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THE DINOFLAGELLATE GENERA *OXYTOXUM* AND *PYROPHACUS* FROM POLLUTED INSHORE WATERS OF KARACHI, PAKISTAN

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Abstract

The present paper describes the taxonomy, distribution and phenology of five species of *Oxytoxum* and two species of *Pyrophacus* for the first time from two inshore localities of Karachi (Manora channel and Korangi creek) which are highly polluted and surrounded with mangroves. Simultaneous observations on temperature and salinity were taken to see their effect on their occurrence. They occurred preferably in southwest monsoon and fall inter-monsoon seasons characterized by relatively higher water temperatures.

Key words: Dinoflagellates; Taxonomy; Inshore waters; Karachi; North Arabian Sea.

Introduction

Dinoflagellates are one of the major basic components of marine phytoplankton. They are unicellular, flagellated organisms that possess autotrophic and heterotrophic associates (Taylor, 1987; Schnepf & Elbrächter, 1992). They are excellent indicators of water masses and their rate of occurrence is related to temperature and salinity and not to nutrients (Graham, 1942; Wood, 1954).

Oxytoxum Lindemann and *Pyrophacus* Stein belong to order Gonyaulacales and families Oxytoxaceae Lindemann (1928) and Pyrophaceae Lindemann (1928) respectively. Unlike some other genera of dinoflagellates they are not widely distributed throughout the world (Gomez *et al.*, 2008). *Oxytoxum* was one of the most ubiquitous dinoflagellate genera in north Arabian Sea (Taylor, 1976). The genus *Oxytoxum* was first described by Stein (1883) from the Atlantic Ocean. Taylor (1976) kept three genera *Centrodinium*, *Corythodinium* and *Oxytoxum* in family Oxytoxaceae. Morphologically differentiation between the genera *Corythodinium* and *Oxytoxum* are still unresolved. Gomez *et al.*, (2008) described 19 species of *Oxytoum* from southern Pacific Ocean.

Oxytoxum cells are normally longer than broad and biconical to ovoid in shape (Dodge, 1985). It has small epitheca with pointed apices and larger hpotheca. Sulcal lists are without projections and girdles do not have protruding list. The variability of morphology within the genus comes from the apical and antapical terminations of the cells. The outer surface of the thecal plates are also extremely varied in its ornamentation. It has never been reported to produce toxins and bioluminescence (Burns & Mitchell, 1982; Dodge, 1985). *Pyrophacus* is a small lens shaped cell and have equal size rounded epitheca and hypotheca (Dodge, 1982). Balkis & Koray (2001) described three species of *Pyrophacus* from eastern Mediterranean Sea. Gomez (2005) described forty two species of *Oxytoxum* and three species of *Pyrophacus* from the world oceans.

A large amount of work has been carried out on the taxonomy and ecology of *Oxytoxum* and *Pyrophacus* all over the world (Kofoid & Skogsberg, 1928; Wood, 1968; Taylor, 1976; Dodge, 1982; Sournia, 1986; Balech, 1988; Steidinger&Tangen, 1997). *Oxytoxum* and *Pyrophacus* of tropical and subtropical areas in particular have been studied by many workers (Lebour, 1925; Steidinger & Williams, 1970; Wood, 1954; Okolodkov, 1993; Okolodkov & Gárate-Lizárraga, 2006). The North Arabian Sea has, however, attracted the attention of marine biologists only in the recent decades.

Nooruddin (1967) gave a list of dinoflagellates from the Karachi coast but did not provide any taxonomical description. Early investigations were made in the North Arabian Sea mainly by Gul & Saifullah (2007; 2009; 2011) and Saifullah *et al.*, (2008). Recently, Gul & Nawaz (2014) described first reports of 13 species of *Protoperidinium* and *Podolampas* from Pakistan coast. However, a large number of genera and species from the area are yet to be described and studied. The present study attempts to explore the occurrence of remaining species and their taxonomy and distribution from the North Western Arabian Sea shelf of Pakistan during the period January 2006- December 2007.

