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FIRST REPORT OF THE ATHECATE, CHAIN FORMING DINOFLAGELLATE *COCHLODINIUM FULVESCENS* (GYMNODINIALES) FROM PAKISTAN

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Abstract

A harmful dinoflagellate *Cochlodinium fulvescens* is reported for the first time from northern boarding of Arabian Sea, Karachi, Pakistan. During a phytoplankton monitoring program between May 2002-July 2003, *Cochlodinium* was observed from two localities of Manora Channel. Highest abundance was recorded during October 2002 at 926 cells l^{-1} during a period of high temperature and high salinity value. Taxonomical characters were studied by using light microscopy, epiflouresence microscopy and scanning electron microscopy. Identification of *Cochlodinium fulvescens* was confirmed by the presence of granular shaped chloroplast, different cell structure, large size, and intermediate position of sulcus between the cingulum and degree of torsion makes more than 1.5 turns. Emergence of the *C. fulvescens* in northern Arabian Sea has great concern from a fisheries viewpoint since this species has been implicated in considerable economic losses worldwide.

Introduction

Cochlodinium catenatum Okamura 1916, *C. convolutum* Kofoid et Swezy 1921, *C. polykrikoides* Margalef 1961, *C. fulvescens* Iwataki, Kawami et Matsuoka 2007 are chain forming, athecate, planktonic species of dinoflagellates producing possible unidentified ichthyotoxin. Catastrophic blooms, first described from Puerto Rico (Margalef, 1961), are reported to have caused fish kills in tropical and subtropical areas of the Western Pacific Ocean to Indian Ocean, Mediterranean, and Arabian Sea (Margalef, 1961; Kim, 1998; Yuki & Yoshimatsu, 1989; Matsuoka & Iwataki, 2004; Azanaza & Baula, 2005; Iwataki *et al*., 2007; Anton *et al*., 2008; Matsuoka *et al*., 2008; Richlen *et al*., 2010).

Cochlodinium fulvescens is newly described out of 40 species of the genus *Cochlodinium* (Kofoid & Swezy, 1921) based on granular shaped chloroplast and sulcus running at the middle of the cingulum (Iwataki *et al*., 2007). *Cochlodinium fulvescens* is a bloom forming dinoflagellate first found in Asian waters (Iwataki *et al*., 2007) and United State of America (Kudela *et al*., 2008). Other chain forming species such as *C. catanetum* and *C. polykrikoides* are known to form harmful algal blooms which have been implicated to shell fish farms, reef fishes, and invertebrates from coral reef areas (Guzman *et al*., 1990; Richlen *et al*., 2010). Blooms from Korea (Kim, 1998; Anon., 2004), Canada (Whyte *et al*., 2001), California (Kudela *et al*., 2008) and Oman (Richlen *et al*., 2010) have caused major economic losses. Recently a massive 7 months long bloom of *C. polykrikoides* was first reported from the Arabian Gulf region, which spread over 1200 km coastline of the United Arab Emirates (Richlen *et al*., 2010). The present work is the first report of *C. fulvescens* from northern Arabian Sea bordering Pakistan.

Materials and Methods

Study area: The study area is located in the coastal waters of Karachi bordering northern Arabian Sea. The coastal area is part of Karachi port and heavily influenced by coastal eutrophication and urban non-point pollution. Two stations were selected for sampling St. A (24˚49'.46"N 66˚57'.55"E) was located within Manora Channel (Karachi Port), whereas St. B (24˚47'.51"N 66˚58'.52"E) was at the mouth of the channel in the near-shore waters (Fig. 1).

Sampling techniques: Triplicate samples were collected from 1m depth using Niskin water sampler (1.7 L) during May 2002-July 2003. Water samples from each station were collected bimonthly through three separate casts and samples were recorded as, for example, My_1, My_2 and so on. Samples for physico-chemical parameter, microscopic and cell abundance analysis were drawn from the same water sampler and fixed appropriately.

