POPULATION DYNAMICS OF *PSEUDO-NITZSCHIA* SPECIES (BACILLARIOPHYCEAE) IN THE NEAR SHORE WATERS OF DAR ES SALAAM, TANZANIA

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ABSTRACT

The genus Pseudo-nitzschia is a chain-forming diatom comprising about 30 species some of which are known to produce domoic acid (DA) that causes amnesic shellfish poisoning (ASP). The current study aimed at assessing the population dynamics of Pseudo-nitzschia in the near shore waters of Dar es Salaam. Samples were collected between August 2008 and July 2009 from two stations. The first station was located in the open waters off Mbudya Island and the second was located at a point in-between Kunduchi and Mbudya Island. Three Pseudo-nitzschia species, i.e., Pseudo-nitzschia pungens, Pseudo-nitzschia seriata and Pseudo-nitzschia cuspidata were encountered with Pseudo-nitzschia pungens being the most abundant. The concentration of Pseudo-nitzschia species ranged from none in various samples to a highest value of 16 cells/L recorded in September 2008. There was no significant difference in the abundance of Pseudo-nitzschia spp. between site 1 and 2 and between the northeast (NE) and southeast (SE) monsoon periods. The abundance of Pseudo-nitzschia spp. was below concentrations reported elsewhere to cause problems to shellfish consumers implying that shellfish collected near Dar es Salaam may be ASP free. However, further studies are required to ascertain DA production in coastal waters of Tanzania.

Key words: *Pseudo-nitzschia* dynamics, amnesic shellfish poisoning, Tanzania.

INTRODUCTION

The genus *Pseudo-nitzschia* H. Peragallo 1899 (Class Bacillariophyceae) is one of the most common diatom genera among marine phytoplankton covering a wide range of salinity and temperature in the coastal and oceanic waters; in polar, temperate, subtropical and tropical areas (e.g. Hasle 1972, Kaczmarska *et al*. 1986). It is a chain-forming diatom comprising about 30 species of which 12 are known to produce toxins (Bates 2000, Bates and Trainer 2006, Lundholm *et al*. 2006). Potentially toxic species of *Pseudo-nitzschia* produce domoic acid (DA), which causes amnesic shellfish poisoning (ASP) (e.g., Bates 1998, Bates *et al*. 1998). Studies show that DA production by *Pseudo-nitzschia* is during the stationary growth phase though *P. australis* constitute an important exception as DA production in this species may begin during the exponential growth phase (see e.g., Bates 1998 and references therein).

Blooms dynamics of *Pseudo-nitzschia* species have been related to several environmental variables such as high nutrient concentrations, water temperature and grazing by zooplankton (Parsons *et al*. 2002, Lundholm *et al*. 2004, Huang *et al*. 2009). For example, Huang *et al.* (2009) reported that grazing by zooplankton was probably the most important factor controlling *Pseudo-nitzschia pungens* bloom dynamics in Zhelin Bay, China. It is thus important to precisely identify the conditions that promote the development of *Pseudo-nitzschia* blooms in order to minimize the possible public health and economical effects in our coastal waters.

Occurrence of *Pseudo-nitzschia* spp. in the coastal waters of the Western Indian Ocean has been reported before (Bryceson 1977, Lugomela and Semesi 1996). A survey of potentially harmful marine microalgae in the western Indian Ocean also identified *Pseudo-nitzschia pungens* in Kilifi, Kenya, and *Pseudo-nitzschia* cf. *cuspidata* in St. Paul Bay, Reunion (Hansen *et al*. 2001). Thus, the presence of *Pseudo-nitzschia* spp. in the region poses a threat to human health and to the developing aquaculture industry should a bloom occur. Nevertheless, there are no reports on incidences of amnesic shellfish poisoning in the Western Indian Ocean.

