# Annual patterns, distribution and long-term trends of *Pseudo-nitzschia* species in the northern Benguela upwelling system

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Phytoplankton data obtained from a 14-year data set off the coast of Namibia were analysed. The temporal distribution of species belonging to the diatom genus, *Pseudo-nitzschia*, was examined using samples collected quasi-monthly at onshore and offshore locations. This data set revealed that *Pseudo-nitzschia* blooms occurred sporadically along the central coast of Namibia, and cell concentrations frequently exceeded 200 000 cells $L^{-1}$, with levels close to or exceeding 1 000 000 cells $L^{-1}$ noted occasionally. Two relatively cold years (2005 and 2010) and three relatively warm years (2001, 2003 and 2011) were found to be exceptional for high biomass *Pseudo-nitzschia* blooms, so possible drivers (wind, temperature and nutrients) were evaluated. *Pseudo-nitzschia* species abundance was associated with moderate temperature (13–16°C) and nutrient conditions. Climatology showed an increase in *Pseudo-nitzschia* blooms during austral summer, reaching a maximum in May, June or July. These blooms seem to initiate following periods of high total phytoplankton concentrations. Wind was determined to play an important role in controlling these blooms. Low biomass *Pseudo-nitzschia* blooms were observed during months with high or irregular winds that coincided with periods of upwelling, whereas high biomass blooms were associated with the decrease of wind after the main wind peak at the end of summer.

KEYWORDS: *Pseudo-nitzschia* species; phytoplankton; northern Benguela upwelling system (nBUS); seasonality; temperature; wind; nutrients

## INTRODUCTION

The Angola–Benguela frontal zone (ABFZ) separates the tropical ecosystem in the north from the Benguela upwelling system (BUS) further south (John *et al.*, 2001). The BUS itself is divided by the Lüderitz upwelling cell at 27°S into the northern and southern BUS. The northern BUS (nBUS) is influenced by two main central water masses. From the north, nutrient-rich and oxygen-poor South Atlantic Central Water (SACW) is transported southward in the Angola Current onto the Namibian shelf. Well-oxygenated but nutrient-poor Eastern SACW (ESACW) is formed in the Cape Basin and is transported northwards along the southwest African shelf edge by the Benguela Current (O'Toole *et al.*, 2001; Shannon and O'Toole, 2003; Shannon *et al.*, 2006; Mohrholz *et al.*, 2008).

The nBUS, located off Namibia, is viewed as the most productive marine ecosystem worldwide, compared to the Pacific large upwelling systems, namely the California Current Large Marine Ecosystem off the west coast of North America and the Humboldt Current Large Marine Ecosystem off the coast of Peru and Chile (Carr and Kearns, 2003; Messié *et al.*, 2009; Lachkar and Gruber, 2012). It is part of the Benguela Current Large Marine Ecosystem (BCLME) and is situated in the centre of this LME. The very productive coastal upwelling conditions in the nBUS are driven by strong along-shore south-east trade winds and are enabled by a relatively wide continental shelf, low eddy activity and shallow mixed layer (Lachkar and Gruber, 2012).

This high productivity is very important and is contributing to the well-established fishing industry in this region; however, sporadic blooms of potentially harmful algal species may have various negative effects on fisheries and the marine ecosystem in general. It has been reported that the BUS can be either a hypoxic or an anoxic marine environment (Bremner, 1980; Bailey, 1991). These phenomena in the nBUS are either enhanced by low oxygen water transported with the poleward undercurrent from the Angolan Gyre (Mohrholz *et al.*, 2008) or by the sinking of organic matter due to the influence of the Lüderitz upwelling cell (Chapmann and Shannon, 1987). Consequently, an increase in these negative effects could have potentially severe economic implications on the mariculture and fishery industry in the nBUS as has already been documented for the sBUS (Cockcroft *et al.*, 2000). High biomass phytoplankton blooms cause a threat to the already sensitive marine environment in terms of low oxygen levels associated with bloom termination and degradation of algal biomass (Monteiro *et al.*, 2006), whereas toxic phytoplankton blooms might have a further negative influence on both wild and farmed fisheries as well as marine mammals inhabiting this region.

Historically and also more recently, many toxic dino-flagellate taxa as well as toxic species of the marine diatom genus *Pseudo-nitzschia* have been reported for the nBUS (Kollmer, 1963; Pieterse and Van der Post, 1967; Kruger, 1980; Vavilova, 1990; Hansen *et al.*, 2014; National Marine Information and Research Centre, NatMIRC, unpublished data). *Pseudo-nitzschia* has been reported as a cosmopolitan taxon that occurs throughout the World's oceans. The growth and distribution of *Pseudo-nitzschia* species have been shown by other authors to be influenced by salinity (Thessen *et al.*, 2005), nutrient concentrations (Caroppo *et al.*, 2005), pH (Lundholm *et al.*, 2004) and photoperiod (Fehling *et al.*, 2005, 2006). In addition, coastal upwelling events, mesoscale eddies and anthropogenic inputs of nutrients have been identified as possible causes of *Pseudo-nitzschia* blooms (Parsons *et al.*, 2002; Kudela *et al.*, 2005; Anderson *et al.*, 2008; Trainer *et al.*, 2012). Although these taxa are regularly reported in the nBUS since 1936, no annual data sets exist for these species' distribution or for their potential to cause toxic bloom events. Therefore, a 14-year phytoplankton species time series data set was extracted and analysed for *Pseudo-nitzschia* species concentrations off Namibia in the nBUS.

The primary aim of this study was to analyse this 14-year data set for the occurrence and spatial distribution of *Pseudo-nitzschia* species and to identify any annual or seasonal patterns. The relationship between *Pseudo-nitzschia* species' presence and certain physical and/or chemical parameters in the water column was also examined. During the study period, it was found that warm water intruded periodically from the Angolan region and has properties similar to the El Niño occurring off the west coast of South America (Shannon *et al.*, 1986; Bartholomae and van der Plas, 2007; Louw *et al.*, 2016). The warm water intrusion into the nBUS is sometimes referred to as the Benguela Niño. The effects of this warm water on *Pseudo-nitzschia* populations and its adverse impact on strong upwelling were investigated. The mesoscale physical events (e.g. upwelling, relaxation of wind) responsible for the large spatial/temporal *Pseudo-nitzschia* blooms were also studied and evaluated as predictors of the highest probability of *Pseudo-nitzschia* blooms appearing on an annual basis.

