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SEASONAL ABUNDANCE, BIOVOLUME AND GROWTH RATE OF THE HETEROTROPHIC DINOFLAGELLATE (*NOCTILUCA SCINTILLANS*) FROM COASTAL WATERS OF PAKISTAN

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Abstract

The seasonal variation of *Noctiluca scintillans* was investigated from coastal areas of Karachi (Manora Channel), Pakistan. Total cell abundance ranged between < 100 cells l^{-1} to 1340 cells l^{-1} during the sampling period May 2002-July 2003. High cells densities were recorded during May 2003. No green or red water discolouration was observed. Cell size biovolume ranging between 100 µm-600 µm was used to convert to carbon biomass 4.5-6.8 pg C $cell^{-1}$. Greatest *Insitu* growth rate of *N. scintillans* (µ day^{-1}) during 2006 was calculated at 0.82 µ day^{-1}.

Introduction

Noctiluca scintillans is non-photosynthetic, non-toxic and bioluminescent species of dinoflagellates. This species commonly forms blooms in coastal and eutrophic areas of the Arabian Sea including Oman (Al-Zari *et al*., 2007), Saudia Arabia (Mohamed & Mesaad, 2007), Pakistan (Saifullah & Chaghtai, 1990; Chaghtai & Saifullah, 2006), India (Venugopal *et al*., 1979; Devassy & Nair, 1987; Sargunam *et al*., 1989; Sahayak *et al*., 2005) and from Indo-Pacific Ocean e.g. Hong Kong (Morton & Twentymaan, 1971) and from Mediterranean Sea, Northern Adriatic Sea (Umani *et al*., 2004) and Australia (Dela-Cruz *et al*., 2002)

N. scintillans forms both green and red coloured tidal streaks along the continental shelf (Saifullah & Chaghtai, 1990) and coastal areas of Pakistan (Chaghtai & Saifullah, 2006). *N. scintillans* is also associated with fish kills due to increases of ammonia level in the seawater by excess of its high cell concentration caused for saphocation (Elbrachter & Qi, 1998; Okaichi & Nishio, 1976). The organic matter and mangrove vegetation along the Karachi coast was a reason for five year blooms of *Noctiluca* in Pakistani waters (Chaghtai & Saifullah, 2006). Only Morton & Twentymaan (1971) have published a report on potential toxin production in strain of *N. scintillans* from Japan. However the production of any toxic compound has not been confirmed. The *Cochlodinium fulvescence* is another non-toxic dinoflagellates which has been recently reported from the Manora Channel and could be possible of future brown tide events (Munir *et al*., 2013).

Water currents and winds prevail the transportation of such large plankton species such as *Noctiluca scintillans.* These coastal forcings can help in transportation of these algae to large extend and some symbionant toxic algae attached to the body of non toxic *Noctiluca* are the reason to distribute the toxins into trophic food web (Chaghtai & Saifullah, 2006, Escalera *et al*., 2007). Such type of symbionant toxic algae e.g. *Pedinomona* are also seen in strains of *Noctiluca* species of Pakistani waters by Chaghtai & Saifullah (2006).

Noctiluca is an important species of Pakistani waters with respect to blooms but its seasonal cycle, temporal variation in abundance and growth rate from the coast of Pakistan are still questionable. This paper addresses the abundance, distribution and carbon-biomass and oceanographic conditions favorable for *Noctiluca* along the Pakistani coast.

Materials and Methods

Sampling area and procedure: Samples were collected from Manora Channel, Karachi coast of Pakistan during the phytoplankton survey 2002-2003 from two stations; Stn. A (24˚49'46"N/66˚57'.55"E) and Stn. B (24˚47'.51"N/66˚58'.52"E). During sampling period, the temperature and dissolve oxygen recorded for correlation to the abundance of *N. scintillans* cells. Samples were fixed with Lugol's solution and settled into cylindrical chamber (50 mL) according to Utermöhl technique for 24 hr. The settled chamber plate was examined under the light microscopy at 20x magnification, and cell count and sizing of *Noctiluca* measured. Cell count was converted into liters value and sizes were also measured to calculate the biovolume (μm^3) and surface area by (Hillebrand *et al*., 1999) and carbon biomass derived by formula (Eppley *et al*., 1970). Biovolume was calculated by $V = Pi/6 * d^3$ for sphere shape dinoflagellates.