There is however no study, which has analyzed the seasonal distribution of *Pseudo-nitzschia* species along the Tanzanian coastal waters as well as factors regulating such distribution. Such information is useful as baseline for future studies and monitoring of environmental changes in coastal ecosystems as well as assessing potential risks to human health and aquaculture industry in the country. Thus, the current study aimed at assessing the abundance and temporal distribution of *Pseudo-nitzschia* spp. in relation to environmental parameters in coastal waters near Dar es Salaam, Tanzania.

MATERIAL AND METHODS

Samples for *Pseudo-nitzschia* identification and quantification were collected twice per month for the period of one year (August 2008 to July 2009) at two locations in the near shore waters off Kunduchi, Dar es Salaam. The first sampling station was located in the open waters off Mbudya Island (06^{o}38'10.24"S and 39^{o}15'14.60"E) with a depth of about 30 m while the second station was located at a point between Kunduchi Marine biological station and Mbudya Island (06^{o}39'28.05"S and 39^{o}14'17.69"E) with a depth of about 20 m. The two stations were chosen due to their easy access from the laboratories at Kunduchi (Figure 1). The hydrography in the study area is strongly influenced by the monsoon trade winds. The northeast (NE) monsoon, which blows from December to April and characterized by high temperatures, low wind speed and calm seas as well as the southeast (SE) monsoon, which blows from June to October and characterized by low temperatures, higher wind speeds and rougher seas (Bryceson 1977).

Water transparency and salinity were measured *in situ* using a Secchi disc (25 cm in diameter) and a refractometer (Otago, Japan), respectively. Water temperature, water conductivity, dissolved oxygen and pH were also measured *in situ* using a multi-parameter water quality checker (Horiba U 10, Japan). Water samples for determination of inorganic nutrients (phosphate and nitrate) were collected from near the surface (0.5 m) using a 1.5 L Niskin bottle and filtered through 0.2 µm pore sized Millex-GS Millipore filters (France). The filtered water samples were kept in 100 mL acid-cleaned plastic vials and immediately kept cool on ice for transport to a deep-freezing facility. In the laboratory, analyses for NO_3^-, and PO_4^{3-} were done using a Shimadzu spectrophotometer (Japan) as described by Parsons *et al*. (1984). Near surface water samples (0.5 m) for phytoplankton biomass (Chl.-*a*) were collected using a 1.5 L Niskin bottle. In the laboratory, 1.5 L volumes of water samples were filtered through membrane filters (0.45 µm pore size). The filters were then kept in glass centrifuge tubes and 10 mL of 90% acetone added to extract the pigments. In the laboratory, the amount of Chl.-*a* in the samples was determined using a spectrophotometer (SHIMADZU, Japan) as described by Parsons *et al*. (1984).