## METHOD

### Study area

The 23°S cross-shelf transect runs seaward from the central Namibian coast close to Walvis Bay harbour on the downstream side of the main Lüderitz upwelling cell

(Supplementary Material 1). Nine stations were sampled regularly from onshore to offshore for physical, chemical and biological parameters from 2001 to 2014. The closest station is located two nautical miles (NM) from the coastline, followed by 5, 10, 20 and continuing every 10 NM in an offshore direction until reaching the furthest station at 70 NM. This transect covers the entire shelf, from coast to the shelf edge. The 10 NM shelf station (Supplementary Material 1) was chosen as the location to study *Pseudo-nitzschia* species' seasonality and possible trends. This station is situated close to Walvis Bay harbour and historically lies within the fisheries grounds off Namibia. Therefore, an environmental time series extending for about one and a half decades exists for this station (2000–2014).

## Sampling, sample preparation and measurements

### Physical and chemical parameters

Oceanographic measurements were obtained using a Seabird CTD fixed to a rosette containing 5 L PVC Niskin bottles. Environmental parameters, such as temperature ($\pm 0.005$ K), were recorded throughout the water column. Water samples for nutrients, chlorophyll-a (Chl-a) and phytoplankton microscopic analyses were collected with Niskin bottles during each CTD cast. The CTD was maintained regularly and calibrated externally and internally according to the manufacturer's instructions.

Remotely sensed wind data (23.1°S, 13.9°E) from the QuikScatterometer (QuikSCAT) and the Advanced Scatterometer (ASCAT) (Bentamy and Fillon, 2012) for the periods of January 2000–November 2009 and March 2009–November 2014, respectively, were used.

Water samples for nutrient analyses (silicate, nitrate, phosphate and ammonium) were filtered on-board through a 0.45-µm cellulose acetate membrane and frozen at −20°C until analysed. Nutrient concentrations were measured using a Bran & Luebbe Traacs 800 autoanalyser

according to instrument-specific protocols based on standard colorimetric methods (Grasshoff *et al.*, 1999).

## Biological parameters

### Phytoplankton counting

Surface water samples were collected for quantitative phytoplankton counting and a subsample of ~200 mL was preserved by adding 5 mL of 40% formaldehyde (Throndsen, 1978) for counting in the laboratory. Table I shows the dates and frequency of sampling for the 14-year time series (2001–2014).

Cell counts were made with a Zeiss Axiovert 200 inverted light microscope (Utermöhl, 1958). Prior to counting, the formalin-preserved samples were settled in a 25-mL chamber for 24 h. Cells identified as *Pseudo-nitzschia* spp. were designated as belonging to either the 'delicatissima' (width <3 µm) or the 'seriata' (width >3 µm) size class, according to Hasle (1965); however, for the purpose of this study, total *Pseudo-nitzschia* species were considered. The concentrations of *Pseudo-nitzschia* species were calculated using the equation given by Utermöhl (1958) and by counting of ~400 cells with a precision of ±10%. Occasionally, in cases of extreme blooms, >500 cells were enumerated with a small increase in precision. Where *Pseudo-nitzschia* species occurred at low concentrations, 50–200 cells were counted which provides a precision range of 15–30% for quantitative estimates (Andersen and Throndsen, 2004).

## Data analysis

The temperature at 15 m depth was used as a measure of upwelling intensity, specifically the 13°C isotherm (Hagen *et al.*, 2001), whereas the 14°C (Bartholomae and van der Plas, 2007) and 16°C thresholds were used to describe thermocline strength (i.e. stratification of the water column).

The long-term yearly medians were determined by calculating the median for each year and then the median of

*Table I: Survey dates on the 23°S transect during the 14-year sampling period (2001–2014): sampled—light grey (black—Pseudo-nitzschia species not found), not sampled—white*

| Month | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jan | | | | | | | | | | | | | | |
| Feb | | | | | | | | | | | | | | |
| Mar | | | | | | | | | | | | | | |
| Apr | | | | | | | | | | | | | | |
| May | | | | | | | | | | | | | | |
| Jun | | | | | | | | | | | | | | |
| Jul | | | | | | | | | | | | | | |
| Aug | | | | | | | | | | | | | | |
| Sep | | | | | | | | | | | | | | |
| Oct | | | | | | | | | | | | | | |
| Nov | | | | | | | | | | | | | | |
| Dec | | | | | | | | | | | | | | |

these medians were calculated. Similarly the long-term monthly medians were determined by calculating the median for each month and then the median of these medians was calculated. Box and whisker plots were used to summarize these data. The box represents the inter-quartile range (IQR, or middle 50%) and the whiskers extend to the most extreme data points (upper and lower), which is no more than 1.5 times the distance of the IQR from the ends of the box. Data points outside of the whisker range are defined as outliers (indicated with an 'O') and those points that are more than 3 times the distance of the IQR away from the ends of the box are defined as extreme outliers (indicated with an asterisk).

The statistics are presented using the American Psychological Association (APA) style. Descriptive statistics, including mean, median, skewness, kurtosis and standard deviation were used to describe the distribution of the data. Analysis of variance on the log-transformed data was used to test for significant difference in mean monthly and yearly values. Pearson's correlation test was used to test for correlations between two variables.

## RESULTS AND DISCUSSION

### Temporal trends for *Pseudo-nitzschia* species

Temporal trends of phytoplankton taxa in the nBUS were elucidated along with the dynamics of *Pseudo-nitzschia* species in this environment. *Pseudo-nitzschia* species numerically dominated the phytoplankton community at some of the sampling stations, representing a significant component of the food available for many secondary producers that comprise an integral part of the regional food web. The strong presence of *Pseudo-nitzschia* spp. also enhances the potential for trophic transfer of domoic acid, a potent neurotoxin produced by certain members of this genus (La Barre *et al.*, 2014).

