

Blooms of *Cochlodinium polykrikoides* (Gymnodiniaceae) in the Gulf of California, Mexico

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Abstract: *Cochlodinium polykrikoides* was the species responsible for the discoloration that occurred between September 15th and 27th, 2000 in a shallow coastal lagoon located in the southern part of the Bahía de La Paz, on the west side of the Gulf of California. Blooms of *C. polykrikoides* were observed four days after two rainy days with a seawater temperature of 29 to 31°C. Nutrient concentration ranges during the bloom were 0.165-0.897 µM NO₂+NO₃, 0.16-3.25 µM PO₄, and 1.0-35.36 µM SiO₄. Abundance of *C. polykrikoides* ranged from 360 x 10³ to 7.05 x 10⁶/cells l⁻¹. Biomass expressed in terms of chlorophyll *a* was high, ranging from 2.7 to 56.8 mg/m³. A typical dinoflagellate pigment profile (chlorophyll *a* and *c*, peridinin, diadinoxantin, and β-carotene) was recorded. In this study, the red tide occurred in front of several fish and shrimp-culture ponds. No PST toxins were found in the samples. However, 180 fish were found dead in the infected fish-pond; the gills were the most affected part. *C. polykrikoides* is a cyst-forming species that recurs in this area. New blooms were observed in November 2000 and September-November 2001 in the same area. Anthropogenic activities, such as eutrophication caused by water discharge in this shallow lagoon, and nutrient enrichment in the culture ponds, as well as effects from precipitation and wind stress, could have favored the outbreak of this dinoflagellate.

Key words: *Cochlodinium polykrikoides*, blooms, caged fish mortality, Bahía de La Paz, Gulf of California.

Palabras clave: *Cochlodinium polykrikoides*, proliferaciones, mortalidad de peces en cautiverio, Bahía de la Paz, Golfo de California.

According to Hallegraeff (1993), a world-wide increase of algal blooms has occurred near the end of the 20th century. This increase has also been observed in México, particularly on the eastern shoreline of the Gulf of California, where bloom-forming species have been recorded for the first time (Cortés-Altamirano and Alonso-Rodríguez 1997). For the western shoreline of the Gulf of California, few algal blooms have been reported. The main blooming species are the phototropic protozoan *Mesodinium rubrum* Lohmann 1908, the dinoflagellates *Scrippsiella trochoidea* (Stein) Loeblich III 1976 and *Noctiluca scintillans* (Macartney) Kofoid et Swezy 1920 (Gárate-Lizárraga *et al.* 2001,

2002), and recently the diatoms *Rhizosolenia debaryana* Peragallo 1892 (Gárate-Lizárraga *et al.* 2003) and *Chaetoceros debilis* Cleve 1894. *Cochlodinium polykrikoides* Margalef 1961 is a bloom dinoflagellate newly reported for this area (Gárate-Lizárraga *et al.* 2000), whose distribution is restricted to warm and tropical waters (Steidinger and Tangen 1996). Blooms caused by *C. polykrikoides* have been associated with massive fish kills, and is regarded as a potentially toxic dinoflagellate in Korea (Kim 1997). The purpose of this paper is to understand environmental conditions where *C. polykrikoides* proliferate in a shallow coastal lagoon located in the southwest coast of the Gulf of California.