Light and scanning electron microscopy: Water samples were fixed with 1% Lugol's solution and prepared (Utermohl, 1958) for examination under inverted microscope. Cell concentration was determined using 50 mL chamber plate and reported as cells l^{-1}. Identification and micrographs were taken under Epiflourescence Microscopy (Olympus BX-51, Japan) and Scanning Electron Microscopy (SEM; JEOL 5600LV). For SEM, Lugol's fixed samples were desalted using a gradient from seawater to freshwater, dehydrated in acetone (10-100% gradient series) and coated with 1.5 nm of platinum-palladium using a Denton sputter-edge coater (Moorestown, USA). The cell size was measured in light microscope using ocular scale and SEM employing computer soft image analysis.

Physico-chemical parameters: Temperature (bucket temperature using thermometer), salinity (refractometer), and chlorophyll a (Chl a; Strickland & Parsons, 1972) were also measured and correlated with *C. fulvescens* abundance and biomass.

Statistical analysis: Pearson correlation coefficient, linear regression and t-test were calculated by software Minitab16 version and Excel program 2007.

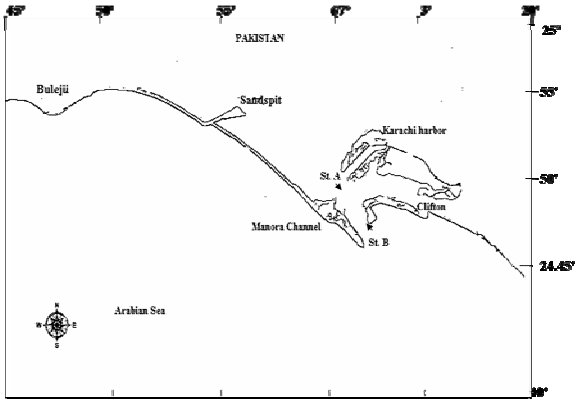


Fig. 1. Coastal map of Pakistan, two sampling points (St. A) and (St. B) marked by arrows from Manora Channel, Karachi.

Results

Morphology: Cells round to ovoid, large in size (35-58 µm in length and 25-40 µm in width), solitary, chain form consisting 2 to 4 (Fig. 2A-E). In SEM, epicone is conical and hypocone is subspherical (Fig. 2C). Cell surface was marked with pores and cell is showing torsion of cingulum twice which is approximately 1.5 turns (Fig. 2C). Sulcus is somewhat deep due to Lugols fixation and running at intermediate position (Fig. 2C). In stained cells, nucleus was located at the anterior side of the epicone (Fig. 2D) and chloroplast was granular and scattered on cingulum and periphery of the cells in lateral view (Fig. 2, D-E).