The 14-year time series (2001–2014) for *Pseudo-nitzschia* species showed repeated bloom events over the Namibian shelf (Fig. 1). The highest cell concentrations were present for the 10–30 NM stations. Harmful phytoplankton cell counts are used in different European countries as threshold or trigger levels to indicate the probability of harmful effects on mariculture resources. Accordingly, the 200 000 cells L$^{-1}$ concentration for total *Pseudo-nitzschia* was adopted for Namibia (Anderson, 1996) in this article and identified as the threshold above which levels of this potentially toxic diatom genus should be seen as a concern for contamination of shellfish resources, effects of domoic acid on wildlife or as a potential for high biomass bloom development. *Pseudo-nitzschia* bloom development (cell counts > 200 000 cells L$^{-1}$) occurred onshore and offshore, whereas high concentrations were often found as close as the 2 NM station. Blooms were present on multiple occasions and in certain cases continued for several months; i.e. multiple bloom developments as in the case of 2003 (10 NM), 2004 (offshore), 2005, 2009, 2010, 2011 and at the beginning of 2014, over the shelf.

In the years 2001, 2003, 2005, 2007, 2009, 2010, 2011 and 2014 notably extreme *Pseudo-nitzschia* blooms occurred, with cell counts close to or exceeding 1 000 000 cells L$^{-1}$ for the 10, 20 and 30 NM stations (Fig. 1 and Supplementary Material 2). No, or low biomass, *Pseudo-nitzschia* blooms were recorded during surveys in 2002, 2004 and 2006 at these three onshore stations. High biomass *Pseudo-nitzschia* blooms were found simultaneously at these three stations during March 2001, July 2003, May 2005, July 2010 and June 2011. This provided some insight as to the potential spatial extent of *Pseudo-nitzschia* blooms across the shelf.

### Variability of *Pseudo-nitzschia* species off Walvis Bay (10 NM station)

The *Pseudo-nitzschia* cell concentrations recorded at this station deviated significantly from a normal distribution,
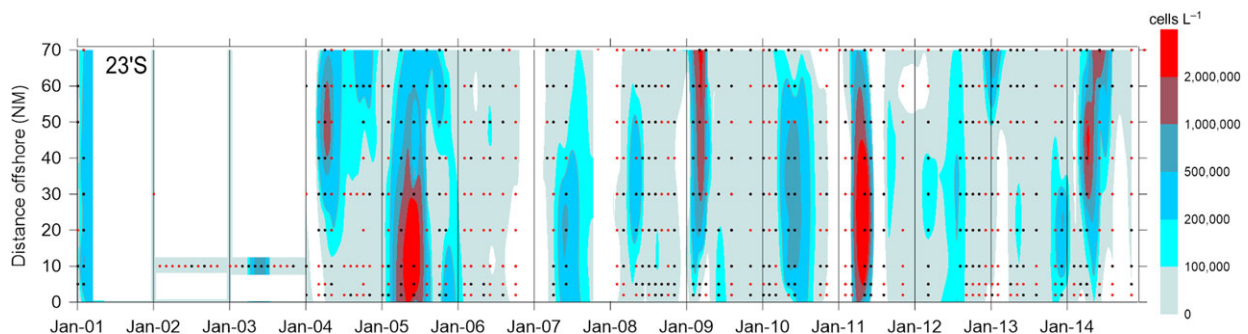


**Fig. 1.** *Pseudo-nitzschia* species concentration (cells L$^{-1}$ in surface seawater (±2 m)) distribution along the 23°S transect over the shelf from 2001 to 2014 (black dots—*Pseudo-nitzschia* species found; red dots—stations sampled).

with skewness of 9.139 (SE = 0.243) and kurtosis of 87.52 (SE = 0.481) with $N = 99$. The high standard deviation indicates that the variances were extremely high for these measurements throughout the time series (see Supplementary Material 3). The mean value was influenced heavily by three extreme outliers in the data collected during July 2003, May 2005 and July 2005.

Notable bloom events for *Pseudo-nitzschia* species, with concentrations higher than 200 000 cells L$^{-1}$, were recorded in 2001, 2003, 2005 and years from 2007 to 2012 for the 10 NM station. However, 2001, 2003, 2005, 2010 and 2011 were exceptional years, with concentrations exceeding 500 000 cells L$^{-1}$ and at times >1 000 000 cells L$^{-1}$ at the 10 NM station.

The annual box and whisker plots (Fig. 2) show that the medians were only higher than the long-term median (11 055 cells L$^{-1}$) for 2001, 2007, 2009–2012 and 2014 when blooms were detected. The extremely high cell counts during July 2003, and especially in May and July 2005, were exceptional occurrences, since the median values for 2003 and 2005 were below the long-term median. Analysis of variance on the log-transformed data showed that the main effect of year was not significant, $F(12, 42) = 1.387$, $P = 0.210$.

From the monthly box and whisker plots (Fig. 3), it is apparent that the median *Pseudo-nitzschia* species concentrations for the months of April, May, June and July were above the long-term monthly median (4009 cells L$^{-1}$). Bloom events were detected during the months of March,
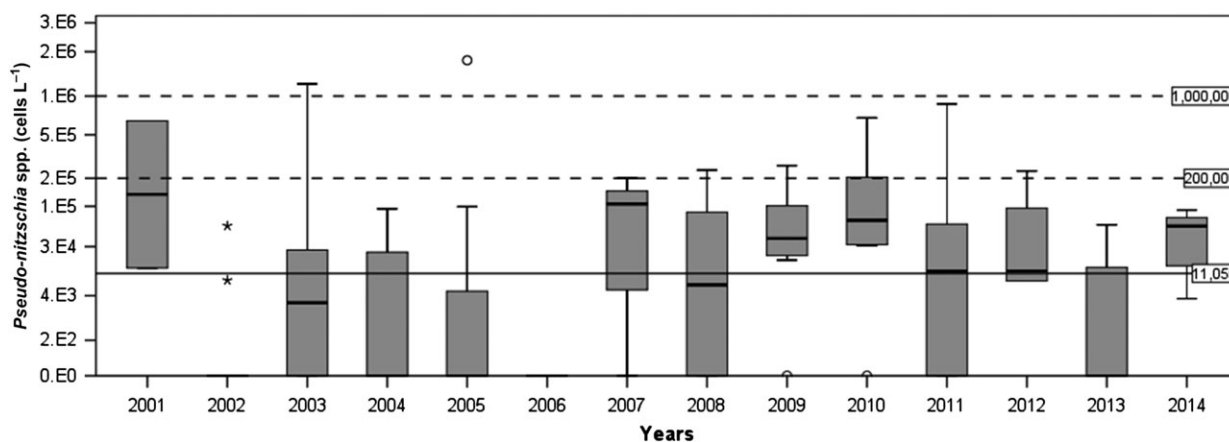