MATERIALS AND METHODS

From September 15th to 28th 2000, several red patches were observed in the inner part of the coastal lagoon, La Ensenada de La Paz (24.13° N, 110.39° W) (Fig. 1). Samples of these patches were collected near the surface and fixed with a few drops of lugol. Live cells were used for proper identification. Water samples were also taken with Van Dorn bottles for the determination of nutrients (NO₃, NO₂, PO₄, SiO₂) and photosynthetic pigments. Seawater temperature was measured with a thermometer (Kahlsico). Nitrates, nitrites, phosphates, and silicates were measured according to Strickland and Parson (1972). Cell counts were performed in 5 ml settling chambers, using a phase contrast inverted microscope. The photosynthetic pigments were determined by high performance liquid chromatography (Vidussi *et al.* 1996). Identification and quantification of pigments were made, as described in Bustillos-Guzmán *et al.* (1995). Because paralytic shellfish toxins (PST) have been reported for *Cochlodinium* type-78 Yatsushiro (Onoue and Nosawa 1989), PST analysis was performed. Samples from a red tide of *C. polykrikoides* were collected for toxin analysis and were filtered through GF/F Whatman filters, frozen, and a post-column oxidation and fluorescence detection system (Hummer *et al.* 1997, Yu *et al.* 1998). Precipitation data were obtained from the Comisión Nacional del Agua (CNA), México. Recurrent blooms were observed during November 2000 and September-November 2001. Samples for identification purposes were taken in these last episodes, which started in the same area that had previous outbreaks and had expanded to Bahía de La Paz and the area near Isla Espíritu Santo (Fig. 1).

RESULTS

C. polykrikoides was the species responsible for seawater discoloration that occurred from September 15th to 27th, 2000 in La Ensenada de La Paz. Most specimens of *C.*

polykrikoides were forming chains of four organisms and rarely of two cells. *C. polykrikoides* is an unarmored, marine, planktonic dinoflagellate with a distinctive spiral-shaped cingulum, which is deep and excavated (Fig. 2) and occupies an area about 0.6 times the cell length, and descends in a distinct left-handed spiral of 1.8-1.9 turns around the cell. Cells range in size from 30-42 µm in length and 22-30 µm in width. A red stigma was observed dorsally in the epitheca.

C. polykrikoides blooms were observed four days after two days of rain. The reddish patches were found mainly in the shallow areas (1-4 m to bottom), and they occurred

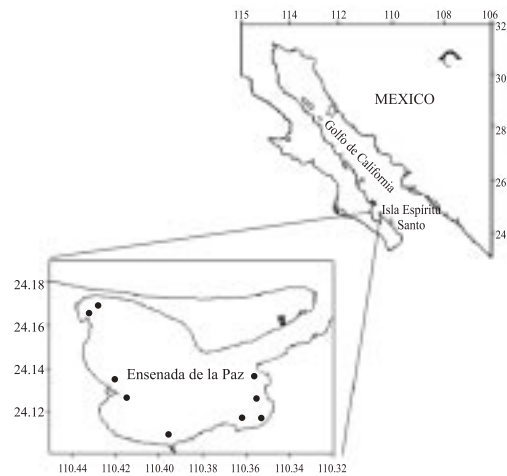


Fig. 1. Sampling sites and study area.

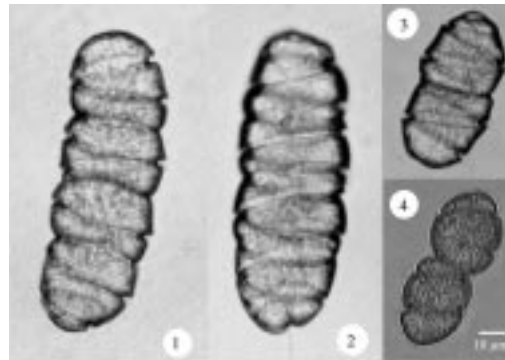


Fig. 2. 1, 2) Two different chains of *Cochlodinium polykrikoides*. 3) Chain of two cells. 4) *Cochlodinium* sp. found during winter.

where water temperature ranged from 29 to 31°C (Fig. 3). Nutrient concentrations during the bloom ranged from 0.165-0.897 μM NO_2+NO_3 , 0.16-3.25 μM PO_4 , and 1.0-35.36 μM SiO_4 (Table 1).

Abundance of *C. polykrikoides* ranged from 360×10^3 to 7.05×10^6 /cells l^{-1} (Fig. 4). Biomass, expressed in terms of chlorophyll ranged from 2.7 to 56.8 mg/m^3 , and the carotenoid dinoflagellate fingerprint, the peridinin, ranged from 0.68 to 32.03 mg/m^3 (Fig. 4). A typical dinoflagellate pigment profile (chlorophyll *a* and *c*, peridinin, diadinoxantin, and β -carotene) was observed in *C. polykrikoides* (Fig. 5).

