.5	28.1	6.3	8.4	10.6	16	
						Sediment			17	Sand
	13:01	NR in Shackle-fort Channel	3.0	2.5	32.1	7.7	8.4	10.5	18	
				1.2	32.1	7.7	8.4	9.7	19	
				0.5	32.0	7.7	8.4	9.7	20	
						Sediment			21	Sand
	14:00	FM Fort Macon	5.3	5.0	32.2	7.9	8.4	9.8	22	
				2.5	32.3	7.9	8.4	9.6	23	
				0.5	32.1	7.9	8.4	9.6	24	
						Sediment			25	Sand
	14:45	MP Morehead City Port	15[a]/12[b]		33.4	8.2	8.4	9.1	26	
			10[a]/8[b]		33.2	8.1	8.4	9.2	27	
			5		32.6	8.0	8.4	9.4	28	
			0.5		31.0	7.7	8.4	9.7	29	
						Sediment			30	Sand

[a] H.L. depth.
[b] Sample depth.

Table 2. Bogue Sound

Date	Time	Station	Depth Total	(m)	$S^{o}/_{oo}$	T°C	pH	D.O	Bottle Number	Sediment Type
2/10/88	07:55	B7	4.1	4.0	29.3	6.8	7.8	10.5	31	
				2.0	29.3	6.8	7.8	10.3	32	
				0.5	29.3	6.8	7.8	10.3	33	
							Sediment		34	Sand
	09:09	B19	2.2	2.0	28.6	6.3	7.8	11.0	35	
				0.5	28.7	6.4	8.3	10.7	37	
				1.0			Mid 1.0		36	
							Sediment		38	Sand
		B39	4.0	4.0	29.7	6.1	7.9	12.4	39	
				2.0	29.6	6.1	7.9	10.7	40	
				3.5	29.7	6.1	7.9	10.6	41	
							Sediment		42	Sand

Table 3. Morehead and Beaufort Harbors

Date	Time	Station	Depth Total	(m)	$S^{o}/_{oo}$	T°C	pH	D.O	Bottle Number	Sediment Type
2/10/88	11:48	B3	4.5	4.0	31.0	7.7	7.9	10.0	43	Very hard bottom
				2.0	30.7	7.6	8.0	9.9		
				0.5	30.5	7.6	8.0	9.9	44	
							Sediment		45	
	12:18	B38	3.8	3.0	31.6	7.8	7.9	10.4	46	
				1.5	31.5	7.8	7.9	9.9		
				0.5	3.5	7.9	7.9	9.8	47	
							Sediment		48	
	12:36	B28	5.1	5.0	29.3	7.5	7.9	10.6	49	Lined bottom
				2.5	27.4	7.4	7.9	10.4		
				0.5	27.0	7.4	7.09	10.4	50	
									51	
	12:51	B-1	4.1	4.0	31.2	7.8	7.9	10.4	52	Hard bottom
				2.0	31.2	7.8	7.9	10.0		
				0.5	30.2	7.8	8.0	9.9	53	
							Sediment		54	
	13:15	B-2	3.8	3.0	29.7	7.3	7.9	10.5	55	Mud
				1.5	29.1	7.3	7.9	10.3		
				0.5	28.0	7.4	7.9	10.3	56	
							Sediment		57	
	13:20	B-2A	0.5						58	Mud
			4.0						59	
							Sediment		60	
	13:46	Taylor's Creek by Fish Factory (TC)	4.4	4.0	29.4	7.5	7.9	10.5	61	Mud
				2.0	29.3	7.5	7.9	10.2		
				0.5	29.1	7.6	8.0	10.2	62	
							Sediment		63	
	14:09	Shackle-ford's Bank West (SBW)	6.6	6.0	32.8	8.4	7.9	9.8	64	Hard bottom
				3.0	31.5	8.1	7.9	9.8	65	
				0.5	30.2	7.9	7.9	9.9	65	
							Sediment		66	
	14:39	SBE Shackle-ford Bank East	7.3	7.0	26.4	6.7	7.9	11.2	67	Hard bottom
				3.5	26.1	6.8	7.9	10.9		
				0.5	26.0	6.9	8.0	10.8	68	
							Sediment		69	

