Rev. Biol. Trop., 29(1): 31-38, 1981

The Dinoflagellate red tide in Golfo de Nicoya, Costa Rica

by

Paul E. Hargraves* and Roxana Víquez**

(Received for publication May 23, 1980)

Abstract: Red tides in the Golfo de Nicoya, Costa Rica, are caused by *Cochlodinium catenatum* Okamura. The maximum concentration of cells was 80×10^6/L. Four-celled colonies dominate in natural samples, while in culture, unicells and pairs are more common. Measurements of primary productivity were impractical because of cell autolysis. Environmental conditions controlling initiation and termination of blooms are unknown. The red tide is apparently non-toxic.

The occurrence of red tides in the Golfo de Nicoya has been observed locally for many years, but formalized study of the bloom phenomenon is little studied and poorly documented. Red tides in coastal tropical waters in general have only recently attracted the interest of biological oceanographers (Ferraz-Reyes *et al.*, 1979; Margalef, 1961; Reyes-Vazquez *et al.*, 1979). Although some information exists on the distribution of red tides along the Caribbean and Pacific coasts of Central America (Hayes and Austin, 1951) they do no attract much attention unless they cause toxicity to molluscs, fish, or people. If the causative organism is a non-thecate dinoflagellate, thus being poorly preserved, it is even less likely to be studied.

Oceanographic conditions in the Golfo de Nicoya, an estuarine system controlled by salinity variation, have been documented by Peterson (1960). Runoff during the rainy season results in a surface outflow which is replenished by more saline water flowing in at deeper levels. During the dry season, winds and tides break down stratification, and salinities characteristic of adjacent oceanic areas result. Seasonal variation in mean water temperature rarely exceeds 3-4 C, with a mean minimum of about 25 C in October-November and a mean maximum of about 29 C in May.

During late February and early March 1979, extensive brick-red discoloration of the near shore waters at Puntarenas were observed. These red tide slicks, often

* Graduate School of Oceanography, University of Rhode Island, Kingston, R. I. 02881

** Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica.

200m wide and 2-3 km long, appeared to extend throughout the central part of the Golfo de Nicoya. Examination of water samples from these slicks demonstrated that the causative organism was a photosynthetic non-thecate dinoflagellate tentatively identified as *Cochlodinium catenatum*. Materials collected during March 1979 form the basis of this report.

MATERIAL AND METHODS

Samples from the surface and 1m depth were collected in- and outside the red tide areas using BOD bottles and unmetered 10µm and 65µm mesh plankton nets. Water temperature at the surface was 28 C and salinity was 32.2%. Light/dark bottle experiments with neutral density screening of 50, 10, 1, and 0% light transmission were conducted on two sets of samples in an attempt to measure productivity rates.

Single cell isolates were made into 125ml polycarbonate flasks and 18x150mm pyrex test tubes using the drawn pipet method described by Boleyn (1967). A variety of culture media were used to stimulate growth of the organism. The composition of some of these is detailed in Table 1. Most were based on the "f" formula devised by Guillard and Ryther (1962). Initial cultures were

TABLE 1

Enriched culture media made with Golfo de Nicoya prefiltered water. Concentrations are in micromolar equivalents or by weight

	f/2-Sit	F/20-Si	F/2-a	CM
NH_4Cl	-	-	50µm	-
$NaNO_3$	882µm	88.2µ m	-	88.2µ m
$NaH_2PO_4 . H_2O$	36 µm	3.6µm	3.6µm	9.0µm
$Na_2SiO_3 . 9H_2O$	-	-	10.0µm	-
$CuSO_4.5H_20$	0 .04µm	0.004µm	0.04µm	0.008µm
$ZnSO_4.7H_2O$	0.08µm	0.008µm	0.08µm	0.002µm
$CoCl_2.6H_20$	0.04µm	0.004µm	0.04µm	0.008µm
$MnCl_2.4H_20$	0.9µm	0.09µm	0.9µm	0.2µm
$NaMo0_4.2H_20$	0.03µm	0.003µm	0.03µm	0.06µm
Ferric sequestrene	12µm	1.2µm	12.0µm	2.5µm
vitamin B12	0.5µg	0.05µg	0.5µg	0.5µg
biotin	0.5µg	0.05µg	0.5µg	0.5µg
Thiamine-HCL	0.1mg	0.01mg	0.1mg	0.1mg
TRIZMA (pH 7.8)	-	25.0mg	250mg	250mg
soil extract (1ml/L)	yes	no	yes	no