In this investigation, no PST toxins were found in samples of *C. polykrikoides*. The algal extracts did not showed any peaks corresponding to PST on the HPLC chromatograms. During the first outbreak, 27 dead pargo fish (*Lutjanus argentiventis* Peters 1869) were found in the infected fish pond, with only their gills infected. In the outbreak occurring in September-November 2001, about 180 caged fish died (60 adult *Pomadasys macracanthus* (Hunter) Jordan 1888; 90 juvenile and 20 adult *Diapterus peruvianus* Cuvier 1830, and 10 adult *L. argentiventis*).

TABLE 1
Concentration of nutrients during the bloom of *C. polykrikoides* at La Ensenada de La Paz

Date	NO_3+NO_2	$\text{PO}_4 \mu\text{M}$	$\text{SiO}_4 \mu\text{M}$
15/09/00	0.159	3.254	10.117
15/09/00	0.162	2.437	9.042
16/09/00	0.194	2.246	7.703
18/09/00	0.897	0.460	1.014
20/09/00	0.851	0.395	7.233
22/09/00	0.514	0.161	19.392
26/09/00	0.290	0.428	35.360
27/09/00	0.165	0.507	17.362
Mean	0.404	1.236	13.403
SD(\pm)	0.313	1.206	10.607

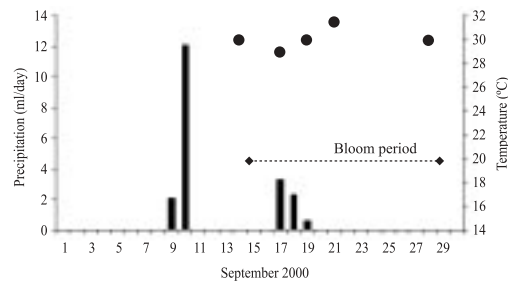


Fig. 3. Sea surface temperature values and variation of precipitation (ml/day) during September 2000 at a stationary sampling station.

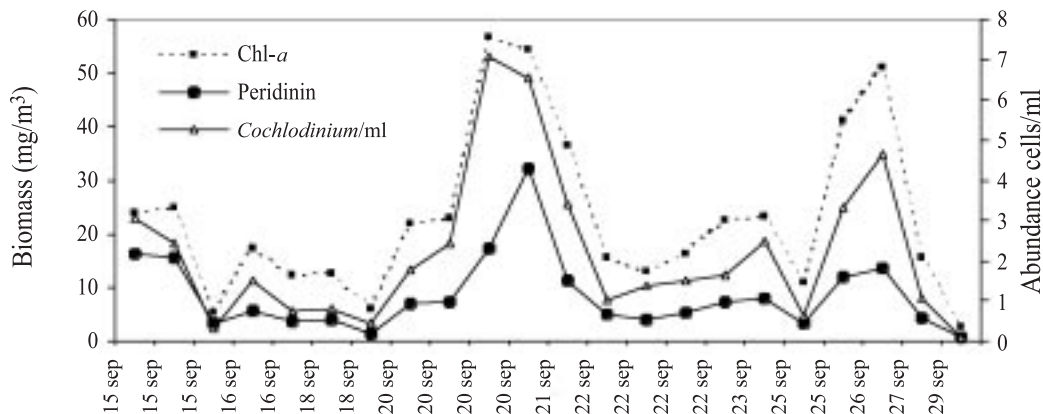


Fig. 4. Variation of abundance, chlorophyll *a* and peridinin in *C. polykrikoides* during a bloom.