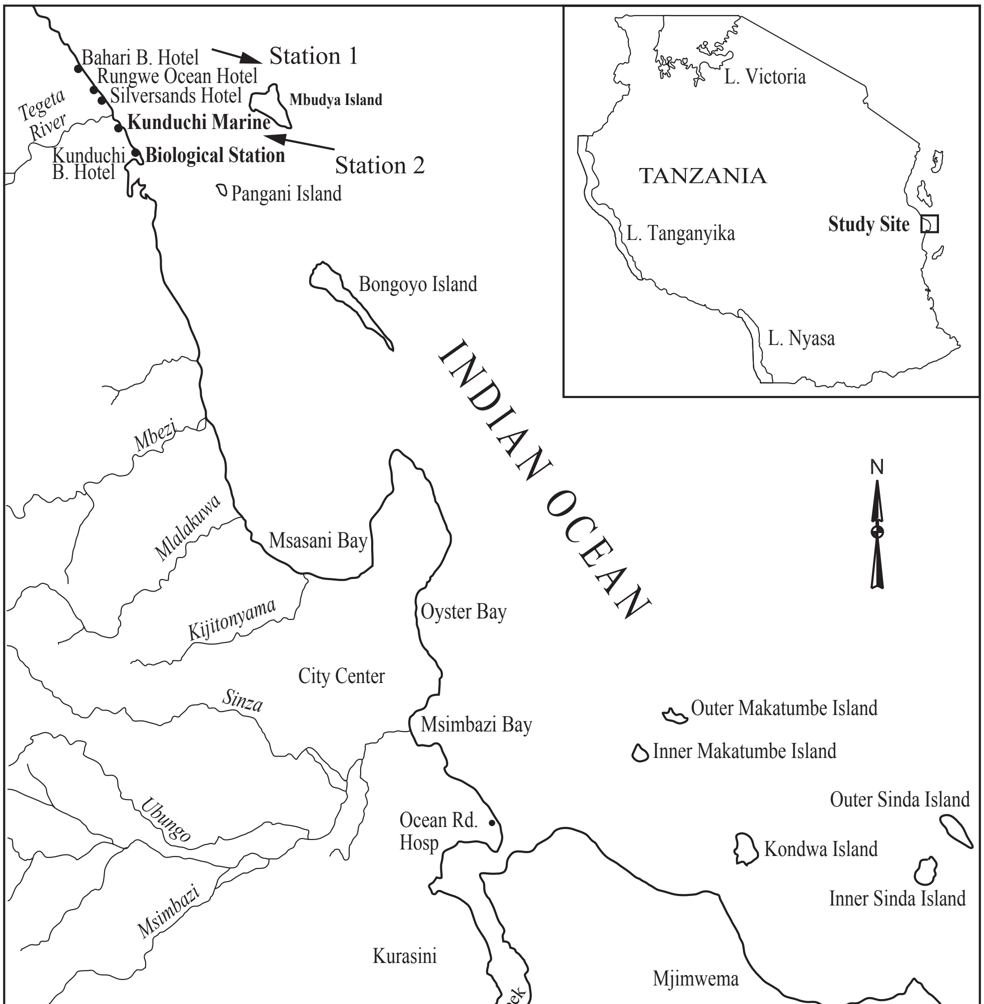

Figure 1: Map of Dar es Salaam showing the sampling sites (arrows) in the open waters off Mbudya Island (Station 1) and between Mbudya Island and Kunduchi Marine Biological station (Station 2).

Samples for identification and determination of *Pseudo-nitzschia* abundance were collected by vertically hauling a 20 μm mesh sized plankton net from a 20 m depth up to the surface. The samples were then kept in plastic vials and fixed using formaldehyde to a final concentration of 4%. In the laboratory, identification of *Pseudo-nitzschia* species was done using a light microscope following the description given by Hasle and Syverstesen (1997), Skov *et al.* (1999) and Priisholm *et al.* (2002). Counting of *Pseudo-nitzschia* cells was done under a light microscope using a Sedgewick Rafter Cell as described by Woelkerling *et al.* (1976).

To compare the differences between the two stations and sampling seasons, data were statistically tested using a parametric t-test and where the assumptions for parametric tests were not met, data were analysed using the respective non-parametric Mann Whitney U test. For seasonal analyses, the months of December to April were considered to represent the Northeast (NE) Monsoon period while the months of June to October represented the Southeast (SE) Monsoon. Correlations were tested using the Pearson Correlation test. In all cases, significance was determined at the 95% confidence level. A Graph Pad In-Stat Demo programme was used for the statistical data analyses.

RESULTS

Environmental parameters

Secchi depth readings were generally low at station 2 compared to station 1 and tended to increase with time reaching peak values between November and January. The values ranged from 3.8 m as recorded at station 2 in August to maximum of 23.5 m in November at station 1 (Figure 2A). Significantly higher secchi depth values were obtained at station 1 compared to station 2 ($t = 3.10$, $p = 0.005$). However, there was no significant difference in secchi depth values between the NE and SE Monsoon periods ($t = 1.596$, $p = 0.125$). Water temperature values at station 1 and 2 were close to each other and increased from low values in August to highest values during March (Figure 2B). There was no significant difference in water temperature between station 1 and 2 ($U' = 90.0$, $p = 0.312$). However, significantly higher water temperatures were recorded during the NE monsoon period compared to the SE monsoon ($U' = 131.0$, $p = 0.0007$).