maintained at room temperature under cool-white continuous light (at Universidad Nacional) and later at 25 C under cool-white light on a 12:12 light: dark cycle (University of Rhode Island). Preliminary measurements of growth rate under the latter conditions at 100 μE. m^{-2} sec^{-1} were estimated using the formula:

$$k = \frac{\log_2 (N_i/N_o)}{t_i - t_o}$$

RESULTS

The organism: It was initially apparent that the red tide organism belonged to the genus *Cochlodinium.* Catenate (chain-forming) species in this genus are rather uncommon. The first one, *C. catenatum* Okamura, was described in Kofoid and Swezy (1921). The original description of *C. catenatum* includes discussion of reddish-brown discoloration of the water and a fish kill. Kofoid and Swezy described a similar organism from La Jolla, but state that their organism is holozoic in nutrition, discounting Okamura's description of yellow-brown chloroplasts.

Margalef (1961) described a photosynthetic catenate *Cochlodinium* from the coast of Puerto Rico, *C. polykrikoides,* which occurred mostly as unicells or pairs, rarely as four-cell chains, and was considerably larger than the *Cochlodinium* from Golfo de Nicoya, although both are photosynthetic. In most other characteristics the two are similar. Silva (1967) describes a photosynthetic catenate *Cochlodinium* from the coast of New Jersey (USA) which she calls *C. heterolobatum.* This species is very close to the Golfo de Nicoya organism, particularly in the occurrence of single cells in young cultures, chain formation in older cultures, and the presence of large red-orange bodies in the hypocone in old cultures. The differences between *C. heterolobatum* and *C. catenatum* are not great. We have not been able to locate the original description of *C. catenatum* by Okamura. It is possible that the organism described by Kofoid and Swezy (1921) is not *C. catenatum.* Their organism does not show a pronounced asymmetry of the hypocone, is colored differently, and is not photosynthetic. Until the comparison between Okamura's *C. catenatum* and Silva's *C. heterolobatum* can be made, we tentatively call the Golfo de Nicoya organism by Okamura's name, *C. catenatum* on the basis of priority, with the realization that *C. heterolobatum* might be a later synonym. If *C. heterolobatum* can be reliably demostrated as a separate species, that name would apply to our material. Conversely, all these names might belong to the same species, in which case *C. catenatum* again has priority. A comparison of the morphological characteristics of these various organisms is shown in Table2, and photographs of representative cells from cultures in Figure 1.

Field observations: Data presented in Table 3 were collected on 12 March 1979 off the public beach area at the tip of Puntarenas peninsula. A subsequent visit to the same area on three other dates in March and April revealed no traces of *Cochlodinium.* Thus the exact time of initiation and termination of this red tide can not be determined with certainty.

The red tide slicks were a deep brick-red, oriented parallel to the shoreline in most cases, and up to 200 m wide and as much as 2-3 km long. The concentration of cells (Table 3-A) decreased rapidly from the center of the slick ($65\text{-}80 \times 10^6$ cells/liter) with distance and depth. Although synoptic depth sampling was not possible, it appears that the red tide is a surface phenomenon, based on a decrease

of cells at 1 meter to 60% or less of surface concentrations. Other constituents of the phytoplankton which were of minor importance by comparison included the dinoflagellate genera *Ceratium, Pyrodinium,* and *Prorocentrum;* and the diatom genera *Ditylum, Rhizosolenia,* and *Nitzschia,* among others.

TABLE 2

Morphological characteristics of catenate Cochlodinium *species*

	Nicoya material	*C. catenatum** Okamura	*C. heterolobatum* Silva	*C. polykrikoides* Margalef
cell length (μ m)	25-36	35	26-45	50
cell width (μ m)	25-31	28-35	23-34	ca.45
L/W ratio	1.0-1.2	1.0-1.3	1.1-1.3	1.2
cross-section	round	round	round	round
girdle turns	1.6	1.5	1.8	1.5
sulcus turns	0.6	0.5	0.8	?
epicone	rounded, slightly attenuate	rounded apex	rounded, larger than hypocone	cupuliform
hypocone	asymmetric	subhemisphere, notched apex	asymmetric	asymmetric
cells/colony	1-8	1-4	1-4	1-4
nucleus	anterior, dorsal	near center	anterior, dorsal	anterior
stigma	anterior, dorsal	?	anterior, dorsal	anterior, dorsal
trichocysts	+	?	+	+
chloroplasts	many	none	many	many
color	yellow/ brown	green/ yellow	yellow/ green	yellow

*according to Kofoid and Swezy (1921)

Four-cell colonies made up over two-thirds of the natural population (Table 3-B). Most of the remainder were pairs and eight-cell colonies. No single cells were seen.