During the survey of Feb 2006, an experiment was conducted to measure the growth rate by cell changes in seawaters from two station's Stn. A and Stn. B. This experiment was designed for one day and analyzed the cell division per day with natural communities during light-dark cycle for 24 hr. Three measuring protocol was followed: 1) unfractionated water (Control), 2) fractionated water through 150 µm, and 3) fractionated water through 10 µm at interval of 24 hr. The water temperature and salinity was recorded >24ºC and >37 psu at time t0 and >25ºC-40 psu at time t24 from both sites (Stn. A & Stn. B).

Results and Discussion

During May 2002-July 2003, *Noctiluca scintillans* (Fig. 1) was observed with maximum cell densities was >180-1340 cells l^{-1}. Monthly mean temperature ranged between >20-31ºC and DO was ranged into 1.9-5.1 mg l^{-1} at Stn. A and 3.0-5.3 mg l^{-1} at Stn. B (Table 1). Low abundance occurred at Stn. A than Stn. B. The cell concentration (cells l^{-1}) ranged as (20-180 cells l^{-1}) with mean ± S.D (42 ± 55) at Stn. A and (80-1340 cells l^{-1}) with mean ± S.D (210±335) at Stn. B (Table 1).

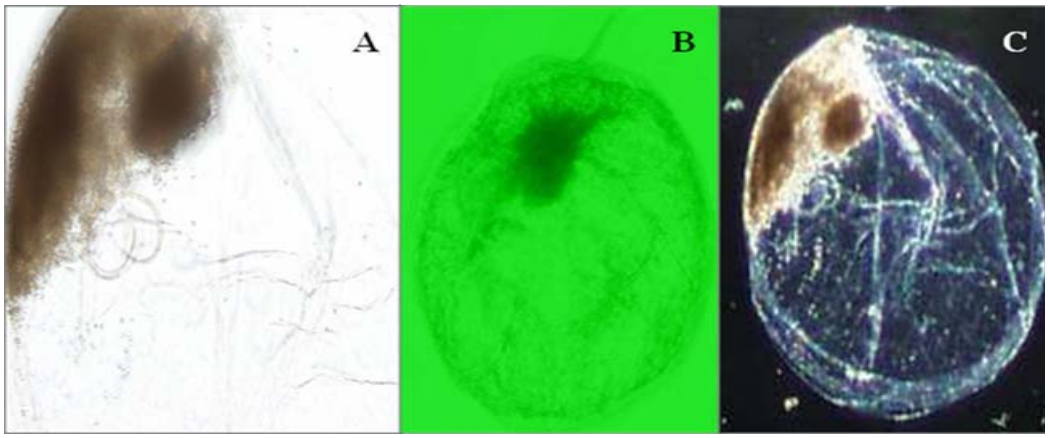

Fig. 1. A-C. Light microscopy of *Noctiluca scintillans* from Karachi coast of Pakistan.

Table 1. Seasonal variation in abundance, distribution of *N. scintillans* and environmental conditions (Temperature and DO) from Manora Channel.