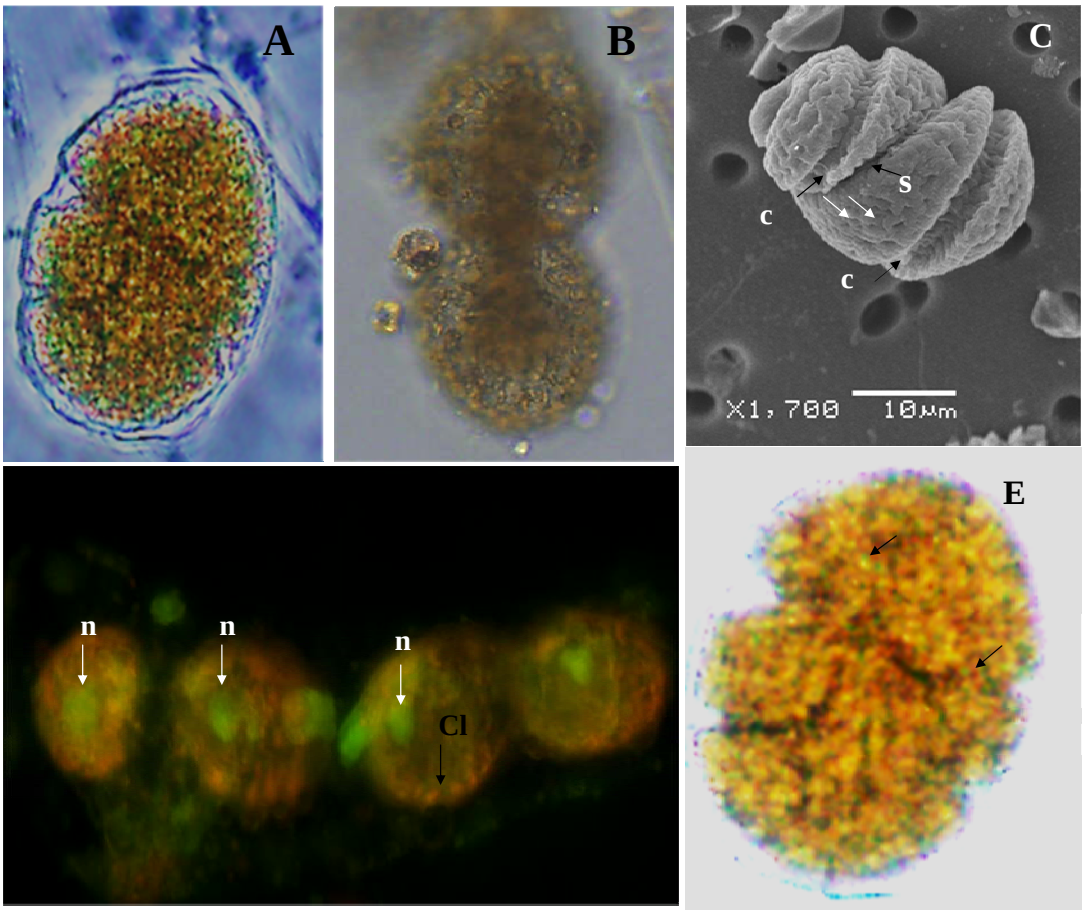


Fig. 2. Light and Scanning electron microscopy of *Cochlodinium fulvescens* showing single to 2 chain cells (A-B), epicone is conical, hypocone is subspherical. Cell surface is porous marked with white arrows (C). Cingulum (c) runs twice marked with white arrows and Sulcus (s) at intermediate position marked with black arrow (C). Stained cell showing 4 chains with nucleus (n) located at the anterior end of the epicone marked with white arrows (D) and granular shape marked with black arrow (D, E). Scale bar = 10 µm.

Ecology: Mean values of monthly variables (temperature, salinity, chlorophyll a) were recorded during May 2002 to July 2003 (Table 1, 2) from two stations. For both temperature and salinity, no difference was noted between the two stations. Chl a was greater at St. B than at St. A. Average value of temperature, salinity and chl a were correlated with the occurrence and abundance of *C. fulvescens* from the period October 2002 to July 2003 at St. A and September 2002 -February 2003 at St. B (Table 3). The occurrence of *C. fulvescens* (October 2002 to July 2003) was correlated with maximum temperature. High salinity values were recorded in November and low in April. Maximum chl *a* concentration was observed in October 2002 whereas minimum values were recorded in

July 2003. At station B, during the occurrence of *C. fulvesens* (September 2002-February 2003), maximum temperature was recorded in October and minimum in November, whereas salinity was high during January and low in October, maximum chl a concentration was observed in October and minimum in February.

Total cell densities ranged between 20-160 cells l^{-1} (Average±S.D; 59.6±45.3). High cell densities were observed in April (106 cells l^{-1}) and in July (160 cells l^{-1}) Fig. 3. Whereas chl *a* values were 18.21 µg l^{-1} and 4.81 µg l^{-1} respectively, corresponding with 31.8 ºC temperature and 35.7 psu salinity. At the station B, abundance of *C. fulvescens* ranged between 20-926 cells l^{-1} (Average ±S.D; 244.7±295). The maximum abundance of *C. fulvescens* was observed during October (600-926 cells l^{-1}) and in December (313 cells l^{-1}), whereas minimum abundance (20 cells l^{-1}) was observed during February (Fig. 3). Whereas chl *a* values were 50.51 µg l^{-1} to 19.66 µg l^{-1} corresponding with 24.7-30ºC temperature and 35.7 -40 psu salinity respectively.