Initial attempts at measuring the primary productivity of *Cochlodinium* were made using the light/dark bottle method of oxygen measurement with additional bottles of neutral density screening to reduce ambient light. Incubation time was two hours, centered around noon (Table 4). The results indicate that despite a dense population of highly active photosynthetic organisms, no net photosynthesis took place in any of the bottles. Microscopic examination indicated that after a two-hour incubation period the concentration of active cells had decreased to 1.2-1.6 x 10^6 cells/L, a decrease of about 98% of the living cells. Thus the effect of enclosing concentrated *Cochlodinium* cells ("bottle" effect) is to induce mass

TABLE 3

Field data on Cochlodinium *red tide*

A. Cell concentrations of *Cochlodinium,* per liter, triplicate samples

	center of slick	edge of slick	outside slick
surface	$65\text{-}80\times10^6$	$30\text{-}35\times10^6$	$1.2\text{-}1.4\times10^6$
1 meter	$43\text{-}47\times10^6$	$18\text{-}21\times10^6$	$0.2\text{-}0.6\times10^6$

B. Distribution of colony size, surface, center os slick

	cells per colony					
	1	2	3	4	6	8
number of colonies	0	68	8	324	4	64
percent of total	0	14.5%	1.7%	69.2%	0.9%	13.7%

mortality and decay of the population. The effect was apparently independent of light and was instead autoinhibitory.

Laboratory culture: Of the culture media listed in Table 1, best growth took place in the f/2 mixture from which silicate and Trizma were deleted, and a small amount of soil extract added. Autoclaving nutrients separately in teflon containers had a small beneficial effect on growth. The medium (CM) was satisfactory for maintenance of *Cochlodinium* but growth was very slow. Substitution of ammonia for nitrate as a source of nitrogen did not significantly increase growth, perhaps because silicate was also present in this medium: added silicate is not necessary for dinoflagellate growth, and an unknown amount of silicate normally leaches into the medium from glass tubes during autoclaving. Small increases in growth rate were noted when salinity of the medium was reduced from $35^o/oo$ to $30^o/oo$.

TABLE 4

Productivity experiment, measured as oxygen evolution; two-hour incubation centered around noon. Neutral density screening providing light transmission (%) as indicated. Expressed as ml/L oxygen

	Replicates	Mean
Initial concentration	8.32; 7.67; 8.14	8.04
Light (100%) bottle	6.97; 6.86; 7.02	6.95
50% bottle	7.34; 7.04; 7.22	7.20
10% bottle	7.21; 7.05; 7.30	7.19
1% bottle	6.62; 6.41; 6.39	6.47
dark bottle	6.15; 6.08; 6.11	6.11

Colony size was different from natural population in cultured material. Single cells and pairs dominate in fresh cultures, with four-celled colonies appearing only as

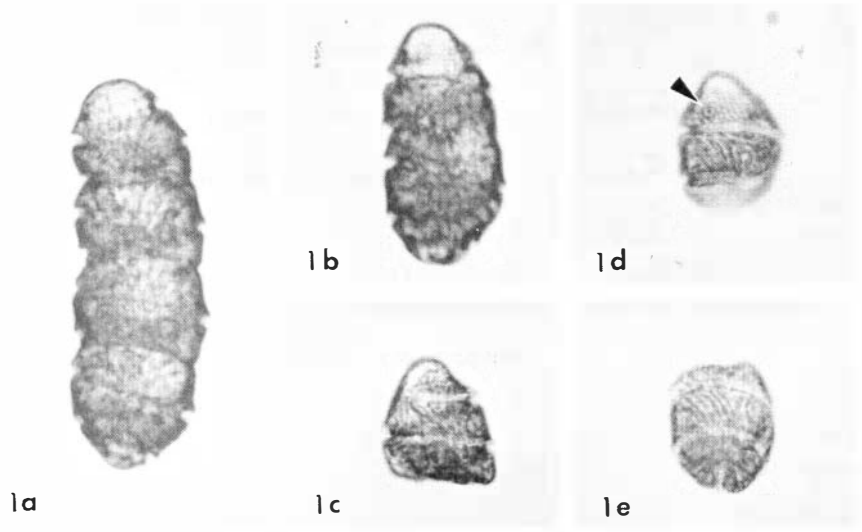

Fig. 1. Morphology of red tide *Cochlodinium*, from cultured material. All x 500.
a) four-celled chain showing slight flexion of colony
b) two-celled chain with more attenuated apex of upper cell
c) unicell, ventral side, showing asymmetry of antapex
d) unicell, dorsal side, showing multiple chloroplasts and location of stigma (arrow)
e) unicell, oblique dorsal view; chloroplasts have a slightly spiralled configuration