Materials and Methods

Phytoplankton samples were collected from two inshore areas of Sindh coast, Manora Channel (Karachi harbour) and Korangi creek, Karachi (Lat. 24°, 48' N; Long. 66°, 58.8' E) (Figs. 1a,b). Both localities were polluted and surrounded by mangrove vegetation belonging to *Avicennia marina*. Sampling was carried out fortnightly from January, 2006 to December, 2007 for two consecutive years in these localities. The former locality, also called Karachi Harbour, lies on the western part of the city. It is highly polluted with oil and also with industrial effluents received through the Lyari River. It is approximately 2km long and about 0.5km wide and is surrounded by mangrove vegetation belonging to species *Avicennia marina* (Forssk.) Vierh. Samples were collected from three stations (Fig. 1a). The other coastal site Korangi creek is located about 25Km away from the centre of the city on the eastern side. It forms a part of the complex creek system intervening many mangrove islets and is characterized by pollution of organic wastes especially that from cattle. It is 7km long and 0.6km wide. Sampling was carried out at three stations equidistant from each other (Fig. 1b). All stations were 6-9 meters deep. Seawater temperature

and salinity at surface were measured with a thermometer and refractometer respectively.

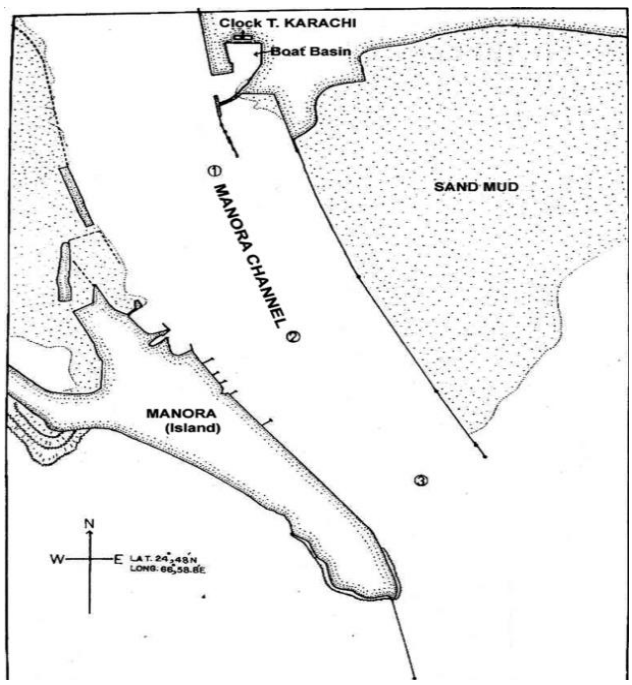


Fig. 1a. Map of Manora channel showing sampling stations 1, 2, 3.

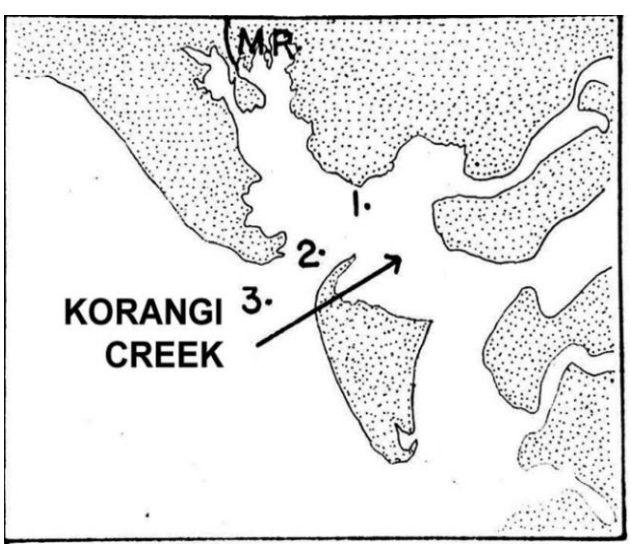


Fig. 1b. Map of Korangi Creek showing sampling stations 1, 2, 3.

