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RESEARCH NOTE

Occurrence of *Cochlodinium fulvescens* (Gymnodiniales: Dinophyceae) in the southwestern Gulf of California

Ocurrencia de *Cochlodinium fulvescens* (Gymnodiniales: Dinophyceae) en el suroeste del Golfo de California

Ismael Gárate-Lizárraga[1]

[1]Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Departamento de Plancton y Ecología Marina, Apartado postal 592, La Paz, B.C.S. 23000, México. igarate@ipn.mx

Abstract.- The naked dinoflagellate *Cochlodinium fulvescens* was rarely observed in Bahía de La Paz since 2008. Sporadic observations were made in 2010 and 2012. The species re-appeared from October 2012 to April 2013. Abundance of *C. fulvescens* ranged from 600 to 45,800 cells L^{-1} during this period in seawater temperature at 22-27°C and salinity of 35.25-35.75. This species appeared as single cells or two-celled chains and co-occurred with *Cochlodinium polykrikoides* at the end of the bloom. *C. fulvescens* usually occurs in autumn to spring and *C. polykrikoides* usually occurs in spring to autumn. The main distinguishing morphological characters between these two species are the relative position of the cingulum and sulcus and the morphology of chloroplasts. This provided reliable identification of live cells. Other *Cochlodinium* species, such as *C. faurei*, *C. pulchellum*, and *C. rosaceum* were also observed in the bay.

Key words: Dinoflagellates, *Cochlodinium fulvescens*, *Cochlodinium polykrikoides*, southwestern Gulf of California

INTRODUCTION

The genus *Cochlodinium* belongs to the order Gymnodiniales Lemmermann, 1910. *Cochlodinium* is an unarmored, marine planktonic dinoflagellate with a distinctive spiral-shaped cingulum of 1.5 or more gyres around the cell. Some photosynthetic *Cochlodinium* species form cell chains. According to the recent world-wide review of marine dinoflagellates (Gómez 2011), there are 35 species of *Cochlodinium*. *Cochlodinium fulvescens* Iwataki, Kawami & Matsuoka, 2007 was recently described as a new species (Iwataki *et al*. 2007). Taxonomy and distribution of *Cochlodinium* species have occasionally been studied in Mexico. Twelve species of *Cochlodinium* have been found in Pacific coastal waters of Mexico (Gárate-Lizárraga *et al*. 2004, 2009a, b; 2011; Cortés-Lara *et al*. 2004, Okolodkov & Gárate-Lizárraga 2006, Morquecho-Escamilla & Alonso-Rodríguez 2008, Gárate-Lizárraga 2013). In these reports, massive blooms of *Cochlodinium polykrikoides* Margalef, 1961 have been recorded along the west coast of Mexico (Morales-Blake *et al*. 2001, Gárate-Lizárraga *et al*. 2004, 2009b). *Cochlodinium fulvescens* has been reported in lagoons and bays along the southern Gulf of California (Morquecho-Escamilla & Alonso-Rodríguez 2008, Gárate-Lizárraga *et al*. 2009a). Recently, other species of *Cochlodinium*, including *C. convolutum* Kofoid & Swezy, 1921, *C. helicoides* Lebour, 1925, *C. helix* Schütt, 1895, *Cochlodinium pulchellum* Lebour, 1917, and *Cochlodinium virescens* Kofoid & Swezy, 1921, have been recorded for the first time (Gárate-Lizárraga *et al*. 2011, Gárate-Lizárraga 2012). The purpose of this study was to describe the seasonal occurrence of *Cochlodinium fulvescens* in Bahía de La Paz, Gulf of California.