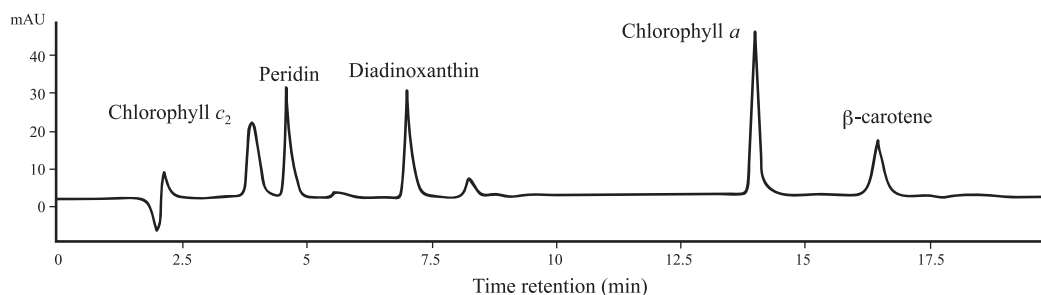


Fig. 5. Pigment profiles of *C. polykrikoides*.

DISCUSSION

C. polykrikoides is an unarmored, marine, planktonic dinoflagellate. This species is synonymous with *C. heterolobatum* Silva (Silva 1967). Proper identification of *C. polykrikoides* was difficult because this species became rounded, contracted, or disrupted with the fixative agent (lugol). Hallegraeff and Fraga (1996) and Cho *et al.* (2001) mentioned that *C. polykrikoides* can be mistaken for *Gymnodinium catenatum* Graham 1943 and *Gyrodinium impudicum* Fraga and Bravo 1995. Cortés-Altamirano and Alonso-Rodríguez (1997) recorded several blooms of *G. catenatum* during Autumn 1997 in Bahía de Mazatlán and later re-identified it as *G. impudicum* (Cortés-Altamirano *et al.* 1999). Cortés-Altamirano (2002) again re-identified this species as *Cochlodinium catenatum*. In this study, we used the description of Margalef

(1961) to identify *C. polykrikoides*, as well as Fukuyo *et al.* (1990) and Steidinger and Tangen (1996). Fig. 6 shows original drawings of *C. catenatum* and *C. polykrikoides* made by Okamura (1916) and Margalef (1961). The epicone of *C. catenatum* is anteriorly subhemispherical and the epicone of *C. polykrikoides* is “cupuliform”. The principal difference between them is the presence of stigma. Margalef (1961) reported only one large and complex stigma and Okamura (1916) reported some small reddish stigma. Description of *Cochlodinium* found in our study coincides with Margalef’s description. Genetic studies are recommended to clearly separate this two species or re-instate *C. polykrikoides* to *C. catenatum*, which was described first.

Distribution of both *Cochlodinium* species is quite similar. *C. catenatum* is distributed in Japan (Okamura 1916), California (Kofoid and Swesy 1921, Venrick 2000), Costa Rica, and Panamá (Guzmán *et al.* 1990) where it was implicated in coral mortality. *C. polykrikoides* is distributed in Puerto Rico (Margalef 1961), Chesapeake Bay, Barnegat Bay, New Jersey (Silva 1967), York River, Virginia (Ho and Zubkoff 1979), Japan (Fukuyo *et al.* 1990), Guatemala (Rosales-Loessener *et al.* 1996), Korea (Kim 1998, Kim *et al.* 1999), and now in the Gulf of California.

Although these two blooms are the only records of this species in the Gulf of California, the genus *Cochlodinium* was firstly recorded in samples collected in January 1942 (Osorio-Tafall 1943). Likewise, Morey-Gaines

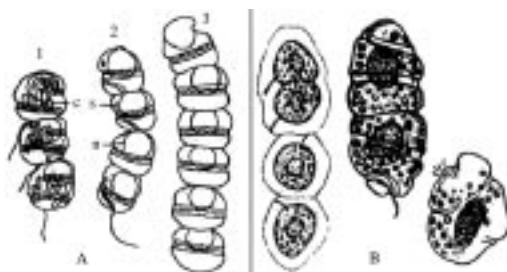


Fig. 6. A. *C. catenatum*; 1) dorsal side showing the chomatophores (c), 2) side view showing the nucleus (n) and stigma (s), 3) portion of chain. B. *C. polykrikoides*.