Salinity values did not show clear trends with time at both stations. Lowest salinity values of 33 was recorded at station 1 in October and at station 2 in December while the highest salinity value of 35 was recorded at station 1 in February, May and July and at station 2 in September, November and March (Figure 2C). Salinity values were not significantly different between the two sampling stations ($t = 0.00$, $p > 0.99$) as well as between the NE and SE monsoon period ($t = 1.09$, $p = 0.29$). The pattern of variations in water conductivity at both station 1 and 2 was similar. Lowest values of 40.7 μS/cm and 41.0 μS/cm were recorded during the month of September and highest values of 47.9 μS/cm and 47.6 μS/cm in March, at station 1 and 2, respectively (Figure 2D). Similarly, there was no significant difference in conductivity values between the two stations ($t = 0.093$, $p = 0.926$) as well as between the NE and SE monsoon periods ($U' = 91.0$, $p = 0.285$).

The amount of dissolved oxygen in the water column at both station 1 and 2 fluctuated in a similar pattern showing low values between January and April and high values in December (Figure 2E). Oxygen levels at station 1 ranged from 5.8 to 7.7 mg/L as recorded in February and December, respectively. Similarly, at station 2 the lowest oxygen value of 5.9 mg/L was recorded in February and the highest value of 7.8 mg/L in December. There was no significant difference in the amount of dissolved oxygen between the two sampling stations ($t = 0.926$, $p = 0.365$) as well as between the NE and SE monsoon ($U' = 80.5$, $p = 0.644$). At station 1, the pH value was lowest (7.9) in June and highest (8.36) in September while at station 2 the pH value was lowest (7.8) in July and highest (8.36) in September and December (Figure 2F). There was no significant difference in pH values between the two stations (t = 0.157, p = 0.876). Significantly higher pH levels were however observed during the SE monsoon period compared to NE period (U' = 119.0, p = 0.007).