Months	St. A (cells l^{-1})	Temperature (°C)	DO mg l^{-1}	St. B (cells l^{-1})	Temperature (°C)	DO mg l^{-1}
May	80	29	2.6	180	29	3.8
Jun	0	30	3.7	0	30	3.5
Jul	120	28	4.5	140	27	4.9
Aug	180	25	3.1	160	25	3.7
Sep	80	29	1.9	80	26	3
Oct	0	30	2.8	0	30	3.4
Nov	0	25	1.9	400	25	3.3
Dec	0	22	2.1	0	23	3.6
Jan	80	24	2.9	360	24	4.7
Feb	80	25	2.4	420	24	4.2
Mar	0	27	3.7	0	27	5.3
Apr	0	29	2.4	0	29	4.1
May	0	29	2.9	1340	29	3
Jun	0	30	3.9	0	30	4.8
Jul	20	32	5.1	80	31	5.2

At Station A, low cell abundance was observed during Jul 2003 when temperature was 32°C and DO was 5.1 mg l^{-1}, and maximum values were observed during Jul 2002 when temperature was 28°C and DO was 4.5 mg l^{-1} (Table 1). The low cell abundance of *Noctiluca* at Stn. B observed in July 2003 when temperature was 31°C and DO recorded as 5.2 mg l^{-1} and maximum abundance was observed in May 2003, when temperature was 27°C and DO was 3.0 mg l^{-1} at Stn. B. It is very important that *N. scintillans* is a typical temperate water species, which initiates the blooms at lower temperature typically found in the Indian Ocean including the south west coast of India (Devassy *et al*., 1978; Naqvi *et al*., 1998), Gulf of Oman (Thangaraja *et al*., 2000; Al-Azri *et al*., 2007; Al Busaidi *et al*., 2008) and Pacific Ocean (Morton & Twentymaan, 1971; Liu & Wing, 2006), Japan (Nakamura, 1998; Tada *et al*., 2004) and Mediterranean Sea (Umani *et al*., 1983). In this study, highest *Noctiluca* densities were observed during periods of lower temperatures (Table 1). Green tide form *N. scintillans* was observed continuously during five years 1999-2004 at both coasts of the Karachi and Balochistan Pakistan with maximum cell densities reaching bloom level (about 2.4 x 10^6 cells l^{-1}) and 2.3 x 10^3 (500 µm) cells during periods of low temperature (19-22ºC; Saifullah & Chaghtai, 2006). Our result indicated that temperature was never below 22ºC and abundance of *Noctiluca* was not high as reported during blooms period (1999-2004) from Pakistan (Saifullah & Chaghtai, 2006) (Table 3). At the high cell concentration during May 2003, the heterotrophic *N. scintillans* was dominant dinoflagellate species in comparison with the autotrophic species (*Ceraitum furca, C. lineatum, Prorocentrum micans, Peridinium stenii* and *Dinophysis caudata*). The autotrophic species were observed in abundances of < 200 cells l^{-1}.

Carbon biomass of *Noctiluca* is important in temperate area, to being heterotrophic depending upon its starved status and food content of prey. Generally, biovolume by cell size is depending upon the volume of the cell produces carbon (Sun *et al*., 2000; Sun & Liu, 2003). To calculate the biovolume and surface area of *N. scintillans*, about 25-30 cells were measured in the range

of 50- 600 µm (Table 2). Small sizes 125-180 µm (mean ± S.D., 157 ± 25.3) dominated during the summer period Jul 2002 and 200-275 µm (mean ± S.D., 241 ± 38.2) in Jul 2003 at Stn. A whereas 50-100 µm (Mean ± S.D., 77.0 ± 21.0) was dominated during early spring season (Aug) at Stn. B. Large cells size 450-600 µm (mean ± S.D., 525 ± 75) dominated during winter season Feb at Stn. A and summer May 2003 at Stn. B. At station A, biovolume and surface area of small cells (1, 338, 037 µm³ ± 67484) converted into carbon biomass (5.80 pg C cell^{-l}) and maximum biovolume and surface area for a diameter of 450-600 µm recorded as (9, 0595, 951 µm³ ± 931697) converted into (6.88 pg C cell^{-l}) during Feb (Table 2). At Station B, biovolume and surface area of small size (2, 698, 43 µm³ + 18971) converted into (4.50 pg C cell^{-l}) in Aug and maximum biovolume and surface of large cell size 450-600 µm (8, 1249, 413 µm³ ± 892525) converted into 6. 83 pg C recorded in May 2003 (Table 2). These carbon values was much higher as compare to diatom community e.g., *Coscinodiscus radiatus*, *Planktoneilla sol* and *Guinardia flaccida* has been reported with maximum carbon value 3.93 pg C cell^{-l} (Naz *et al*., 2013).