**Fig. 2.** Annual box and whisker time series plot for *Pseudo-nitzschia* species concentration (cells L$^{-1}$ in the surface (±2 m)) distribution along the 23°S transect at 10 NM off the coast over the period 2001–2014 (solid line represents long-term median annual concentration of 11 055 cells L$^{-1}$ and outliers are indicated with an 'o' and extreme outliers with an asterisk) ($n = 99$).
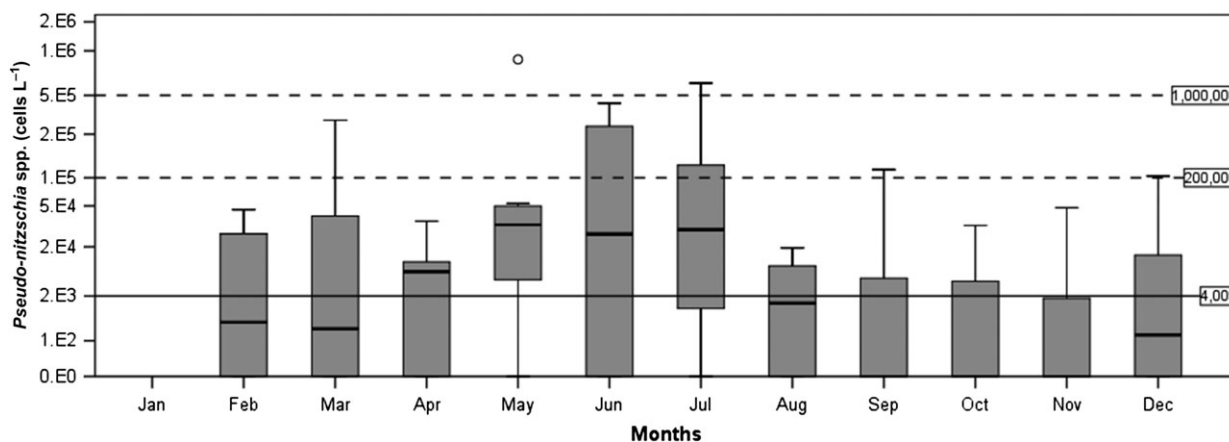


**Fig. 3.** Monthly *Pseudo-nitzschia* species concentration (cells L$^{-1}$ in the surface (±2 m)) on the 23°S transect, at the 10 NM station off the coast of Namibia from 2001 to 2014 (solid line represents long-term median monthly concentration of 4009 cells L$^{-1}$ and outliers are indicated with an 'o') ($n = 99$).

May, June, July, September and December with extreme blooms during May and July. Analysis of variance on the log-transformed data showed that the effect of month was not significant, $F(10, 44) = 1.52$, $P = 0.164$.

## High biomass blooms: *Pseudo-nitzschia* bloom relationship with other phytoplankton blooms and Chl-a

Analysis of this 14-year time series revealed that high concentration *Pseudo-nitzschia* blooms did not co-occur along with high biomass blooms comprising the normal phytoplankton community in the nBUS (Fig. 4a). Concentrations of *Pseudo-nitzschia* species and total phytoplankton showed no correlation, Pearson's $r(55) = 0.135$, $P < 0.326$ (using log-transformed data). It is also evident from Fig. 4b that there was no clear relationship between Chl-a concentrations and *Pseudo-nitzschia* species' blooms. Concentrations of *Pseudo-nitzschia* species and Chl-a showed no correlation, Pearson's $r(43) = -0.179$, $P < 0.252$.