MATERIALS AND METHODS

Bahía de La Paz is the largest bay on the peninsular side of the Gulf of California. The bay constantly exchanges water with the Gulf of California via a northern and a southern opening (Gómez-Valdés *et al*. 2003). As part of a continuing toxic or harmful microalgae monitoring program, 56 phytoplankton bottle samples were monthly collected from January 2010 to April 2013 at a fixed sampling station in Bahía de La Paz (Fig. 1), in the State of Baja California Sur (24°212N, 110°312W). Two-day samplings were performed each month from October 2012 to April 2013. Two horizontal tows, using a 20 µm mesh net with 50 cm diameter, were performed. Samples were immediately fixed with acid Lugol's solution and preserved with 4% formalin. Live phytoplankton samples were used to identify *Cochlodinium* species. *Cochlodinium* cell counts were made in 5 mL settling chambers under an inverted, phase-contrast microscope (Carl Zeiss,

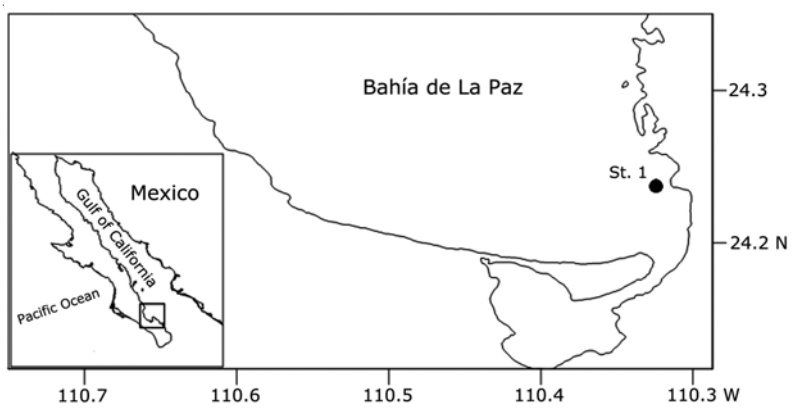

Figure 1. Map of the study area indicating fixed sampling station for microalgae monitoring in Bahía de La Paz / Mapa del área de estudio indicando la estación fija de monitoreo de microalgas en Bahía de La Paz

Oberkochen, Germany) (Utermöhl 1958). Sea surface temperature was recorded with a bucket thermometer. Salinity was measured with a refractometer (Model STX3, Vee Gee Scientific, Kirkland, WA). A compound microscope was used to measure cells and a digital camera (Konus Italia Group, Verona, Italy) was used to record images.

Results and discussion

During this investigation, only 13 samplings were *C. fulvescens* positive, occurring in a temperature range of 22-27°C and salinity of 35.25-35.75 (Table 1). These specimens were either solitary (Figs. 2A-C) or two-cell chains (Figs. 2D-F). No four-cell chains were observed. The specimens fit well with the diagnosis and description of Iwataki *et al*. (2007). Cells are rounded and ellipsoidal in shape (Figs. 2A-C; D-F). The cingulum encircles the cell twice; the sulcus runs down apart from the cingulum (Figs. 2 B-C). A reddish-orange pigmented body is located at the dorsal side of the epicone (Figs. 2 B-C). The nucleus is spherical and located at the anterior part of the epicone (Fig. 2A). Chloroplasts are granular and distributed along the cingulum and the periphery of the cell. The specimens appear pale yellow. Cells were 32-55 µm long and 25-38 µm wide (n = 30). *C. fulvescens* became round (Fig. 2G) when preserved with Lugol's solution.

Table 1. Abundance of *Cochlodinium fulvescens*, temperature and salinity data in 13 samplings performed in Bahía de La Paz, from October 2010 through April 2013 / Datos de abundancia de *Cochlodinium fulvescens*, temperatura y salinidad en 13 muestreos realizados en Bahía de La Paz, de octubre 2010 hasta abril 2013