(1982) reported concentrations of 100×10^3 *Cochlodinium*/l⁻¹ during a red tide of *G. catenatum* in Bahía de Mazatlán in April 1979. Along the Manzanillo coast (state of Colima), some red tides of *C. polykrikoides* were reported by Morales-Blake and Hernández-Becerril (2001). Gárate-Lizárraga *et al.* (2004) found these two species occurring in samples collected in Bahía de Mazatlán in April 2001. The lack of records of *Cochlodinium* in previous works may result from using formaldehyde and lugol, which are not good fixatives (Osorio-Tafall 1943, Gárate-Lizárraga *et al.* 2000, Figueroa-Torres y Zepeda-Esquivel 2001). Recent observations of live phytoplankton from Bahía de La Paz confirm the presence of other *Cochlodinium* species such as *C. schuetti* Kofoid et Swezy 1921 and *C. citron* Kofoid et Swezy 1921 through the year.

Both *C. polykrikoides* abundance (360×10^3 to 7.05×10^6 /cells l⁻¹) and chlorophyll *a* concentrations (2.7 to 56.8 mg/m³) were closely related. These biomass concentrations are the highest recorded in the area. Highest values of chlorophyll *a*, up to 10 mg/m³, have been reported and related to blooms of the protozoan *Mesodinium rubrum* (Martínez-López *et al.* 2001), which seem to be very common in this area (Gárate-Lizárraga *et al.* 2001). Highest values of chlorophyll *a* (17.15 to 41.45 mg/m³) have been also reported for exceptional blooms of the diatom *Rhizosolenia debyana* (Gárate-Lizárraga *et al.* 2003). Blooms of *C. polykrikoides* could be also responsible for local increases in the concentrations of chlorophyll *a*, and seem to be very important in terms of the fertility of the coastal zone.

Jeong *et al.* (2001) found the presence and persistence of *C. polykrikoides* in lower (20°C) and higher (28°C) water temperatures, however, those outbreaks were mainly associated with a lower temperature (25-26°C). *C. polykrikoides* blooms reported here occurred at a higher range (29 to 31°C) and salinities between 28.94 to 32.5 psu. In general, our findings of nutrient concentrations coincide with those found by Lechuga-Devéze *et al.* (1997) and Cervantes-Duarte *et al.* (2001) for mid-summer

(August-September). However, at the beginning of this red tide caused by *C. polykrikoides*, phosphates were higher (2.24-3.25 µM) than found in previous studies. This could be a result of the preceding rainy days, which contributed the phosphates in runoff. These concentrations are in the range considered characteristic of eutrophic waters in Bahía de Mazatlán, which is influenced by municipal sewage effluent (Alonso-Rodríguez *et al.* 2000).

Intensive aquaculture has contributed to eutrophication in some coastal areas. It is probable that nutrients close to shrimp culture ponds during a post-harvest period were unloaded into La Ensenada de la Paz. These eutrophic environments are very susceptible to harmful algal blooms (Kim 1998). Further, nutrient accumulation near the culture ponds can be moved by the action of the local winds and runoff from heavy rains. Anthropogenic activities, such as eutrophication caused by water discharged into La Ensenada de La Paz and nutrient enrichment in the culture ponds, as well as effects of precipitation and wind, could have favored the outbreak of this dinoflagellate. *C. polykrikoides* is a cyst-forming species (Matsuoka and Fukuyo 2000) that is recurrent, and it is plausible that this is the cause of the blooms observed in November 2000 and September-November 2001. Moreover, after Hurricane "Juliette," *C. polykrikoides* expanded to Bahía de La Paz and off Isla Espiritu Santo from September 26th to November 4th, 2001.