Table 2. Diameter (µm), mean cell-biovolume (µm³) and surface area mean per carbon (Pg C cell^{-l}**) of *N. scintillan's* during May 2002-July 2003 from St. A and St. B.**

Study period	Station A sizes range (Mean ± S.D.)	(Mean biovolume µm³ ± surface area)	Per cell carbon (Pg C cell^{-l})	Station B sizes range (Mean ± S.D.)	(Mean biovolume µm³ ± surface area)	Per cell carbon (Pg C cell^{-l})
May	300-500µm (421 ± 78.7)	(36110000 ± 515483)	(6.50)	100-275µm (181 ± 74.4)	(3080035 + 94854)	(5.49)
Jun	-	-	-	-	-	-
Jul	125-180µm (157 ± 25.3)	(1338037 ± 67484)	(5.16)	100-275µm (216 ± 52)	(5958368 + 153075)	(5.76)
Aug	-	-	-	50-100 µm (77.0 ± 21.0)	(269843+18971)	(4.50)
Sep	425-500 µm (456.2 ± 32.5)	(52341510 ± 674119)	(6.66)	275-425 µm (350 ± 106)	(33485156 + 47296)	(6.47)
Oct	-	-	-			
Nov	-	-	-	350-475µm (425 ± 66.1)	(42071094 + 57632)	(6.56)
Dec	-	-	-	-	-	-
Jan	470-525 µm (501 ± 28.4)	(74180456 ± 849272)	(6.80)	400-500 µm (431 ± 46.6)	(43111955+58908)	(6.57)
Feb	450-600 µm (525 ± 75)	(90595951 ± 931697)	(6.88)	335 µm	(19674913 + 35238)	(6. 25)
Mar	-	-	-	-	-	-
Apr	-	-	-	-	-	-
May	-	-	-	450-600 µm (528 ± 78.9)	(81249413 ± 89252)	(6.83)
Jun	-	-	-	-	-	-
Jul	200-275 µm (241 ± 38.2)	(6402656 ± 162888)	(5.80)	150-300 µm (240 ± 55.7)	(7749149 ± 186438)	(5.87)

Table 3. Occurrence and abundance of *Noctiluca scintillans* and water temperature from different areas of the world.

Location	Cells densities	Temperature	References
Mediterranean Sea			
Northern Adriatic Sea	0^6 ind m^{-3}	< 6. 5 ºC	Umani *et al*., 2004
Northern Pacific Ocean			
Alaska (Ice off station)	----	3ºC	Tibbs, 1997
German Bight	10^4-10^5 cells l^{-1}	< 5 ºC	
Indo pacific Ocean			
Jangmak Bay, Japan	100 cells l^{-1}	< 25 ºC	Nakammra, 1988
Tolo Harbor, Japan	10^5 cells l^{-1}	---	Liu & Wang, 2006
Northern Arabian Sea Pakistan			
Clifton beach Karachi	10^4 cells l^{-1}	19ºC	Saifullah & Chaghtai 2006
Korangi Creek, Karachi	10^4 -10^6 cells l^{-1}	20 ºC	Saifullah & Chaghtai 2006
Sandspit, Karachi	10^6 cells l^{-1}	19 ºC	Saifullah & Chaghtai 2006
Karachi harbor, Karachi	10^3 cells l^{-1}	18 ºC	Saifullah & Chaghtai 2006
Miani Hor, Balochistan	10^3 cells l^{-1}	20-22 ºC	Saifullah & Chaghtai 2006
Churna Island, Balochistan	10^3 cells l^{-1}	21 ºC	Saifullah & Chaghtai 2006
Mouth of Manora Channel	1340 cells l^{-1}	29 ºC	This study