## The effects of physico-chemical environmental conditions on *Pseudo-nitzschia* blooms

### Temperature

*In situ* temperature variability and seasonality in the nBUS from onshore to offshore along the 23°S transect for the period 1999–2012 (surface temperature was excluded due to solar radiation effects and its high variability) were discussed in detail by Louw *et al.* (2016). For the purpose of this study, temperatures for the 15 m depth (Fig. 5) extended to 2014 are shown. The 13°C isotherm indicates the upwelling threshold and 16°C corresponds to the intense stratification temperature threshold. The first segment of this time series was relatively warm (Fig. 5a and b) but changed subsequently to a cooler period. The relatively warmer conditions at the beginning of this time series were not classified as a Benguela Niño but instead were considered to be a "normal warm water event." However, the *in situ* temperature increment in 2011 as described by Louw *et al.* (2016) did indicate the presence of a
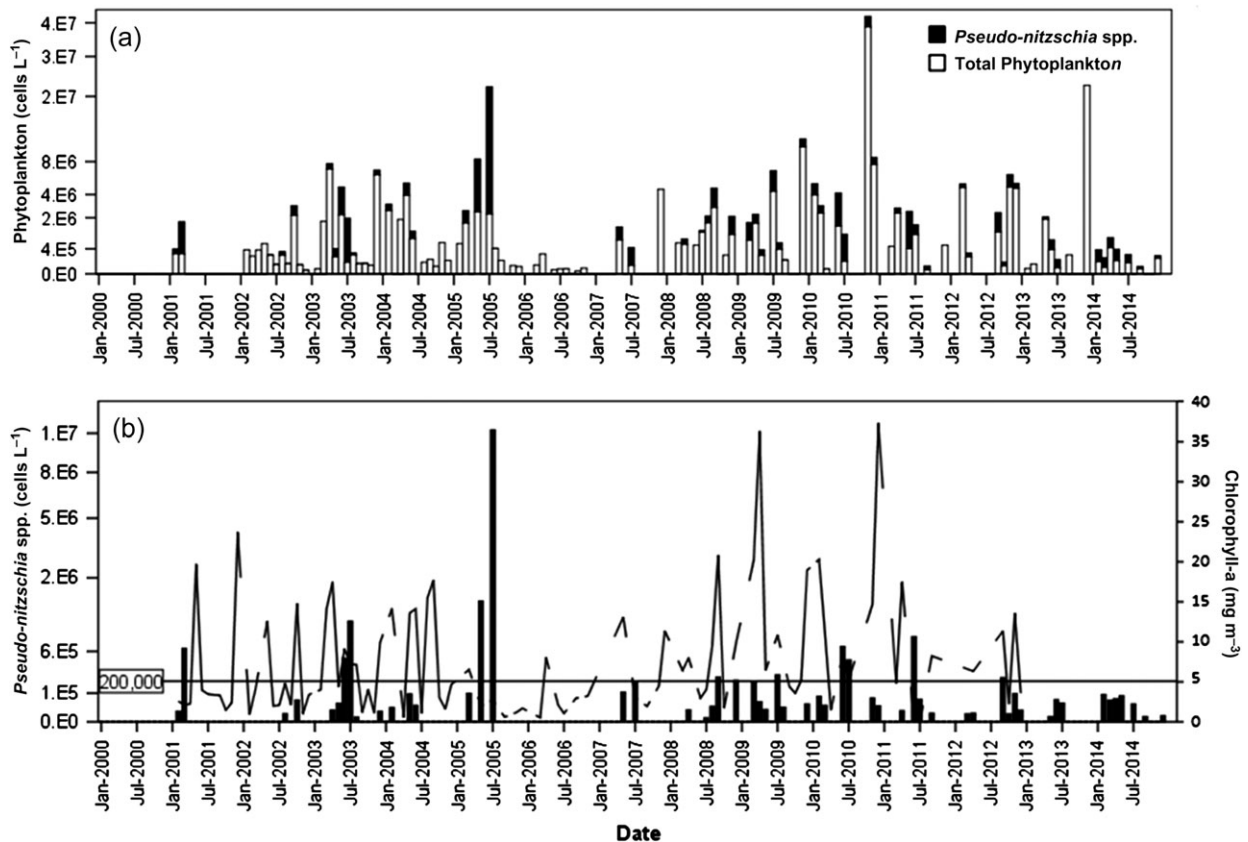


**Fig. 4.** Time series (2001–2014) of *Pseudo-nitzschia* species concentration (cell L$^{-1}$) for the 10 NM station off Walvis Bay on the 23°S transect compared with (**a**) total surface phytoplankton species and total surface *Pseudo-nitzschia* species [indicated by bars] (cell L$^{-1}$) (**b**) total surface *Pseudo-nitzschia* species (indicated by bars) and total surface Chl-a concentration (mg m$^{-3}$) ($n = 99$; indicated by line graph).
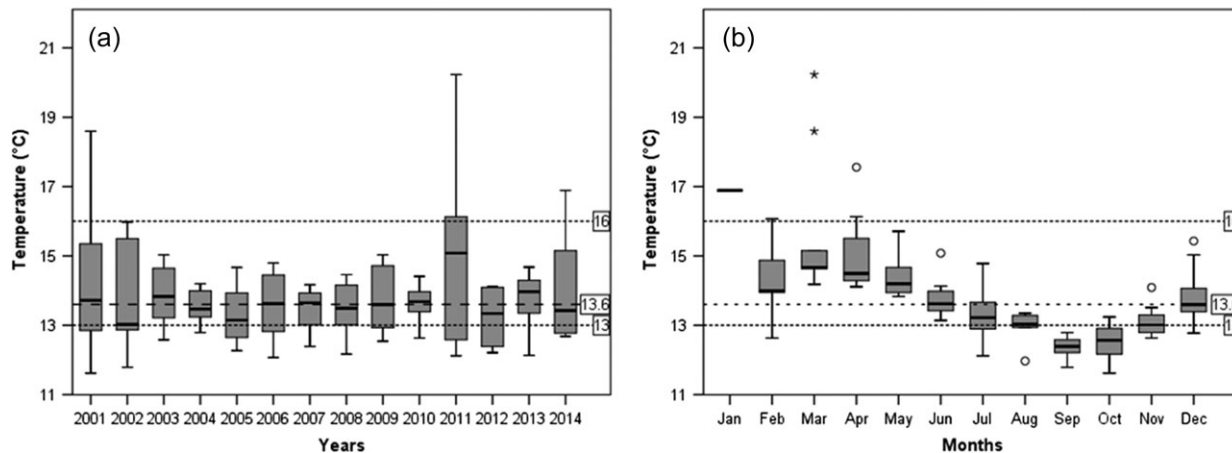
**Fig. 5.** Box plots of the annual temperature (**a**) and monthly temperature (**b**) at 15 m depth on the 10 NM station off Walvis Bay on the 23°S transect from 2001 to 2014. The long-term median is 13.6°C (outliers are indicated with an 'o' and extreme outliers with an asterisk) ($n = 103$).

Benguela Niño, which resulted in the maximum average sea surface temperature measured for this 14-year period, along with poleward flowing warmer water, low nutrients and reduced ambient phytoplankton concentrations. Such warm water events are not unusual in the nBUS (Shannon *et al.*, 1986; Bartholomae and van der Plas, 2007), and are similar to El Niño events in the Pacific Ocean. The Benguela Niño in 2011 was characterized by exceptionally high *Pseudo-nitzschia* concentrations.

Analysis of variance showed that the main effect of year was not significant, $F(13, 89) = 0.749$, $P = 0.712$. The median temperatures for 2001, 2003, 2010, 2011 and 2013 were above the long-term annual median of 13.6°C. For the remaining years, the median temperature was below or equal to the long-term annual median value. However, there were significant statistical differences in temperature between the different months as shown by analysis of variance $F(11, 91) = 11.669$, $P = 0.000$. September was the month that differed significantly from most other months. The highest and lowest median temperatures occurred in March and September, respectively (Fig. 5b), the latter generally corresponding with upwelling. Months with a median temperature lower than the long-term monthly median temperature were July, August, September, October and November.