Sampling dates	Cell abundance (cells L^{-1})	Temperature (ºC)	Salinity
10/16/2010	600-1000	27	-
10/21/2010	1200-1600	26	-
12/13/2010	800-1200	22	-
04/28/2011	400-800	25	-
11/24/2011	400-800	25	-
12/12/2011	800-1400	22	-
04/19/2012	800-1000	24	-
10/28-29/2012	600-1200	27	32.25
12/12-13/2012	4000-6600	24	35.72
01/16-17/2013	7600-10800	23	35.42
02/19-20/2013	8200-11800	23	35.75
03/20-21/2013	6400-19800	22	35.62
04/24-25/2013	27800-45800	24	35.68

Abundance of *C. fulvescens* was low (400-1600 cells L^{-1}) during samplings from 2010 through 2011, reaching peak densities of 4000-6600 cells L^{-1} in December 2012 (Table 1). An increase in abundance occurred from January through April 2013, reaching moderate densities ranging from 27,800 to 45,800 cells L^{-1}. Other *Cochlodinium* species were found in net phytoplankton samples from 2013. Three specimens of *Cochlodinium faurei* Kofoid & Swezy, 1921 were also found in net phytoplankton samples collected in January 2013 (Fig. 2H). The cells are medium-sized with a sub-ovoid to ellipsoid body. Cells were 60-64 µm long and 35-38 µm wide. The cytoplasm is finely granular, but usually very clear and transparent. This finding is the first record for Bahía de La Paz. Four encysted specimens of *C. pulchellum* Lebour, 1917 were observed in net samples in 24 February 2013. They were small-sized, reaching a total length of 38-44 µm, with an asymmetrical fusiform body (Fig. 2 I). This species was also reported earlier in Bahía de La Paz (Gárate-Lizárraga 2011). Two specimens of *Cochlodinium rosaceum* Kofoid and Swezy, 1921 were found in net phytoplankton samples collected in April 2013; it is a medium-sized species with an ellipsoid, rose-