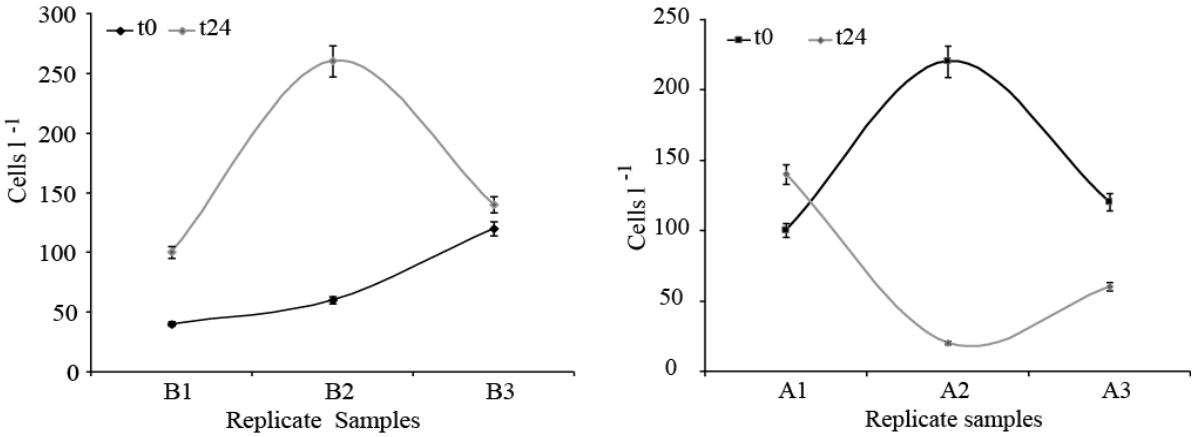

Fig. 2. Cell changes in replicate samples at time to and fixed time t24 from both stations.

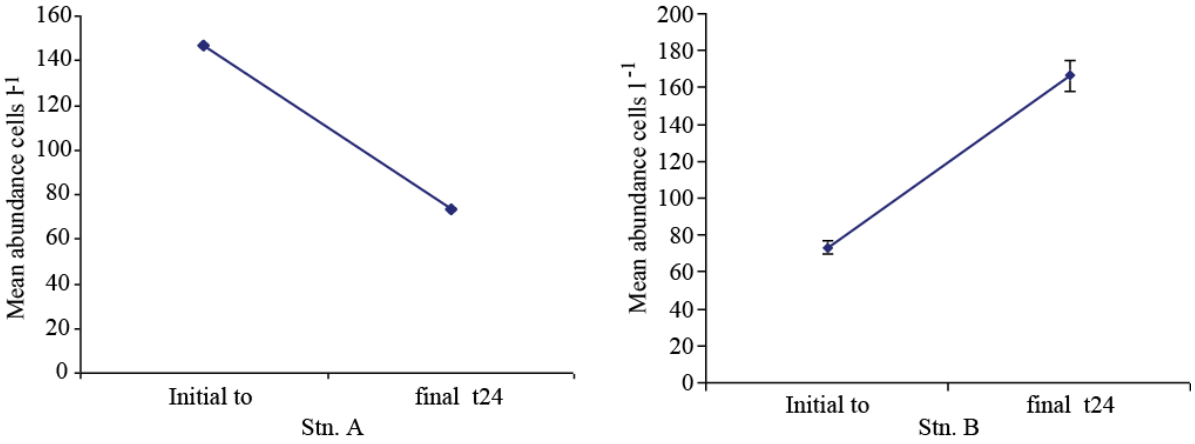

Fig. 3. Average cell densities of *Noctiluca scintillans* at time to and t24 during Feb. 2006.