The relationship between *Pseudo-nitzschia* concentrations and temperature is presented in Supplementary Material 4. There was a non-significant correlation between concentration (log-transformed) and temperature at the 10 NM, station Pearson's $r(49) = 0.152$, $P = 0.296$. It is apparent that 2001, 2003, 2005, 2010 and 2011 were years with exceptionally high *Pseudo-nitzschia* concentrations, whereas 2004 and 2006 were exceptionally low biomass years. Beginning in 2003,

low-intensity upwelling occurred (below the 13°C isotherm), which was accompanied by warmer water temperatures. The anomalous year 2005 may reflect the presence of one or more *Pseudo-nitzschia* species exhibiting higher growth rates at low water temperatures; however, an elevated growth response to another environmental forcing (s) (e.g. nutrients) may also have contributed to the dominance of *Pseudo-nitzschia* in 2005 (see below). Although no direct correlations were found between temperature and phytoplankton, we suggest that this may represent a lag in phytoplankton response (Louw *et al.*, 2016).

## Wind

The nBUS is a wind-driven upwelling system and wind is thus a major factor controlling mixing of the water column and therefore nutrient availability for phytoplankton in the upper layers. Two major wind cycles are observed annually (Fig. 6b) with some small periodic pulses occurring between these cycles (Louw *et al.*, 2016). There was a non-significant correlation between *Pseudo-nitzschia* species concentration (log-transformed) and remotely sensed wind speed at the 10 NM station, Pearson's $r(46) = -0.004$, $P = 0.981$. However, it is apparent that for 2010 and 2014 when the *Pseudo-nitzschia* concentrations were above the long-term median, the wind speed was below the long-term median (Fig. 6a). Analysis of variance showed that the main effect of year was not significant, $F(12, 142) = 1.181$, $P = 0.302$. The months in which *Pseudo-nitzschia* concentrations were above the long-term median (May, June and July) also showed median wind speeds lower than the long-time median (Fig. 6b). There was a statistically significant difference in wind speed between the different months as shown by analysis of variance $F(11, 143) = 4.633$, $P = 00.000$.
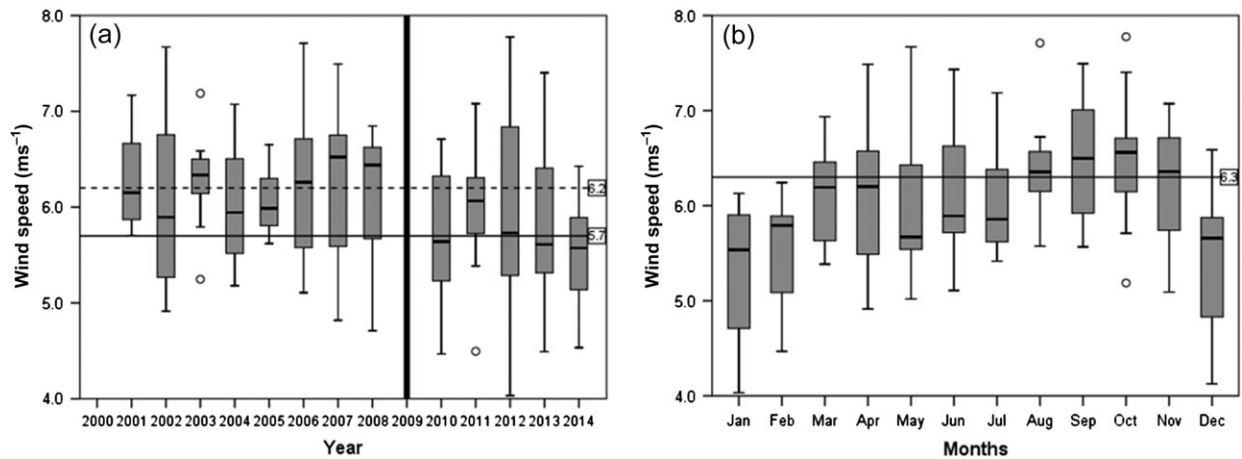
**Fig. 6.** Box plots of annual (**a**) and monthly (**b**) remote sensing wind data for the 10 NM station off Walvis Bay on the 23°S transect from 2001 to 2014. The dotted line represents the annual and monthly median values (The black vertical line in 2009 indicates the change of wind sensors from QuikSCAT to ASCAT. The outliers were indicated with an 'o'.).

The wind speed is normally low during the first quarter of the year in summer months (December–February) and increases steadily to its peak at the end of summer, resulting in breakdown of the thermocline. Consequently, nutrients are introduced into the upper water column and major phytoplankton blooms in the nBUS are initiated. Louw *et al.* (2016) have shown that the probability of such blooms occurring is high between March and May. A slight relaxation of wind occurs from May to July, which is also the period of highest possible *Pseudo-nitzschia* blooms along the nBUS (Fig. 3). Following this period, the wind speed increases again (along with mixing of the water column), reaching a maximum that coincides with an upwelling peak from August to November—the main upwelling period in the central nBUS and a time characterized by very low *Pseudo-nitzschia* abundance over the 14-year time series.

The five years that showed extremely high concentrations of *Pseudo-nitzschia* species at 10 NM of the 23°S transect (2001, 2003, 2005, 2010 and 2011; see also Supplementary Material 3) were examined to assess the response of *Pseudo-nitzschia* species to different environmental conditions and to compare their annual behaviour (Fig. 7). Data for *Pseudo-nitzschia* species presented in Fig. 7a are compared with data obtained for the ambient phytoplankton community, as well as wind speed and associated temperature patterns. *Pseudo-nitzschia* species were clearly most abundant from May to July (Fig. 7a), whereas the main phytoplankton community abundance was highest during the first part of the each year (Fig. 7b). Timing of *Pseudo-nitzschia* bloom initiation for the four years identified above was more or less the same, reaching their highest concentrations in July after the ambient phytoplankton blooms, indicating different

drivers of growth for these respective phytoplankton assemblages. It is also apparent that total phytoplankton biomass, including *Pseudo-nitzschia* abundance, is significantly reduced during periods of highest wind stress, which generally begins after July (Fig. 7c). The effect of high wind speeds over this time frame can be seen in the low water temperature associated with water column mixing and thus dispersion of bloom populations (Fig. 7d). Otherwise, winds were largely moderate for all four years, yet still sufficient to mix nutrients into the upper water column and initiate a phytoplankton bloom.