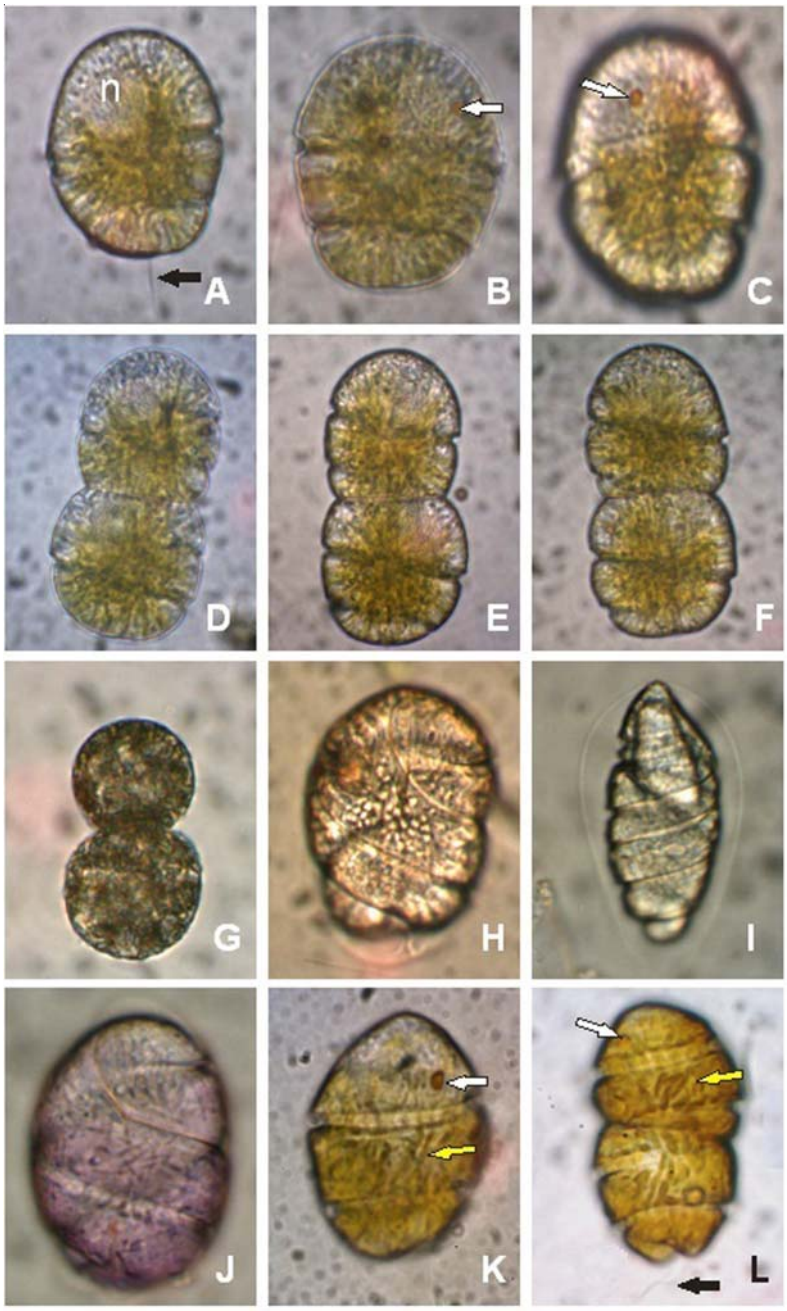

Figure 2. Light microphotographs of *Cochlodinium fulvescens*, *Cochlodinium faurei*, *Cochlodinium pulchellum, Cochlodinium rosaseum* and *Cochlodinium polykrikoides* from Bahía de La Paz. A-C) Three single cells of *Cochlodinium fulvescens* showing the nucleus (n), flagellum (black arrow), and stigma (white arrow); D-F) Three two-celled chains of *Cochlodinium fulvescens*; G) Two-celled chain of *Cochlodinium fulvescens* fixed with Lugol's solution; H) Specimen of *Cochlodinium faurei*; I) Hyaline cyst of *Cochlodinium pulchellum*; J) Specimen of *Cochlodinium rosaceum*; K) Single cell of *Cochlodinium polykrikoides* showing stigma (white arrow) and the rod-shaped chloroplasts (yellow arrow); L) Two-cell chain of *Cochlodinium polykrikoides*, showing the reddish orange pigmented body (white arrow), the rod-shaped chloroplasts (yellow arrow), and longitudinal flagellum (black arrow) / Microfotografías de luz de *Cochlodinium fulvescens, Cochlodinium faurei*, *Cochlodinium pulchellum*, *Cochlodinium rosaseum* y *Cochlodinium polykrikoides* de Bahía de La Paz. A-C) Tres especímenes de *Cochlodinium fulvescens* mostrando el núcleo (n) el flagelo (flecha negra) y el estigma (flecha blanca); D-F) Tres cadenas de 2 células de *Cochlodinium fulvescens*; G) Cadena de 2 células de *Cochlodinium fulvescens* fijada con solución de Lugol; H) Espécimen de *Cochlodinium faurei*; I) Quiste hialino de *Cochlodinium pulchellum*; J) Espécimen de *Cochlodinium rosaceum*; K) Célula solitaria de *Cochlodinium polykrikoides* mostrando el estigma (flecha blanca) y los cloroplastos acintados (flecha amarilla); L) Cadena de 2 células de *Cochlodinium polykrikoides* mostrando el estigma de color rojizo naranja (flecha blanca), los cloroplastos en forma de barras (flecha amarilla) y el flagelo longitudinal (flecha negra)

colored cell (Fig. 2J), and 68-70 µm long and of 32-34 µm wide. Specimens of *C. rosaceum* fit well with the description by Kofoid & Swezy (1921). This is the first record of this species along the Pacific coast of Mexico.