During 2006, cell density increased after 24 hr from 100 cells l^{-1} to 120 cells l^{-1} (average 146) in replicate samples at time t0 and 20-140 cells l^{-1} (Average 73 cells l^{-1}) at time t24 from Stn. A (Fig. 2). Total cell numbers varied from 40 cells l^{-1} to 120 cells l^{-1} (average 73) in replicate samples at time t0 and 100-260 cells l^{-1} (Average 166 cells l^{-1}) at time t24 from Stn. B (Fig. 2). Specific growth rate was negative as -0.69 µ day^{-1} at Stn. A and positive as 0.82 µ day^{-1} at Stn. B (Fig. 3). No cells were observed from the fractionated samples 150 µm and 10 µm (data not shown), supposed to be zero growth rates as measured. Absence of *Noctiluca* cells from fractionated samples indicating the positive growth for >150 µm and >10 µm sizes phytoplankton's (diatom, dinoflagellates). In temperate waters, growth rate of *N. scintillans* was obtained at maximum as 0.07 d^{-1} and 0.40 d^{-1} (Nakamura, 1998a; Buskey, 1995) and high values 0.66 d^{-1} measured from Japan (Tada *et al*., 2004). High growth of *Noctiluca scintillans* during this study indicating that dynamics of bloom condition of *N. scintillans* is more suitable at the low turbid waters of Karachi harbour.

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References

Al-Azri, A., K. Al-Hashmi, J. Goes, H. Gomes, H. Al-Habsi, S. Al-Khusaibi, R. Al-Kindi and N. Al-Azri. 2007. Seasonality of the bloom-forming dinoflagellate *Noctiluca scintillans* in the Gulf of Oman in relation to environmental conditions. STAFO meeting, Kuwait.

Al Busaidi, S.S., K.M. Al Rashdi, H.M. Al Gheilani and S. Amer. 2008. Hydrographical Observations during a red tide with fish mortalities at Masirah Island, Oman. *Agri. Mar. Sci*., 13: 63-72.

Buskey, E.J. 1995. Growth and bioluminescence of *Noctiluca scintillans* on varying algal diets. *J. Plank. Res*., 17: 29-40.

Chaghtai, F and S.M. Saifullah, 2006. On the occurrence of green *Noctiluca Scintillans* blooms in the coastal waters of Pakistan, Northern Arabian Sea. *Pak. J. Bot*., 38(3): 893-898.

Dela-Cruz, J., P. Ajani, R. Lee, T. Pritchard and I. Suthers. 2002. Temporal abundance patterns of the red tide dinoflagellate, *Noctiluca scintillans* along the South-east coast of Australia. *Mar. Ecol. Prog. Ser*., 236: 75-88.

Devassy, V.P. and S.R.S. Nair. 1987. Discolouration of water and its effect on fisheries along the Goa coast Mahasagar. *Bullet. Nat. Inst. Oceano*., 20: 121-128.

Eppley, R.W., F.M.H. Reid and J.D.H. Strickland. 1970. Estimates of phytoplankton crop size, growth rate and primary production. *Bull. Scripps Inst. Oceanogr*., 17: 33-42.

Elbrachter, M. and Y.Z. Qi. 1998. Aspects of *Noctiluca* (Dinophyceae) population dynamics, *In*: Physiological ecology of harmful algal blooms. (Eds.): D.M. Anderson, A.D. Cambella and G.M. Hallegraeff. Springer, London, 662.

Escalera, L., Y. Pazos, A. Morono and B. Reguera. 2007. *Noctiluca scintillans* may act as a vector of toxigenic microalgae. *Harmful Algae*., 6 3): 317-320.

Hillebrand, H., C.D. Durslen, D. Kirschtel, U. Pollinger and T. Zohary. 1999. Biovolume calculation for the pelagic and benthic microalgae. *J. Phycol*., 35: 403-424.

Liu, X.J. and C.K. Wong. 2006. Seasonal and spatial dynamics of *Noctiluca scintillans* in a semi-enclosed bay in the northeastern part of Hong Kong. *Bot. Mar*., 49: 145-150.