The phytoplankton samples were collected by horizontal net hauls of 10 minutes duration using a fine net of mesh size of 40µm. After collection all samples were preserved in 4% formalin. Later phytoplankton material was washed with water and then treated with stains (cotton blue, tryphan blue) for studying the plate patterns. After preparation of slides specimens were studied under a compound microscope. Ocular micrometer was used for measuring the size of the species. Photographs were taken by digital camera and some samples were directly mounted on metallic stubs using duble adhesive tap and coated with gold for five minutes in sputtering chamber and observed under scanning electron microscope SEM (JSM-6380A) for detail identification.

Results

The annual distribution of surface seawater temperature in Manora Channel and Korangi Creek is shown in Fig. 2a,b. It is quite evident that the temperatures in both the localities increased gradually March onwards to high values in summer (SW monsoon season) and in autumn (fall intermonsoon period) and later declining to low values in winter (NE monsoon season). During winter seawater temperature was as low as 20ºC and as high 31ºC in the month of October.

The salinity values of seawater at surface varied between 34.2 psu and 38.0 psu in both localities (Fig. 2a, b). Lower values were recorded during NE monsoon season (winter) and higher values during SW monsoon season (summer) and fall intermonsoon period (autumn).

The following is a taxonomic account of the different species recorded in the areas of study.

Gonyaulacales F.J.R. Taylor, 1980

Oxytoxaceae Lindemann, 1928

***Oxytoxum* Stein, 1883:** Cell elongated, needle-shaped to top-shaped, epitheca narrow and hollow; hypotheca and epitheca often acute with antapical spine; girdle wide and deep.

***Oxytoxum tessellatum* (Stein) Schütt, 1895 Fig. 3**

Schütt (1895), p. 160, pl. 17, fig. 52; Schiller (1937), p.462, figs.526a,b; Wood (1968), p. 62, fig. 163.

Body medium sized; epitheca shorter than hypotheca, conical, low, rounded with spine; hypotheca conical with convex margins, antapex spined; girdle deep, moderately wide; hypotheca surface with rectangular meshes.

Dimensions: Length: 45-65 µm Width: 25-38 µm

General distribution: Sargasso Sea (Hulburt, 1962), Caribbean Sea (Wood, 1968).

***Oxytoxum constrictum* (Stein) Bütschli, 1885 Fig. 4**

Bütschli (1885), p. 1006, pl. 53, fig. 5; Wood (1968), p. 88, fig. 249; Gómez *et al.*, (2008), p. 27, figs. 15-16.

Body small, top shaped; epitheca hemispherical, anteriorly rounded with concave sides; hypotheca hemispherical with margins, constricted near the upper third and with a short, sharp spine; Thecae with numerous longitudinal ridges.

Dimensions: Length: 77µm Width: 42 µm

General distribution: Red sea (Ostenfeld & Schmidt, 1901), Arabian Sea (Cleve, 1903; Schröder, 1906), Indian Ocean (Wood, 1963).

***Oxytoxum parvum* Schiller, 1937 Fig. 5**

Schiller (1937), p. 464, fig. 531; Wood (1968), p. 92, fig. 267; Taylor (1976); p. 127, pl. 24, fig. 239; Gómez *et al.*, (2008), p. 27, figs. 21-22.

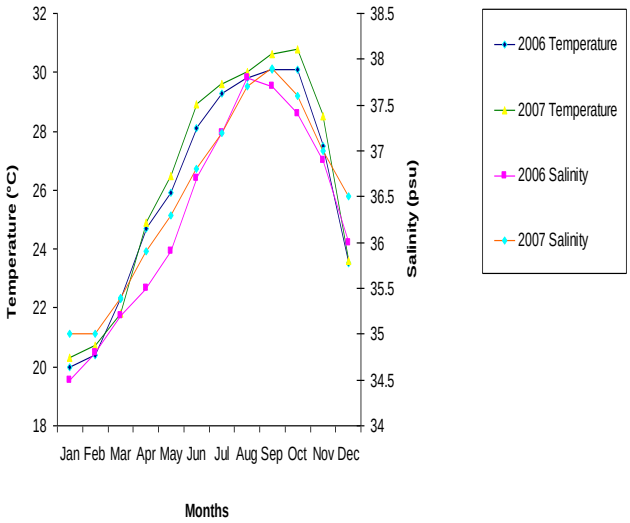


Fig. 2a. Annual variations in temperature and salinity of seawater in Manora Channel during 2006-2007.