During the four years with low biomass *Pseudo-nitzschia* blooms (2002, 2004, 2006 and 2013), cell concentrations rarely reached 100 000 cells $L^{-1}$, with maximum levels present between April and August (Fig. 8a). Total phytoplankton abundance showed similar trends with a peak in April (Fig. 8b), and early summer blooms, normally observed in December, tended to shift towards October/November. The wind pattern during these years was stronger and more variable, with multiple peaks or pulses throughout the year (Fig. 8c). Years with a high abundance of *Pseudo-nitzschia* species were characterized by low to moderate wind speeds during the first half of the year followed by dominance of stronger winds. By comparison, years with a low *Pseudo-nitzschia* abundance exhibited low to moderate wind speeds only from December to February. These patterns and trends suggest strongly that wind is the dominant physical factor controlling *Pseudo-nitzschia* blooms in the nBUS.

*Nutrients*

Surface nitrate ($NO_3^-$), phosphate ($PO_4^{3-}$), silicate ($SiO_2$) and ammonium ($NH_4^+$) measurements showed

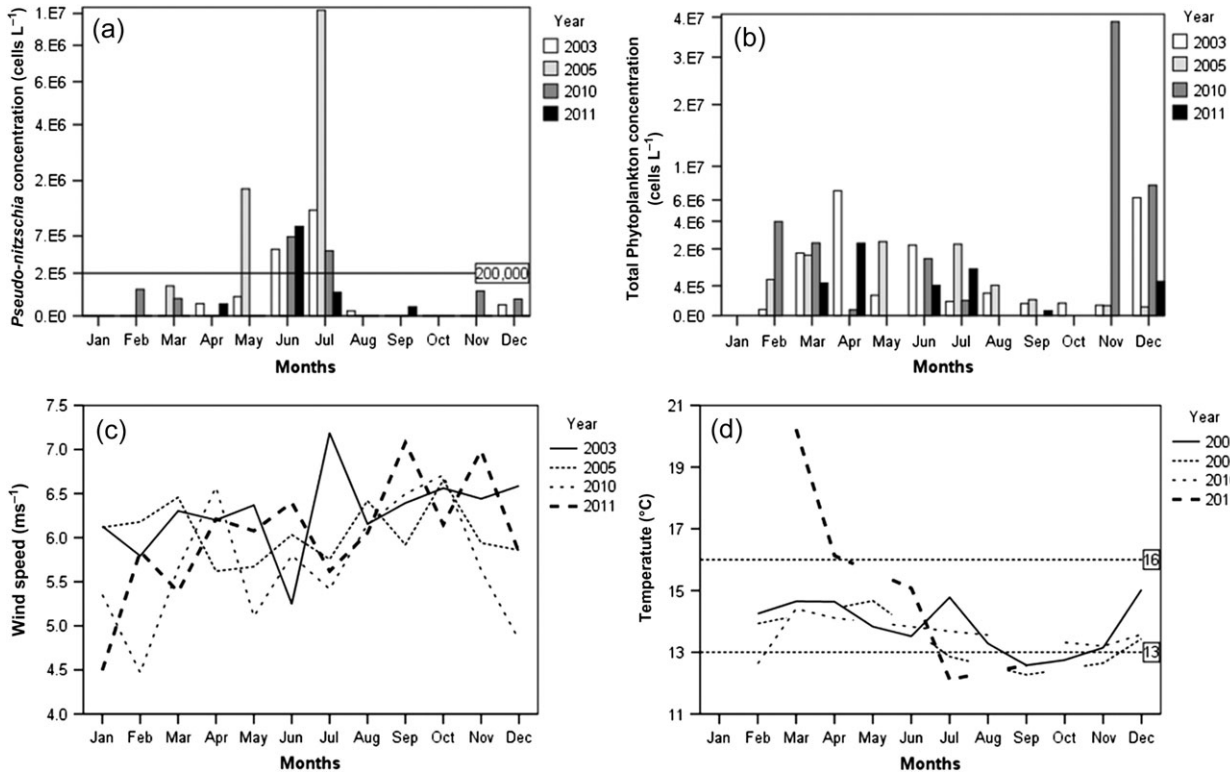**Fig. 7.** (**a**) *Pseudo-nitzschia* species (*n* = 25), (**b**) total phytoplankton (subtracted for the *Pseudo-nitzschia* species) (*n* = 25), (**c**) wind data (*n* = 36) and (**d**) temperature data (*n* = 22) for the 15 m depth for the four anomalous years (2003, 2005, 2010 and 2011).
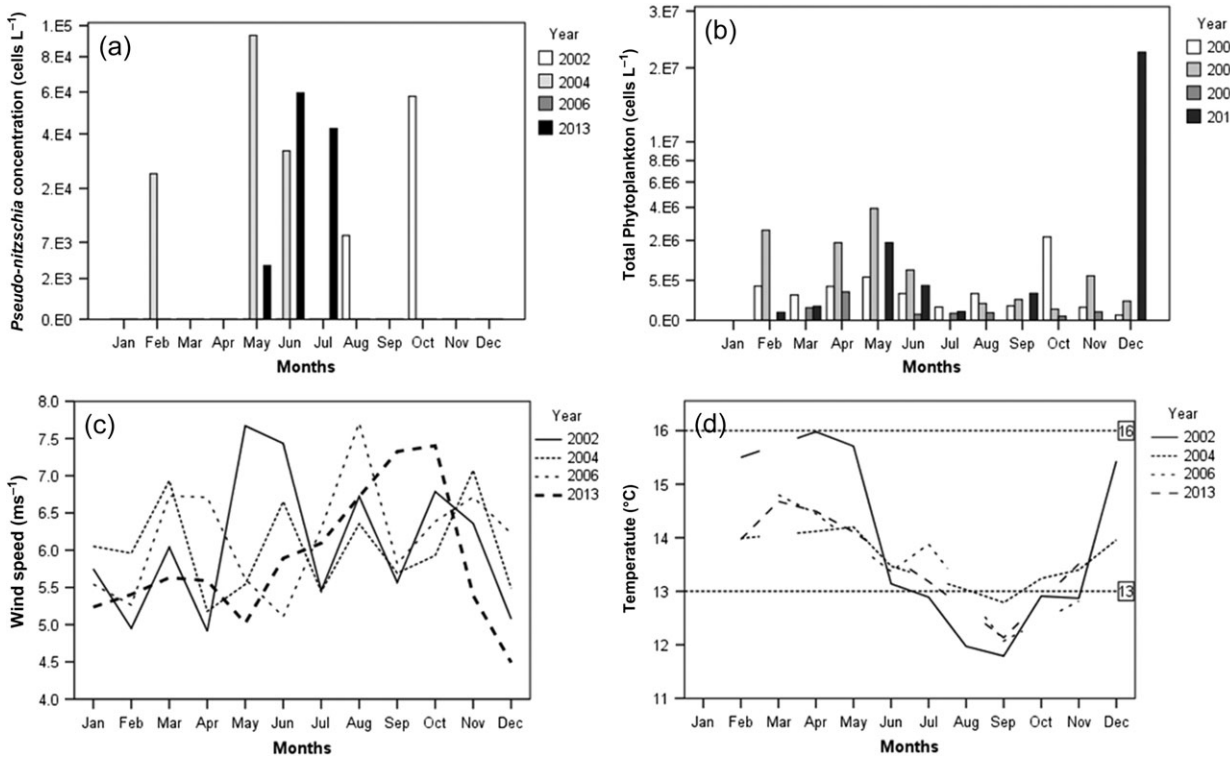