Cochlodinium fulvescens is a bloom-forming dinoflagellate first found in Tachibana Bay (west Japan), Hurun Bay (South Sumatra), Indonesia (Iwataki *et al*. 2007), British Columbia (Iwataki *et al*. 2008), California (Iwataki *et al*. 2008, Curtiss *et al*. 2008, Kudela & Gobler 2012), and the Gulf of California (Morquecho-Escamilla & Alonso-Rodríguez 2008, Gárate-Lizárraga *et al*. 2009a). Recently *C. fulvescens* was also reported in the Bahía de Acapulco (Meave del Castillo *et al*. 2012) and coastal waters of Karachi bordering the northern Arabian Sea (Munir *et al*. 2012). *C. fulvescens* was firstly recorded in Bahía de La Paz during a multi-species bloom in June 2008 (Gárate-Lizárraga *et al*. 2009a) and then in December 2010. Densities of *C. fulvescens* in our study were higher, compared to the report of Gárate-Lizárraga *et al*. (2009a). However, it was lower than the 380,000 to 490,000 cells L^{-1} reported by Morquecho-Escamilla & Alonso-Rodríguez (2008) from *C. fulvescens* blooms in Bahía de Mazatlán (23°11211.83 N, 106°26246.23W).

C. polykrikoides (Figs. 2K-L) is similar to *C. fulvescens* as both species form cell chains and have a nucleus and an eyespot located in the anterior dorsal part of the cell (Iwataki *et al*. 2007, Matsuoka *et al*. 2008). The presence of an eyespot was demonstrated for *C. polykrikoides* by TEM in Iwataki *et al*. (2010). A similar position was reported for the phylogenetically related species *C. fulvescens* (Iwataki *et al*. 2007). *C. polykrikoides* has numerous rod-shaped chloroplasts which extended longitudinally in healthy cells and were brownish-green in color (Fig. 2L). Meanwhile, *C. fulvescens* has granular chloroplasts that are distributed along the cingulum and the periphery of the cell. Blooms of *C. polykrikoides* have become recurrent events in Bahía de La Paz (Gárate-Lizárraga 2013); the most recent occurred from August through October 2013. In samples from October 2012, *C. polykrikoides* occurred with *C. fulvescens*, with densities of 12800-24,600 cells L^{-1} for the former and 1200-1600 cells L^{-1} for the latter. The 2 species were previously coupled in the Bahía de La Paz in June 2008 (Gárate-Lizárraga *et al*. 2009a). There is clear overlap in the preferred temperature range of the 2 species in Bahía de La Paz; *C. polykrikoides* is common at 20-31°C and *C. fulvescens* at 22-27°C. Our results partly coincide with the work of Munir *et al*. (2012), with low densities of *C. fulvescens* at < 26°C and high densities at 31-32°C. Kudela & Gobler (2012) and Howard *et al*. (2012) state that cells of *C. fulvescens* are present at low temperatures (14-18°C). Comparing salinity, *C. fulvescens* was common at 35.25-35.75 in the Gulf of California, but Kudela & Gobler (2012) report that *C. fulvescens* was common at 32.8-33.6 off the coast of California.

In Bahía de La Paz, monitoring of live microalgae specimens that form red tides started in 2000, during a bloom of *C. polykrikoides* (Gárate-Lizárraga *et al*. 2004). Since that time, many microalgae bearing a soft cell membrane, such as dinoflagellates and raphidophytes, have been identified (Gárate-Lizárraga *et al*. 2004, 2009c; Gárate-Lizárraga 2013). Proper identification of several *Cochlodinium* species in Bahía de La Paz is a good example of the importance of working with fresh samples. The morphology of *Cochlodinium* species changes during observation under a light microscope. Likewise, cells tend to form a hyaline membrane around the cell or a temporary hyaline cyst (Fig. 2I); otherwise, they would explode. Specimens showing those features were fixed with Lugol's solution and could not be properly identified. Hence, live samples are important in the study of *Cochlodinium* species and other naked dinoflagellates.

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