Table 1. Monthly mean ± S.D. and range values of water parameters during May 2002-July 2003 at Station A.

	(Summer 2002) May-July	(Raining) Aug-Oct	(Winter) Nov-Feb	(Spring) March-April	(Summer 2003) May-July
Temperature (ºC)	(29 ± 1.32) (28-30)	(28 ± 2.6) (25-30)	(24 ± 1.46) (22-25)	(28 ± 1.21) (27-29)	(30 ± 1.66) (29-32)
Salinity (psu)	(38 ± 1.44) (37-40)	(37 ± 1.0) (36-38)	(38 ± 1.26) (37-38)	(37 ± 1.21) (35-38)	(36 ± 0.58) (36-37)
Chlorophyll a (µg l^{-1})	(12 ± 7.61) (4-20)	(16 ± 12.9) (5-31)	(20 ± 3.9) (7-29)	(13 ± 7.42) (7-18)	(12 ± 8.56) (5-21)

Table 2. Monthly mean ± S.D. and range values of water parameters during May 2002-July 2003 at Station B.

	(Summer 2002) May-July	(Raining) Aug-Oct	(Winter) Nov-Feb	(Spring) March-April	(Summer 2003) May-July
Temperature (ºC)	(29 ± 1.2) (28-30)	(27 ± 3) (25-30)	(24 ± 0.63) (24-25)	(28 ± 1.82) (27-29)	(30 ± 1.08) (29-31)
Salinity (psu)	(38 ± 0.32) (37-38)	(38 ± 1.69) (36-39)	(39 ± 0.43) (39-40)	(37 ± 0.53) (37-38)	(37 ± 1.82) (35-38)
Chlorophyll a (µg l^{-1})	(42 ± 53.4) (8-103)	(22 ± 26.0) (3-50)	(12 ± 4.4) (4-19)	(10 ± 11.7) (6-13)	(10 ± 14.3) (4-30)

Table 3. Pearson correlation (r) and linear regression values calculated between water parameters and *C. fulvescens* abundance at St. A and St. B; Significant level p<0.05* and p<0.001**

x axis	y-axis	r	R^2	F-value	T-value	P-value
Station A						
Temperature (ºC)	Abundance	0.59	35	4.86	2.2	0.05**
Salinity (psu)	Abundance	-0.54	29.2	3.72	-1.93	0.086*
Chlorophyll a (µg l^{-1})	Abundance	0.04	0.2	0.02	0.13	0.899**
Station B						
Temperature (ºC)	Abundance	0.73	51.6	8.45	2.92	0.019**
Salinity (psu)	Abundance	-0.64	45.3	6.64	-2.58	0.033*
Chlorophyll a (µg l^{-1})	Abundance	0.92	86	49.08	7.01	0.000**

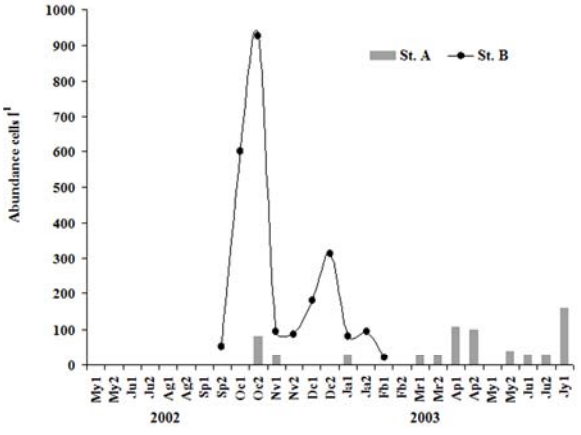
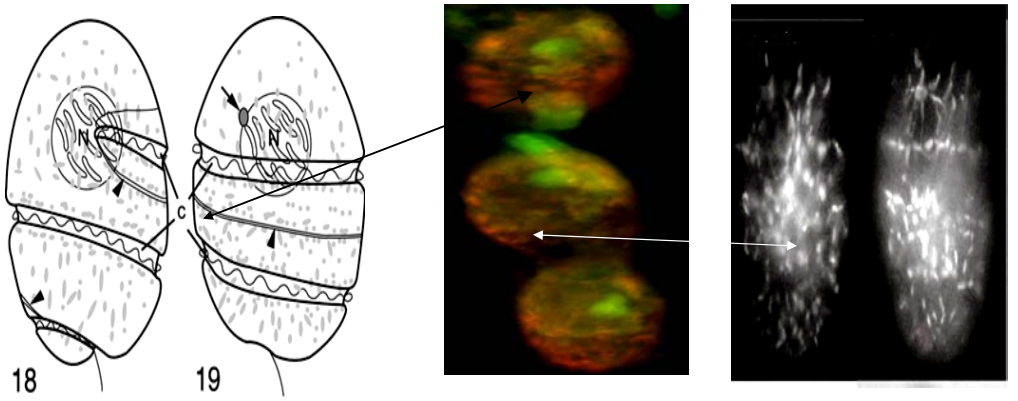