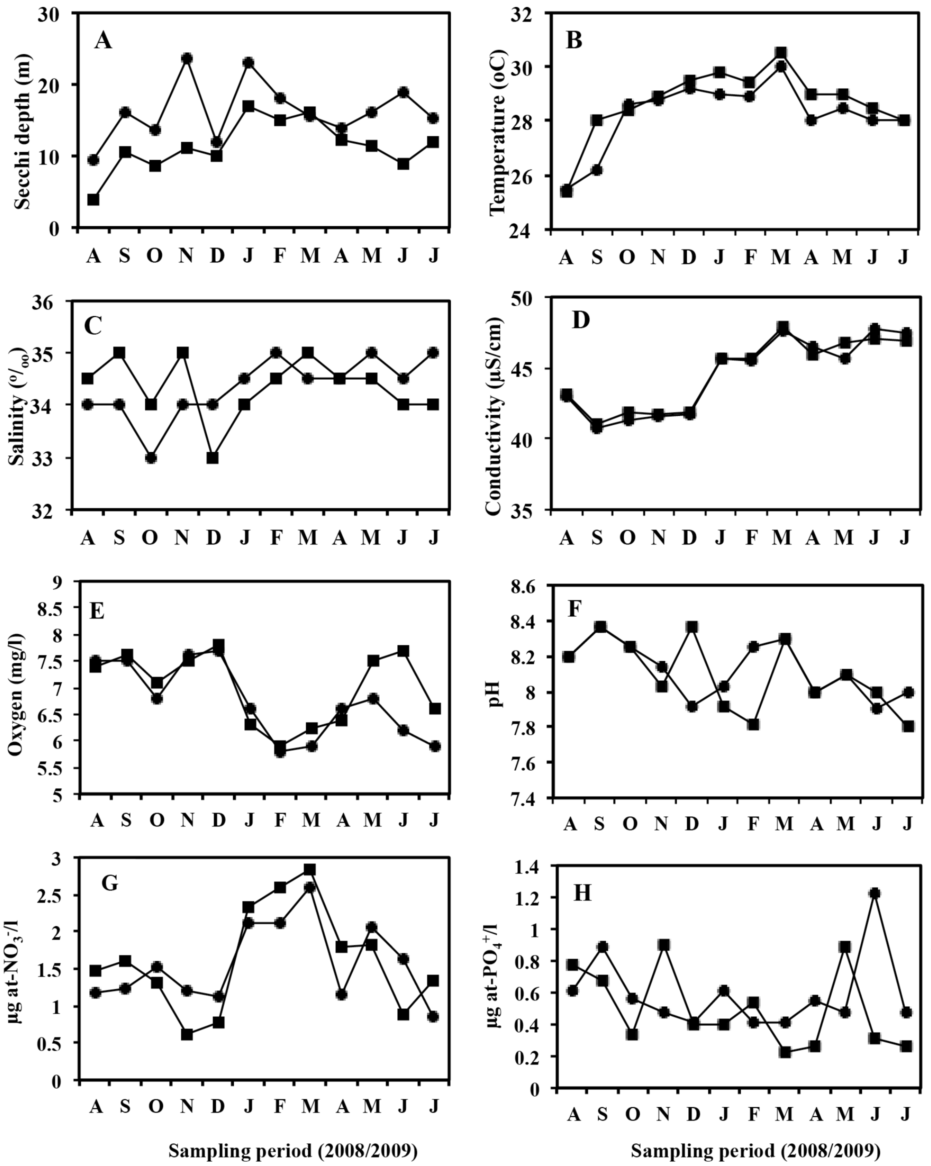

Figure 2: Temporal distribution in environmental parameters at Station 1 (cycles) and Station 2 (squares). A = water transparency; B = water temperature; C = salinity; D = water conductivity; E = oxygen concentration; F = water pH level; G = nitrate concentration; H = phosphate concentration

The concentration of inorganic nitrate (NO_3^-) in the water column was generally low during the period of August to December, rising to higher levels from January to March before decreasing again from April to June (Figure 2G). At station 1, NO_3^- level ranged from 0.85 μg at -NO_3^- as recorded in July to 2.59 μg at-NO_3^- in March while at station 2 it ranged from 0.62 μg at-NO_3^- in November to 2.84 μg at-NO_3^- in March. However, there was no significant difference in NO_3^- concentration between the two sampling stations (t = 0.179, p = 0.860) as well as between the NE and SE monsoon period (t = 1.407, p = 0.173). Levels of inorganic phosphate (PO_4^+) in the water column fluctuated without showing a clear temporal pattern (Figure 2H). Low PO_4^+ levels (0.41 μg at-PO_4^+) were recorded at station 1 in February and March while the highest level of 1.23 μg at-PO_4^+ was recorded in June. At station 2, a lowest value of 0.22 μg at-PO_4^+ was recorded in March and a highest value of 0.90 μg at-PO_4^+ was recorded in November. There was no significant difference in PO_4^+ concentration between the two sampling stations (U' = 96.0, p = 0.178) as well as between the NE and SE monsoon periods (t = 1.487, p = 0.151).