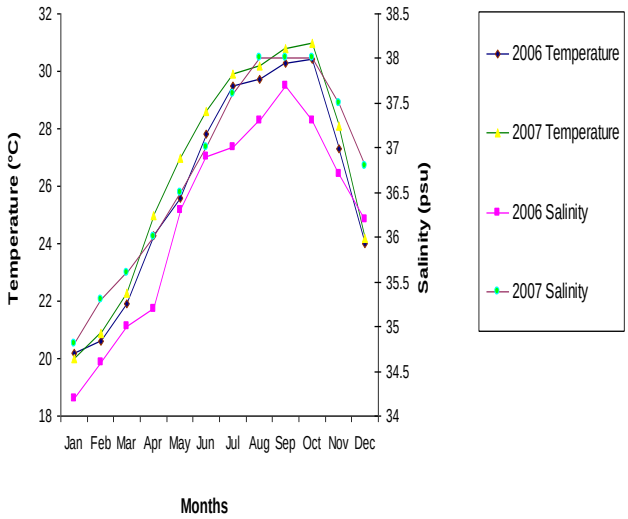


Fig. 2b. Annual variations in temperature and salinity of seawater in Korangi Creek during 2006-2007.

Syn: *O. tenuistriatum* Rampi (1941), p. 63, pl. 2, fig. 14.

Body rotund; epitheca more or less globiform with a conspicucus, acute spine; girdle deep; hypotheca longer than epitheca, swollen below girdle, pointed at base; theca with longitudinal ribs and pores.
Dimensions: Length: 60 µm Width: 21 µm

General distribution: Indian Ocean (wood, 1963).

***Oxytoxum scolopax* Stein, 1883 Fig. 6a,b**

Stein (1883), pl. 5, figs. 1-3; Wood (1968); p. 93, fig. 270; Balech (1971), p. 166, pl. 36, figs. 705-707; Gómez *et al*., (2008), p. 27, figs. 4-5.

Cell elongated epitheca much smaller than hypotheca, broadly conical, apex with pointed spine and base rounded in shape; hypotheca larger with rounded anterior margin and acute posterior, a small spine posteriorly. Thecae with longitudinal rows, intercalary lines after well marked.
Dimensions: Length: 126-135 µm Width: 15-25 µm

General distribution: South Indian ocean, Arabian sea, Red sea (Cleve, 1900; 1901); Red sea (Ostenfeld & Schmidt, 1901); Arabian sea, G. of Aden (Schröder, 1906); New Zealand waters (Burns & Mitchell, 1982).

***Oxytoxum milneri* Murray & Whitting, 1899 Fig. 7**

Murray & Whitting (1899), p. 328, pl. 27, fig. 6; Wood (1963), p. 46, fig. 173.Epitheca short, broadly pointed, then tapering into a long, asymmetrical point; hypotheca deep, conical with convex margins, then forming an acute point; plates areolate with ridged sutures.

Dimensions: Length: 125-130 µm Width: 35 µm

General distribution: Caribbean Sea (Wood, 1968).

Pyrophacaceae Lindemann, 1928

***Pyrophacus* Stein, 1883:** Large sized cell, biconical to lens-shaped, epitheca and hypotheca equal; cingulum narrow and slightly desending; sulcus with few small plates.

***Pyrophacus horologium* Stein emend. Wall & Dale, 1971 Fig. 8a, b**

Stein (1883), p. 24, figs.8-13; Taylor (1976), p. 182, pl. 34, figs. 387, 388, 390; Steidinger & Tangen (1997), p. 523, pl. 5,46; Balkis & Koray (2001), p. 543, fig.2A.