Fig. 3. Seasonal occurrence and abundance cells l^{-1} of *Cochlodinium fulvescens* from both station's St. A (bar) and St. B (line graph).

Discussion

Our data represent the first report on the identification and ecology of *Cochlodinium fulvesens* from northern bordering Arabian Sea. It is a chain forming species and is often difficult to differentiate it from other chain forming species under light microscopy. All the four chain forming *Cochlodinium* species have similar shapes. The scanning electron microscopy and fluorescence microscopy were employed to observe the chloroplast shape, the position of sulcus and cingulum which are masked in Lugol's fixed samples and not readily prominent under light microscopy. Taxonomy of the *Cochlodinium* species is very critical, differentiating features of the four chain forming *Cochlodinium* species are well described on the bases of shape of chloroplast and sizes, torsion of cingulum and sulcus position (Matsuoka *et al*., 2008). *C. fulvescens* and *C. polykrikoides* are closely related species, having

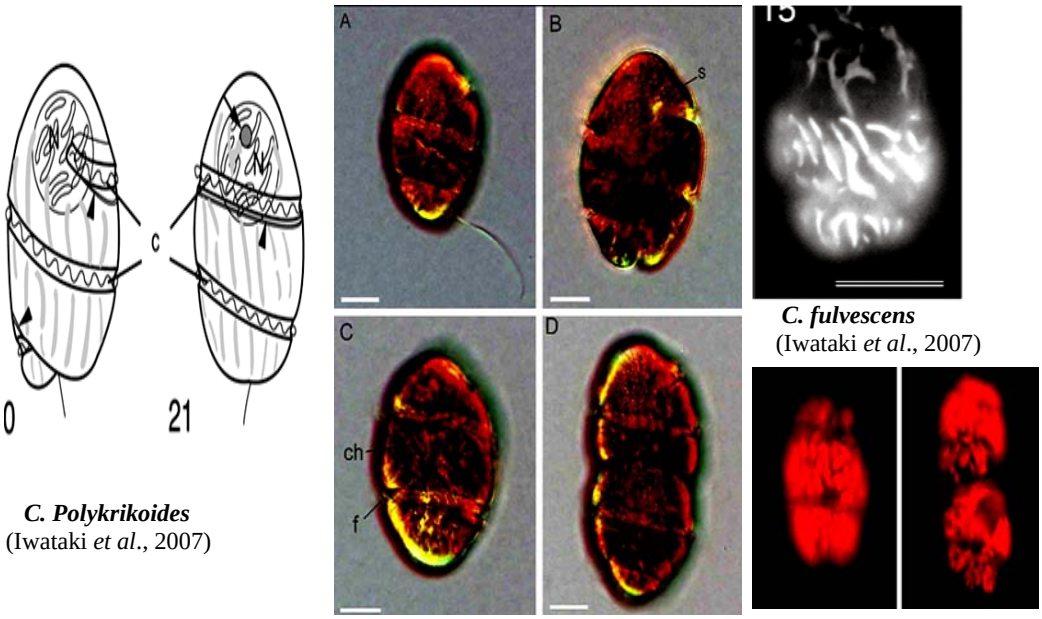
phylogentically sisterhood relationship (Iwataki *et al*., 2007). The only clear morphological character which separates these two species is the shape of chloroplast (Fig. 4). We observed many and granular like chloroplasts which were scattered on the cingulum and periphery of the cell (Fig. 4B) as to newly described species *C. fulvescens* from Asia (Iwataki *et al*., 2007; Matsuoka *et al*., 2008).