Phytoplankton abundance and biomass

Total phytoplankton abundance (number of cells/L) and biomass (Chl.-*a*) fluctuated over time showing a peak in April on abundance at station 1 and a January to March elevation in Chl.-*a* at station 2 (Figures 3A & B, respectively). At station 1, phytoplankton abundance ranged from a lowest value of 140 cells/L as recorded in August to 520 cells/L in April while at station 2 cell numbers ranged was from 198 cells/L in June to 460 cells/L in March (Figure 3A). However, there was no significant difference in phytoplankton abundance between the two stations (t = 0.710, p = 0.486) as well as between the NE and SE monsoon periods (t = 1.289, p = 0.211). Chl.-*a* concentration at station 1 was lowest (0.106 mg/m^3) in October and highest (0.201 mg/m^3) in April while at station 2 Chlorophyll *a* was lowest (0.148 mg/m^3) in November and highest (0.224 mg/m^3) in March (Figure 3B). Similarly, the concentration of Chl.-*a* in the water column did not show significant differences between the two stations (t = 1.942, p = 0.0651) as well as between the NE and SE monsoon periods (t = 0.348, p = 0.732).

***Pseudo-nitzschia* diversity and abundance**

Three *Pseudo-nitzschia* species (i.e., *Pseudo-nitzschia pungens*, *Pseudo-nitzschia seriata* and *Pseudo-nitzschia cuspidata*) were encountered during the current study. Identification of the three morphotypes was on the basis of their cell dimensions and cell shapes. Both the valve and girdle views of *Pseudo-nitzschia pungens* looked symmetrical and lanceolate in shape with cell length ranging from about 80 to 170 μm while the cell width was between 2 to 5 μm. The valve view of *Pseudo-nitzschia seriata* was asymmetrical and somewhat lanceolate while the girdle view looked symmetrical and linear. Cell length ranged from 95 to 150 μm with a width of about 6 to 8 μm. *Pseudo-nitzschia cuspidata* showed pointed ends and a lanceolate shape at both valve and girdle views. Cell lengths ranged from about 50 to 70 μm with a width of about 3 to 4 μm.

In general, *Pseudo-nitzschia pungens* was the most abundant at both sites followed by *Pseudo-nitzschia seriata* while *Pseudo-nitzschia cuspidata* was the least abundant (Figures 3Cand 3D). At station 1 the concentration of all *Pseudo-nitzschia* spp. was lowest (0 cell/L) in October, November and May and high (14.1 cells/L) in September (Figure 3C) while at station 2, *Pseudo-nitzschia* spp. was lowest (0 cell/L) in October, November, January, April and May and highest (15.6 cells/L) in September (Figure 3D). There was no significant difference in the abundance of *Pseudo-nitzschia* spp. between site 1 and 2 (t 0.1957, p = 0.8467) as well as between the NE and SE monsoon periods (U' = 93.5, p = 0.2252).