Lens shaped cell; epitheca & hypotheca equal; girdle even; sulcus short, with few small plates, plates marked with linear marking.

Plate formula: 5′, 0a, 9″, 9c, 9‴, 1p, 3⁗.

Dimensions: Length: 35-52 µm

General distribution: Caribbean Sea (Wood, 1968); Indian Ocean (Taylor, 1976); Eastern Mediterranean Sea (Balkis & Koray, 2001).

***Pyrophacus steinii* (Schiller) Wall & Dale, 1971 Fig. 9a,b**

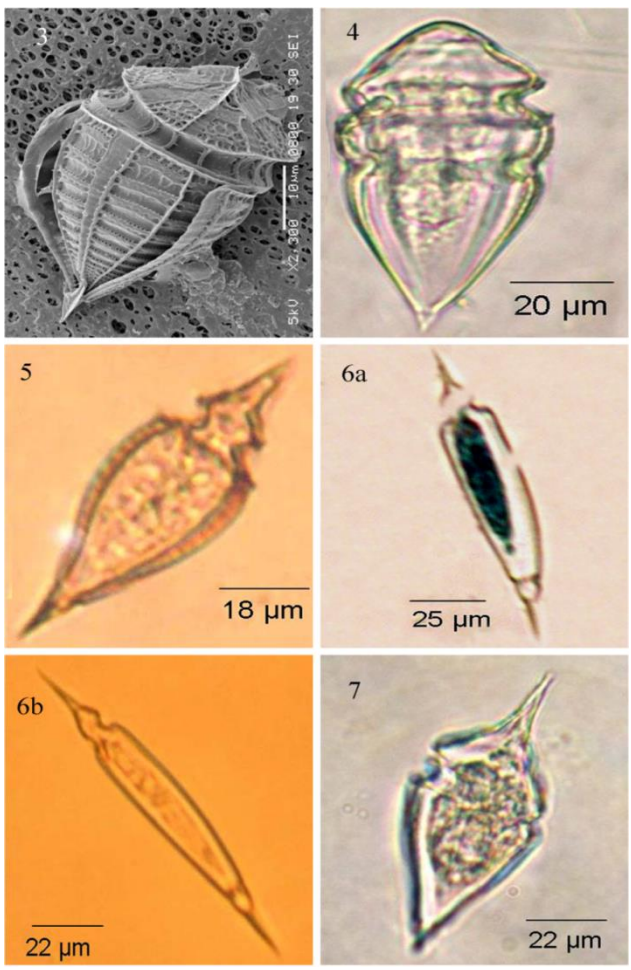
Taylor (1976), p.183, pl. 34, figs. 384, 386, 389; Balech (1988), p.310, figs. 6-9; Steidinger & Tangen (1997), p. 523, pl. 46; Balkis & Koray (2001), p.543, fig.2B.

Cell flattened; lenticular cell with attenuated epitheca; marginal striations on the precingular plates.