C. fulvescens (Iwataki *et al*., 2007)

C. fulvescens (Munir S *et al*.,)

C. fulvescens (Iwataki *et al*., 2007)



C. Polykrikoides
(Iwataki *et al*., 2007)

C. fulvescens
(Iwataki *et al*., 2007)

C. polykrikoidea (Richlen *et al*., 2010)

Fig. 4. Line drawing and fluorescence and light microscopic micrographs showing granular shaped chloroplast between *Cochlodinium fulvescens* (Asia and Pakistan) and longitudinal rod shape *Cochlodinium polykrikoides* (Asia, Gulf of Oman).

In Pakistani waters, maximum abundance of *C. fulvescens* was observed in late spring and early winter season during phytoplankton assemblage at the Mouth of Manora Channel. Maximum cell densities were recorded up to greater than 920 cells l^{-1} at St. B (Mouth of Channel) and less than 160 cells l^{-1} at St. A. (Karachi harbour; Fig. 3). Our findings indicated that *C. fulvescens* is also stenohaline and eurythermal species. Maximum cell densities observed during high temperature at 31-32 ºC and low cell densities found at < 26 ºC from both station's (Fig. 5) which has positive correlation value (r=0.59, r=0.73, t-test, p>0.001) (Table 3) from both stations. Maximum cell densities at low salinity value (<36 psu) and low abundance were observed with high salinity values (>38-40 psu; Fig. 5), which has negative correlation value (r=-0.54, r=-0.64, t-test, p>0.05) from both stations (Table 3). Chl a was most significant factor and high cells densities was observed at maximum chl a (50.5 µg l^{-1}) at St. B but lower value recorded as high cells densities when chl a was low (5 µg l^{-1}) at St. A (Fig. 5), It shows significant correlation values (r= 0.04, r= 0.92; t-test, p>0.001) from both stations (Table 3).