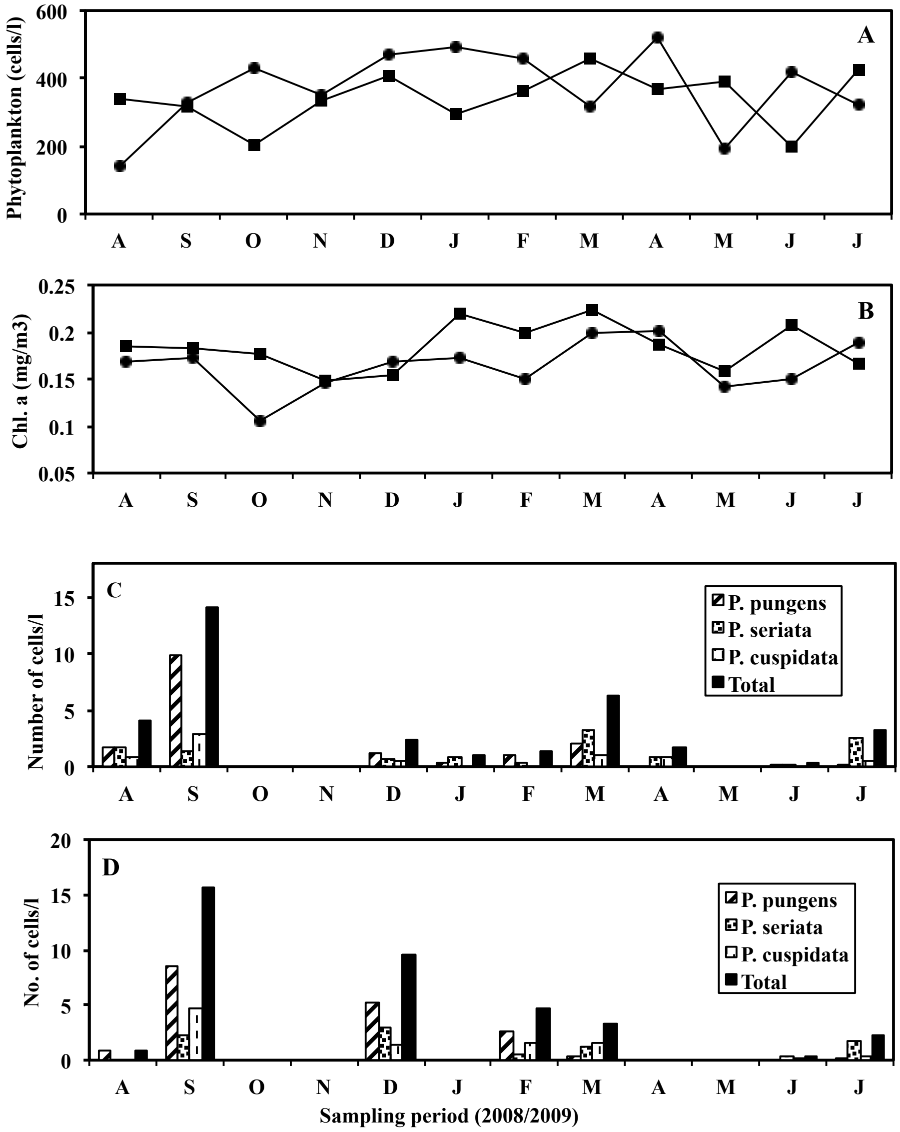

Figure 3: Temporal distribution of phytoplankton density (A) and Chl.-*a* (B) at station 1 (cycles) and station 2 (squares) as well as *Pseudo-nitzschia* density at station 1 (C) and station 2 (D).

Significant correlations were found between the amount of chlorophyll-*a* and water conductivity (r = 0.4595, p = 0.0239). However, there was no significant correlation between Chl.-*a* and any other environmental parameters tested, i.e., secchi depth (r = -0.1089, p = 0.6126), water salinity (r = 0.3114, p = 0.1386), water pH (r = 0.2228, p = 0.2953), amount of dissolved oxygen (r = -0.2657, p = 0. 2095), water temperature (r = 0.1702, p = 0.4266), phytoplankton abundance (r = -0.0861, p = 0.6891) as well as NO_3^- (r = 0.3325, p = 0.1124) and PO_4^+ (r = 0.0757, p = 0.7252). Similarly, There was no significant correlation between the abundance of *Pseudo-nitzschia* spp. with any of the environmental parameters tested i.e., secchi depth (r = -0.1243, p = 0.5627), water salinity (r = 0.0012, p = 0.9956), water pH (r = -0.0045, p = 0.9835), water conductivity (r = -0.3492, p = 0.0945), amount of dissolved oxygen (r = 0.2341, p = 0.2709), water temperature (r = -0.2263, p = 0.2877), Chl.-*a* (r = 0.1736, p = 0.4172), phytoplankton abundance (r = 0297, p = 0.8905) as well as NO_3^- (r = -0.1025, p = 0.6337) and PO_4^+ (r = 0.0789, p = 0.7140) concentration in the water column.