the culture ages. The maximum calculated growth rate (k) was about 0.3 divisions per day; normally it was less. Presumably this is much less than occurs in natural conditions. Growth rate measurements are continuing in various combinations of light, salinity, and nutrients to explain the apparent discrepancy.

DISCUSSION

It is highly likely that the Golfo de Nicoya red tide *Cochlodinium* is the same organism as Silva's *C. heterolobatum*. The important question of whether both are identical to Okamura's *C. catenatum* is at present unresolved. Kofoid and Swezy (1921) believed that their organism from La Jolla, which was yellow/green and not photosynthetic, was the same as Okamura's *C. catenatum*. But Okamura (1920) had mentioned yellow/brown chloroplasts and water discolored reddish-brown, with dying fish. Without seeing the original description, we believe that the Golfo de Nicoya red tide organism is *C. catenatum*, and may well be the same as *C. heterolobatum*.

A similar organism was reported from the coastal waters of Sucre, Venezuela by Reyes-Vásquez *et al.* (1979), and identified as possibly *C. catenatum*. This red tide resulted in nine deaths and 159 illnesses. Okamura's original description of *C. catenatum*, according to Kofoid and Swezy, resulted in a fish kill. The Golfo de Nicoya *Cochlodinium*, according to local fishermen, does not cause illness through shellfish ingestion nor fish kills. When such fish kills occur, they may often be a

result of de-oxygenation of the water rather than release of a toxic metabolite. Considering concentrations of $80x10^6$ cells per liter, and the results of our attempts to measure productivity, it would not be surprising if fish kills occurred at some time in association with the decay of these dinoflagellates.

The transient nature of the *Cochlodinium* red tide precluded further intensive study during 1979. Apparently predictability is only possible within wide limits. We were reliably informed by different people at different times that these blooms occur: at the beginning of the dry season (Jan.); at the beginning of the rainy season (May); through-out the dry season; at the end of the rainy season (Nov.). Our study took place in the middle of the dry season. Clearly an intensive sampling program is needed to define the seasonal occurrence of this organism.

Although growth rate in culture was low, we do not know comparable rates under natural conditions. It is possible that 0.3 divisions per day is a realistic number and that the extensive formation of red tide slicks and patches is a result of physical processes in the water mass. Such mechanisms could include prevailing onshore winds, convergence zones near river discharges, local convection areas (Langmuir circulation), or local current transport influenced by bottom topography.

In summary, an initial attempt has been made to characterize the red tide of Golfo de Nicoya. The causative organism, tentatively identified as *Cochlodinium catenatum* Okamura, reaches very high concentrations, at least during the dry season (Jan.-Apr.) in the vicinity of Puntarenas. The organism is amenable to culture, although growth rates are slow. The environmental conditions leading to initiation and decay of blooms are presently unknown, but should be thoroughly investigated. The organism may have potential as a food organism for aquaculture, if maximum sustainable yields can be considerably increased and if it is non-toxic. Experiments are currently in progress to determine growth rates in culture under a variety of conditions, and to determine its potential toxicity.

ACKNOWLEDGEMENTS

This research was partially supported by the Organization of American States through Fellowship N° BEGES-63708; and National Science Foundation Grant N° OCE76-82280 both and by the Universidad Nacional, Heredia,Costa Rica. The advice, assistance, and encouragement of Claudia Charpentier, Skip French, and Diane Hargraves is gratefully acknowledged.

RESUMEN

El organismo que causa la marea roja del Golfo de Nicoya, Costa Rica, es *Cochlodinium catenatum* Okamura. La concentración máxima de células fue $80x10^6$/litro. Colonias en cadenas de cuatro dominaron en las muestras naturales, mientras que en cultivo las células generalmente estaban aisladas o en parejas. Las mediciones de la producción primaria no son confiables a causa de la autolisis de las células. Las condiciones ambientales que gobiernan el inicio y el final de las afloraciones son desconocidas. Aparentemente esta marea roja no es tóxica.

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