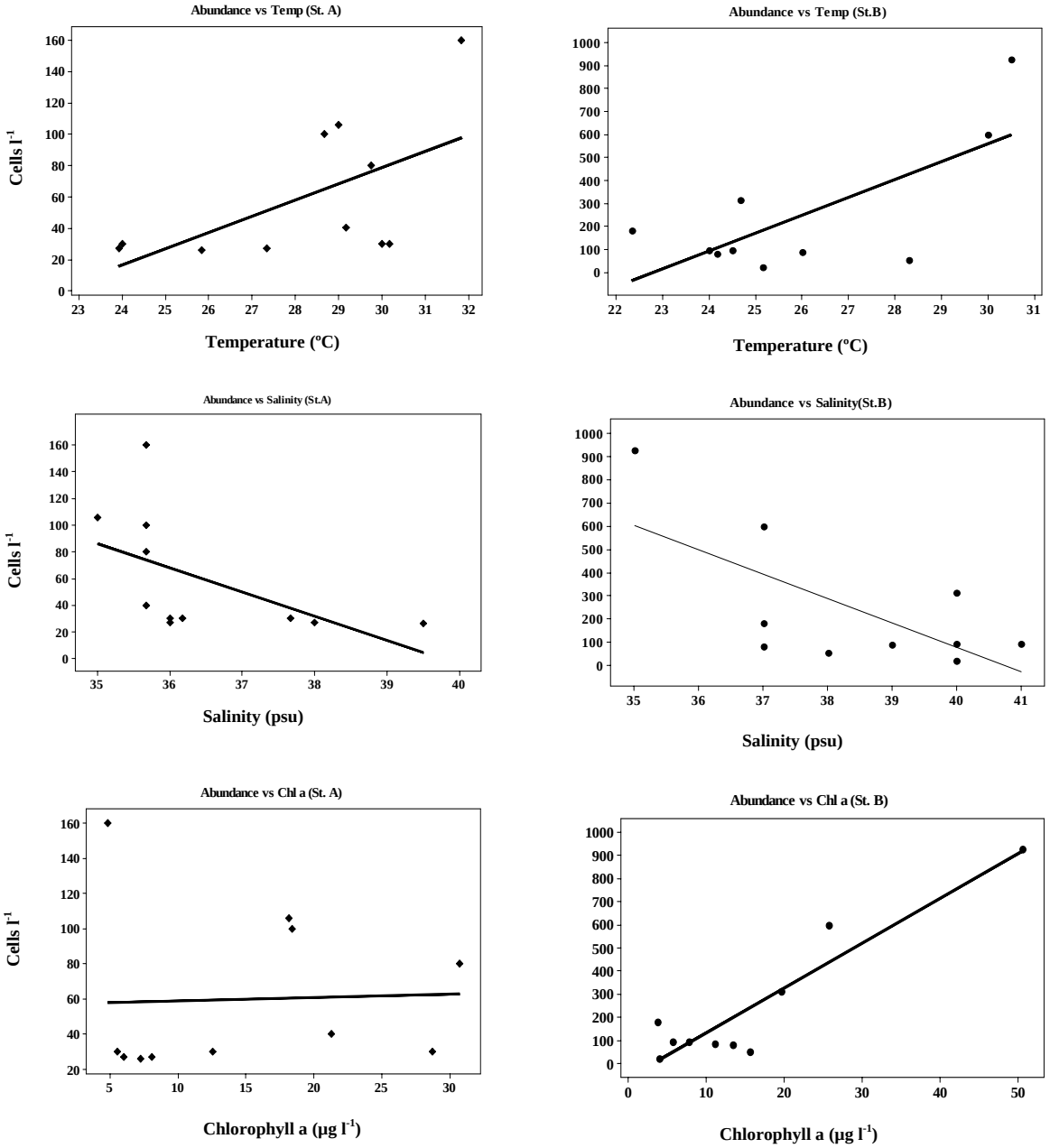


Fig. 5. Relationship between temperature (ºC), salinity (psu), chl a (µg l^{-1}) and abundance of *Cochlodinium fulvescens* (cells l^{-1}) from both stations.

In the Arabian Sea, a recent bloom of *C. polykrikoides* which has been originated at the Port of Dibba Al Hassan, east coast of UAE in Aug 2008, and spread to coasts of Iran and Qatar and reported to have damaged fish farm and coral reefs area (Richlen *et al.*, 2010) and killed tons of fish (Anon., 2008). Only a few harmful algal species of dinoflagellates have been reported to form bloom and fish kills in the northern Arabian Sea bordering Pakistan, for example, *Noctiluca scintillans* forms both green and red tides in Pakistan (Subrahmanyan, 1954; Saifullah & Chaghtai, 1990; Chaghtai & Saifullah, 2006) and *Prorocentrum minimum* (Rabbani *et al*., 1990). However, some toxic species has been reported from the coast of Pakistan (Chaghtai & Saifullah, 2001; Gul & Saifullah, 2010; Munir *et al*., 2011; Gul & Saifullah., 2011) but no record of *C. fulvescens* from northern Arabian Sea is available from previous studies. The dispersion of *Cochlodinium* species throughout the world such as Atlantic Ocean to Mediterranean Sea (Garate-Lizarraga *et al*., 2008), Monetary Bay, Canada (Kudela *et al*., 2008) and GOA, Indian Ocean (Bhatt & Matondkar, 2004), Oman Arabian Gulf (Richlen *et al*., 2010) may be attributed to ballast water. The preliminary baseline data

suggests a need for continuous monitoring of Pakistani coastal waters to indicate possible HAB events to protect the developing fisheries industry of Pakistan.

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