Table 4. The inlet and offshore

Date	Time	Station	Depth Total	(m)	$S^o/_{oo}$	T°C	pH	D.O	Bottle Number
2/11/88	08:49	I1	14.6						
				14.0	31.6	7.9	8.3	9.5	70
				10.0	31.1	7.9	8.3	9.4	71
				6.0	31.1	7.6	8.3	9.5	72
				2.0	28.9	7.3	8.3	9.6	73
				0.5	28.9	7.2	8.3	9.6	74
	08:59	I2	14.0	12.0	32.6	8.1	8.3	9.3	75
				8.0	32.6	8.1	8.3	9.3	76
				4.0	32.4	7.9	8.3	9.2	77
				0.5	31.1	7.6	8.3	9.3	78
	09:13	I4	5.0	4.0	33.9	8.5	8.3	9.6	79
				2.0	32.4	8.1	8.3	9.5	80
				0.5	31.2	8.0	8.3	9.4	81
	09:23	I5	10.7	8.0	33.8	8.2	8.3	9.4	82
				4.0	33.6	8.3	8.3	9.2	83
				0.5	32.0	8.1	8.3	9.2	84
	09:37	I3	8.8	8.0	33.4	8.3	8.3	9.4	85
				4.0	32.6	8.0	8.3	9.3	86
				0.5	31.0	7.8	8.4	9.3	87
	09:51	I8	11.0	10.0	33.7	8.4	8.3	9.2	88
				5.0	33.3	8.3	8.4	9.1	89
				0.5	31.5	7.9	8.4	9.1	90
	10:02	I6	12.2	12.0	33.9	8.6	8.3	9.1	91
				8.0	33.8	8.5	8.3	8.9	92
				8.0	33.7	8.5	8.4	8.9	93
				0.5	33.8	8.5	8.4	8.8	94
	10:13	I7	4.7	4.0	33.7	8.6	8.3	9.4	98
				2.0	34.0	8.6	8.3	9.1	96
				0.5	34.0	8.6	8.4	9.0	97

~ 1/liter were found. Cell counts were made on the whole water sample (in most cases the pooled replicate samples since populations were so low). The sample was settled and decanted. A Zeiss inverted microscope was used to examine the populations using 20x and 40x objectives. Within the sound and straits, populations of Heterocapsca triqueta, Prorocentrum micans, Polykrikos, Dinophysis, Katodinium, Ceratium, and Tintinids were prevalent. Bottom samples yielded Navicula, Nitzschia, Gyrosigma, Bidulphia, and Oxyrrhis. In general, the assemblage was typical of North Carolina waters as the Ptychodiscus containing waters apparently pulled offshore. The scarcity of Ptychodiscus makes it improbable that the motile population would serve as a seed population to reinfect the waters next season. The low water temperatures of North Carolina appear to be a major contributing factor to P. brevis demise as Ptychodiscus is a warm water species. In addition, the chance of another inoculum via a Gulf Stream eddy is highy unlikely.

B. INCUBATION OF SEDIMENT SAMPLES IN THE LABORATORY

Due to the paucity of organisms in the water column, our focus became the incubation of the sediments collected from the former bloom areas. Sediment samples were collected from each of the 26 locations. Each was subdivided and one set incubated at room temperature immediately upon return from the field while the other set was kept at field temperatures in the dark for 1 month (to simulate in situ conditions) prior to gradual warming to 20°C. Motile Ptychodiscus thrives in culture at 20°C and thus this was chosen as the end point temperature. Culture dishes were swirled daily for aeration and the overlying water monitored twice weekly for the appearance of cells. At one location, B2, the sediments were muddy and particularly rich in cysts. At this location, sediments were sonified and individual cysts followed for potential germination. However, no Ptychodiscus was apparent.

The last observation of all sediment samples was on 18 April 1988, approximately 2 months after capture in the field. At this time, no Ptychodiscus has been observed germinating from the sediments although numerous other species were present to indicate the presence of favorable conditions for growth and reproduction in our incubation dishes. The absence of these red tide organisms suggests that Ptychodiscus did not form cysts in North Carolina waters subsequent to its inoculation from the Gulf Stream.

C. CONCLUSIONS

It is unlikely that our laboratory conditions were unsuitable for germination of this species as so many other dinoflagellate species were seen to excyst. In addition, subsequent to our study, there has not been a reoccurrence of red tide conditions in any of the areas subject to blooms in 1987 and designated as areas of high probability for reinfection if benthic populations germinated. This lends credence to our observation that Ptycodisus brevis did not form cysts in North Carolina waters. Most cyst forms loose their viability after the first year. Since Ptychodiscus did not reappear within one year of the initial infection, it is improbable that it will reappear unless another inoculum is received.

In addition, the sediments underlying the bloom areas were mostly sand with the exception of a few locations. Dinoflagellate cysts are usually found in finer grained sediment where retention is greater. Thus, the physical nature of the underlying sediments was not conducive to cyst retention such that if cysts were formed they might not readily be retained. This factor reduces the probability of a large benthic resting population residing in the sediments awaiting better conditions to reseed the water column.

APPENDIX I

STATION LATITUDE AND LONGITUDE

Station	Latitude	Longitude
FM	34°42'	76°41'
MP	34°42'50"	76°41'50"
B7	34°43'	76°47'
B19	34°43'	76°53'
B39	34°42'	76°58'
B3	34°43'	76°44'
B38	34°43'40"	76°41'40"
B28	34°44'50"	76°40'30"
B-1	34°44'20"	76°40'40"
B-2	34°43'35"	76°40'
B-2A	34°43'30"	76°40'
TC	34°42'40"	76°38'30"
SBW	34°41'30"	76°39'
SBE	34°41'20"	76°37'50"
I-1	34°41'30"	76°40'
I-2	34°40'30"	76°40'20"
I-3	34°39'50"	76°40'30
I-4	34°41'	76°41'
I-5	34°40'30	76°41'40"
I-6	34°39'30"	76°38'50"
I-7	34°40'20	76°39'20"
I-8	34°39'20"	76°40'30"