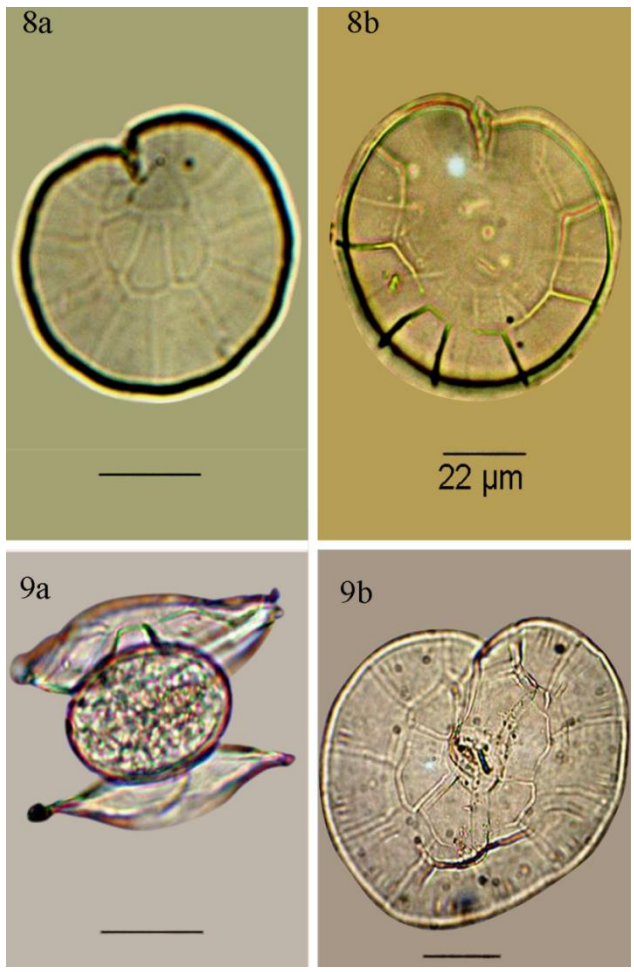
Plate formula: 6-7′, 0a, 11-13″, 11-13‴, 1p, 5⁗.

Dimensions: Length: 30-45 µm

General distribution: Indian Ocean (Taylor, 1976); Eastern Mediterranean Sea (Balkis & Koray, 2001).



Light microscopy (LM) and Scanning microscopy (SEM).
Fig. 3. *Oxytoxum tessellatum*, SEM. Fig. 4. *O. constrictum*, LM. Fig. 5. *O. parvum*, LM. Figs. 6a,b. *O. scolopax*, LM. Fig.7. *O. milneri*, LM.



Light microscopy (LM).
Fig. 8a,b. *Pyrophacus horologium*, LM. Fig. 9a,b. *Pyrophacus steinii* LM. (Scale Bars: Figures 8a, 9ab= 22µm).

Discussion

It is quiet evident that both temperature and salinity showed higher values in surface waters during the southwest monsoon season (Figs. 2a, b). Hassan & Saifullah (1971, 1972) and Saifullah & Hassan (1973) also observed similar situation in their studies in the same areas. The temperatures were high because of larger amounts of solar radiation received during the summer months and the consequent higher salinity values because of increased rate of evaporation during the period. Salinity values showed a wider range of salinity in both localities as compared to the open seawater (Banse, 1968), which may be due to the fact that inshore waters are subject to runoff from the nearby land which in the present case are Lyari and Malir rivers.

Balech (1988) and Sournia (1986) recognized a genus *Corythodinium*; but Dodge and Sunders (1985) synonymized it with *Oxytoxum*. According to Steidinger &Tangen, (1997) both genera have different plate pattern. *Corythodinium* has large pentangular anterior sulcal plate which occupies the epitheca and left handed girdle displacement. The genus *Corythodinium* has also larger epitheca than *Oxytoxum* (Taylor, 1976).

Five species of *Oxytoxum* have been recorded from the areas of study. Gómez (2005) reported forty two species of this genus from the world oceans whereas Burns & Mitchell (1982) described only three species from New Zealand.

Oxytoxum tessellatum (Fig. 3) is a medium sized species which is distributed in warm temprate to tropical waters. It was twice as long as broad. The cingulum showed a displacement of about one cinulum width, deeply excavated, and showed striae at right angle to the margins of the hypotheca (Gómez *et al.*, 2008). At first sight, the main difference between *O. tessellatum* and *O.elegans* was the length of apical spine that is shorter in *O. tessellatum* (Gómez *et al.*, 2008). *O. constrictum* (Fig. 4) is morphologically similar to that described by Wood (1968) and Gómez *et al.*, (2008), while Taylor (1976) did not report it from the Indian Ocean. *Oxytoxum parvum* (Fig. 5) and *O. tenuistriatum* have longitudinal costae as well as finer rows of pores (Taylor, 1976). *O. parvum* and *O. tenuistriatum* differ on the basis of length of apical spines. *Oxytoxum scolopax* (Figs. 6a, b) is very distinctive species. It is elongated in shape with prominent spines on both the apex and antapex and bladder- like swelling at the base of the antapical spine. *O. sceptrum* (Stein) Schröder is not elongated and also posterior swelling is absent (Taylor, 1976). *O. milneri* (Fig. 7) was

characterized by a pointed apex, often long and tapered into asymmetrical point often long. *O. subulatum* also differs with O. *milneri* in having a canula like epitheca, with a truncated tip.