Blooms of *C. polykrikoides* occurring in Japan have provoked massive death in fish populations (Yuki and Yoshimatsu 1989, Kim 1998). In Costa Rica and Panama, blooms of *C. catenatum* were implicated in coral mortality, which was partly attributed to smothering by the mucus produced by the dinoflagellate (Guzmán *et al.* 1990). Recently, a bloom of *Cochlodinium* in Canada caused substantial mortality to cultured salmon, accounting for economic losses of about CAN\$ 2 million (Whyte *et al.* 2001).

Although we did not find evidence of PST in *Cochlodinium* samples, death of juvenile

and adults fish of *L. argentiventis*, *D. peruvianus*, and *P. macracanthus* was observed. Fish mortality occurs because caged fish have less opportunity to avoid harmful algal blooms than wild fish. Ho and Zubkoff (1979) suggested that physical contact, not a released toxin, was the cause of oyster larvae (*Crassostrea virginica* Guilding 1828) deformation and mortality during a *C. polykrikoides* red tide in the York River of Virginia. This dinoflagellate generates reactive oxygen, O_2^- , OH, H_2O_2 . These radicals are factors that can induce fish kills (Kim *et al.* 1999). Kim *et al.* (2002) propose that biologically-active multiple metabolites secreted by *C. polykrikoides*, such as cytotoxic agents and mucus substances, may contribute to fish kills. In this study, the red tide occurred in front of several fish and shrimp-culture ponds. One of them was infected with *C. polykrikoides*, reaching abundances of 8×10^6 /cells l^{-1} . Fishes were examined and gill damage was observed.

Hydrological and phytoplankton monitoring programs are necessary to help prevent future mass kills of marine animals, particularly caged species of economic value. Adequate water quality will be necessary to achieve sustainability of coastal ecosystems. Insight into the mechanisms that determine lethal species composition is needed to predict the possible effect of water quality.

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RESUMEN

Durante el desarrollo de una marea roja ocurrida del 15 al 27 de septiembre del año 2000 en la Ensenada de La Paz, B.C.S. se tomaron muestras de agua con una botella Van Dorn para determinar la temperatura, la especie causante y la cantidad de nutrientes y pigmentos fotosintéticos. Se hicieron análisis de toxinas de *Cochlodinium polykrikoides*, la especie responsable de esta marea roja. La mayoría de los especímenes formaron cadenas de cuatro células y raramente de dos. La abundancia fue de 360×10^3 a 7.05×10^6 /cells l^{-1} . Los florecimientos de *C. polykrikoides* ocurrieron cuatro días después de dos días lluviosos; el intervalo de temperatura fue de 29 a 31°C. La concentración de nutrientes registrada durante este fenómeno fue $0.165-0.897 \mu M NO_2+NO_3$, $0.16-3.25 \mu M PO_4$, y $1.0-35.36 \mu M SiO_4$. El perfil pigmentario reveló la presencia de clorofila *a* y *c*, peridinina, diadinoxantina, y β -caroteno. La biomasa total expresada en clorofila *a* fue alta, oscilando entre 2.7 y 56.8 mg/m³, mientras que la biomasa de *Cochlodinium*, expresada en peridinina, varió entre 0.68 y 32.03 mg/m³. En este estudio, la marea roja se desarrolló cerca de varios estanques de cultivo de peces y camarón. En uno de ellos proliferó *C. polykrikoides*. Los análisis de toxinas PST fueron negativos; sin embargo, durante el incremento de las proliferaciones algunos estanques fueron alcanzados y murieron 180 peces, principalmente pargos (*Lutjanus argentiventis*, *Pomadasys macracanthus*). Las branquias fueron las partes más afectadas. En condiciones desfavorables *C. polykrikoides* forma quistes, lo cual ha provocado su recurrente proliferación en el área, registrándose nuevas proliferaciones en noviembre del 2000 y en septiembre-noviembre del 2001. Actividades antropogénicas como la eutroficación causada por la descarga de aguas residuales y de nutrientes de los estanques de cultivo, pudieran estar favoreciendo la proliferación de este dinoflagelado.

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