DISCUSSION

The phytoplankton species composition found in the study area is generally similar to that reported earlier by e.g., Bryceson (1977) for samples collected off Dar es Salaam and those reported by Lugomela and Semesi (1996) for samples collected off Zanzibar town. In addition, the phytoplankton biomass recorded during the current study was similar to that reported by Lugomela and Semesi (1996), which ranged from 0.12 to 0.5 mg Chl.-*a*/m^3. The current study observed a slight but non-significant increase in Chl.-*a* concentration during the NE monsoon period compared to the SE monsoon period. In general, the East African coast shows elevation in Chl.-*a* during the NE monsoon as compared to the SE monsoon period (Bryceson 1977; Kitheka *et al*. 1996; Lugomela and Semesi 1996). Bryceson (1977) associated the NE monsoon chlorophyll elevation to a number of meteorological and hydrographical factors. These include a relatively higher exposure of the phytoplankton to light due to a shallower thermocline during the NE monsoon. Also the slow East African coastal current during this period that increases the residence time of the water masses on the shelf and shallow environments, therefore acquiring neritic characteristics which are known to support higher production.

During the current study, *Pseudo-nitzschia pungens* was the most common species compared to the other two *Pseudo-nitzschia* species. Indeed, *P. pungen* is considered as among the most cosmopolitan species of the genus *Pseudo-nitzschia* (Casteleyn*et al*. 2008). Unfortunately, all of the three *Pseudo-nitzschia* species (*P. pungens*, *P. seriata* and *P. cuspidata*) are included in the list of the toxin producing *Pseudo-nitzschia* species (Fryxell *et al*. 1997, Priisholm *et al*. 2002, Fryxell and Hasle 2003; Lundholm *et al*. 2003). This suggests a great potential of ASP along the coast of Tanzania should any of these organisms grow in high numbers.

The three *Pseudo-nitzschia* species were found to occur sporadically in the year and at times contributing substantially to the total phytoplankton densities. However, their concentrations reported in this study were far below those of Bates *et al*. (1989) of up to 15 × 10^6 cells/L, which caused intoxication to numerous people in the Cardigan Bay, Prince Edward Island, Canada, in 1987. This may suggest that the near shore waters of Dar es Salaam are currently out of amnesic shellfish poisoning risks. More data is however required to corroborate this conclusion and to further ascertain their population dynamics and their potential for DA production in coastal waters of Tanzania.

The lack of significant correlation between the abundance of *Pseudo-nitzschia* spp. with any of the tested environmental parameters suggests that other variables not determined in this study may be responsible for the observed spatial and temporal fluctuation of the *Pseudo-nitzschia* species. These may include factors such as grazing by zooplankton and viral infections, which are all well known to govern phytoplankton production in the sea. Indeed, grazing by zooplankton was probably the most important factor controlling the *Pseudo-nitzschia pungens* bloom dynamics in Zhelin Bay, China (Huang*et al*. 2009). In the same study, the authors showed that water temperature also had significant linear relationship with the population density of *Pseudo-nitzschia pungens*, with a temperature value of 23.8 °C being an optimum condition for the algal bloom. Water temperature in the current study ranged between 25.4 and 30.5 °C, which could be regarded to be beyond the optimum condition for *Pseudo-nitzschia pungens* growth. In another study, Lundholm *et al*. (2004) showed that the growth of 11 *Pseudo-nitzschia* species stopped at pH values of 8.7 to 9.1. However, for *P. delicatissima* and *Nitzchia navis-varingica* the upper pH limit for growth was 9.3 and 9.8, respectively (Lundholm *et al*. 2004). This suggests that many *Pseudo-nitzschia* species are capable of tolerating elevated pH values well above the average seawater value of 8.2.

This is the first comprehensive report on the seasonal distribution of *Pseudo-nitzschia* species along the coast of Tanzania. The paucity of information about *Pseudo-nitschia* in this area may mainly be due to lack of sampling programmes and limited expertise for proper identification of the organism. However, as shellfish constitute an important fishery along the coast of Tanzania, a need to evaluate the possibility for ASP in the area remains pertinent. Thus, more studies to corroborate the current study and ascertain DA production by *Pseudo-nitzschia* species in coastal waters of Tanzania are required.

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