The genus *Pyrophacus* Stein is characterized by a lens shaped cell with narrow cingulum and short sulcus (Balkis & Koray, 2001). It belonged to the family Pyrophaceae Lindemann and only three species of *Pyrophacus* have been recorded from the world's oceans (Gomez, 2005). *Pyrophacus horologium* (Figs. 8a, b) is biconical while *Pyrophacus steinii* (Figs. 9a, b) is flattened in shape (Steidinger & Tangen, 1997). *Pyrophacus horologium* is usually smaller than the other species of the genus with a transdiameter of less than 100 µm and the surface markings consist of densely scattered microgranules. An additional diagnostic feature of *P. steinii* is the presence of marginal striations on the precingular plates in addition to thecal granules and pores (Taylor, 1976).

Species diversity (number of species) was low from December to May but from June to November number of taxa gradullay increased. During NE monsoon and spring inter monsoon seasons three and four species were recorded and peak value of seven taxa were examined in fall inter-monsoon season. Manora Channel (Lower Harbor) was more diverse in number of species than Korangi Creek., which may be due to the fact that it is more polluted and richer in nutrients than the latter (Saifullah *et al.,* 2002; Shoaib *et al.,* 2017). Biodiversity and standing crop of dinoflagellates are both related to nutrient concentrations (Alkawari & Ramaiah, 2010). The higher the concentration of the nutrients the greater the diversity of species and also their abundance. *Oxytoxum* and *Pyrophacus* occurred more frequently on the Manora channel than on Korangi creek (Table 1).

High temperature and low salinity values favor growth of dinoflagellates (Qasim *et al.,* 1972; Joseph & Pillai, 1975; Dodge & Marshall, 1994; Arzu & Gönülol, 2016). Yoo (1991) observed that change in salinity; pH and nutrient concentrations also cause variation in the abundance and composition of dinoflagellates. The temperature range of occurrence of all the species are shown in table 1. It is evident that most species were eurythermal occurring in a range showing a difference of more than 5°C, while few species *Oxytoxum milneri* and *O. tessellatum* were stenothermal occurring in very narrow range (Table 1). As regard the salinity tolerance it appears that all the species were stenohaline.

Table 1. Distribution of species at different ranges of temperature, salinity and in different seasons and areas.

No.	Name of species	Ranges		Seasonal occurrence		Southwest monsoon (Jun-Aug)	Fall inter monsoon (Sept-Nov)	Different areas	
		Temperature (°C)	Salinity (psu)	N-E Monsoon (Dec-Feb)	Spring inter monsoon (Mar-May)			Manora Channel	Korangi Creek
1.	*Oxytoxum constrictum*	21.36-29.06	36.61-36.65	+	+	+	+	+++	++
2.	*Oxytoxum milneri*	24.50-26.02	36.37-36.48	+	+	+	+	++	++
3.	*Oxytoxum parvum*	21.36-29.06	36.61-36.65	-	-	-	+	++	++
4.	*Oxytoxums colopax*	24.01-29.00	36.25-36.58	+	+	+	+	++	-
5.	*Oxytoxum tessellatum*	24.46-27.58	36.30-36.56	-	-	+	+	+	-
6.	*Pyrophacushorologium*	23.44-27.76	35.44-36.60	-	+	+	+	++	+
7.	*Pyrophacussteinii*	22.90-27.30	36.31-36.40	-	-	+	+	++	+

+ Present, - Absent, +++ Very common, ++ Common

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