Morton, B. and R.P. Twentyman. 1971. The occurrence and toxicity of a red tide caused by *Noctiluca scintillans* (Macartney) Ehrenb, in the coastal waters of Hong Kong. *Envir. Res*., 4: 544-557.

Mohamed, Z.A. and I. Messad. 2007. First report on *Noctiluca scintillan* blooms in the Red Sea off the coasts of Saudi Arabia: consequences of eutrophication. *Oceanologia*., 49(3): 337-351.

Munir, S., T. Naz, Z. Burhan, P.J.A. Siddiqui and S.L. Morton. 2013. First report of the athecate, chain forming dinoflagellate *Cochlodinium fulvescens* (Gymnodiniales) from Pakistan. *Pak. J. Bot*., 44(6): 2129-2134.

Nakamura, Y. 1998. Biomass, feeding and production of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *J. Plank. Res*., 20(11): 2213-2222.

Naz, T., Z. Burhan, S. Munir and P.J.A. Siddiqui. 2013. Biovolume and Biomass of common diatom species from the coastal waters of Karachi, Pakistan. *Pak. J. Bot*., 45(1): 325-328.

Okaichi, T. and S. Nishio. 1976. Identification of ammonia as the toxic principle of red tide of *Noctiluca miliaris*. *Bull. Jpn. Soc. Sci. Fish*., 23: 25-30.

Sargunam, C.A., V.N.R. Rao and K.V. K. Nair. 1989. Occurrence of *Noctiluca* bloom in Kalpakkam coastal waters, East coast of India. *Ind. J. Mar. Sci*., 18(4): 289-290.

Saifullah, S.M. and F. Chaghtai. 1990. Incidence of *Noctiluca scintillans* (Macartney) Ehrenb., blooms along Pakistan's shelf. *Pak. J. Bot*., 22: 94-99.

Sun, J., D. Liu and S. Qian. 2000. Estimating biomass of phytoplankton in Jiaozhou Bay 1. Phytoplankton biomass estimated from cellvolume and plasma volume, *Act. Ocean. Sini*., 19(2): 97-110.

Sun, J. and D.Y. Liu. 2003. Geometric models for calculating cell biovolume and surface area of phytoplankton, *J. Plank. Res*., 25: 1331-1346.

Sahayak, S., R. Jyothibabu, K.J. Jayalakshmi, H. Habeebrehman, P. Sabu, M.P. Prabhakaran, P. Jasmine, P. Shaiju, G. Rejomon, J. Thresiamma and K.K.C. Nair. 2005. Red tide of *Noctiluca milliaris* off south of Thiruvananthapuram subsequent to the 'stench event' at the southern Kerala coast. *Curr. Sci. Ind.*, 89(9): 1472-1473.

Tibbs, J.F. 1967. *On some planktonic Protozoa taken from the track of drift station.* ARLIS, 1960-61. Arctic, 20: 247-54.

Thangraja, M., A. Al-Aisri and H. Al-Shaqsi. 2000. *Phytoplankton Blooms, Red Tide Phenomena and Recorded Impacts in Oman*. Marine Science and Fisheries Center Annual Report, Ministry of Agriculture and Fisheries Sultanate of Oman. pp. 22.

Tada, K., S. Pithakpol and S. Montani. 2004. Seasonal variation in the abundance of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *Plank. Biol. Ecol*., 51(1): 7-14.

Umani, S.F., A. Beran, S. Parlato, D. Virgillo, T. Zollet, A. De Olozabal, B. Lazzarini and M. Cabrini. 2004. *Noctiluca scintillans* Macartney in the Northern Adriatic Sea: long-term, dynamics, relationships with temperature and eutrophication, and role in the food web. *J. Plank. Res*., 26(5): 545-561.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitative Phytoplankton-Methodik. *Ass. Intern. Limnol. Théor*., 9: 1-38.

Venugopal, P., P. Haridas, M. Madhu-Pratap and T.S.S. Rao. 1979. Incidence of red water along south Karala coast. *Indian .J. Mar. Sci*., 8: 94-97.

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