**Fig. 8.** (**a**) *Pseudo-nitzschia* species, (**b**) total phytoplankton (subtracted for the *Pseudo-nitzschia* species), (**c**) wind data and (**d**) temperature data for the 15 m depth for the four low abundance bloom years (2002, 2004, 2006 and 2013).
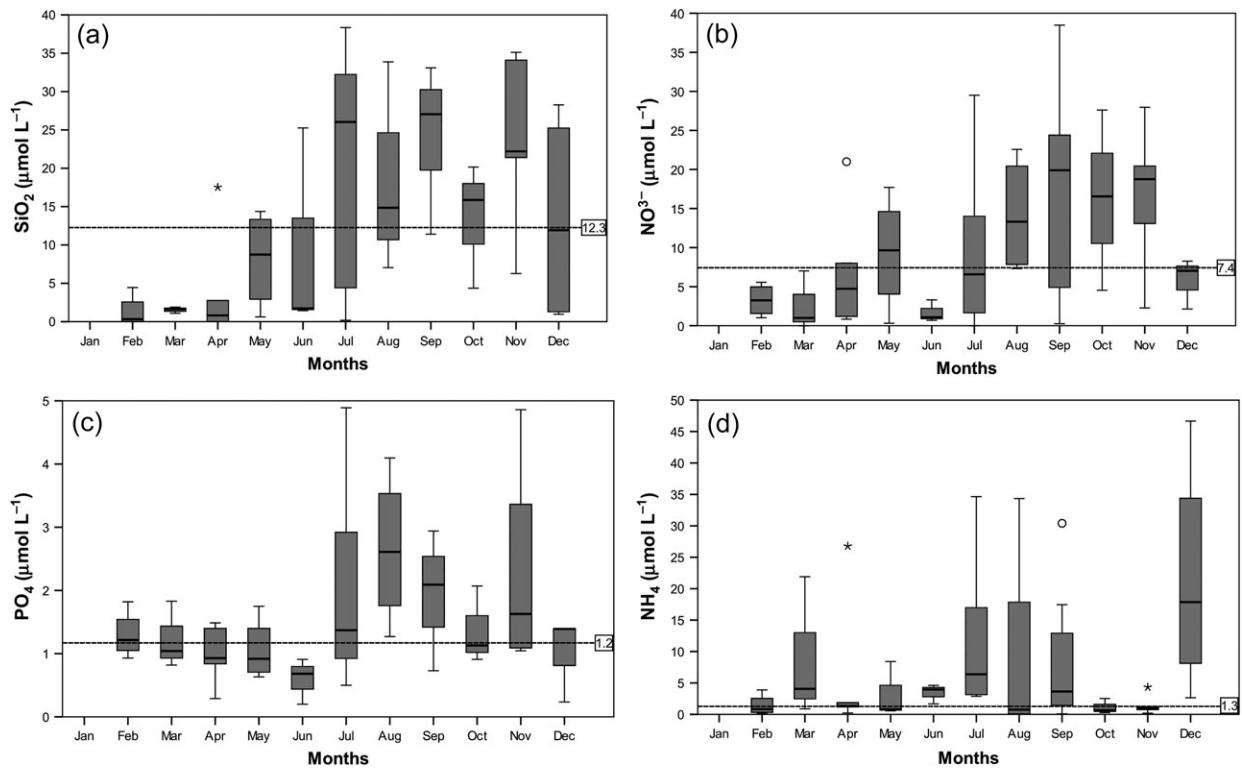
**Fig. 9.** Surface box plots of the monthly concentrations (µmol $L^{-1}$) of (**a**) silicate ($SiO_2$) ($n = 49$), (**b**) nitrate ($NO_3^-$) ($n = 48$), (**c**) phosphate ($PO_4^{3-}$) ($n = 47$) and (**d**) ammonium ($NH_4^+$) ($n = 48$) for the 10 NM station off Walvis Bay on the 23°S transect from 2001 to 2011 (the dotted line represents the annual and outliers are indicated with an 'o' and extreme outliers with an asterisk).

that concentrations of these nutrients were low during the austral summer; however, regular small peaks of nutrients were visible during this period (Fig. 9). By comparison, the major nutrient loading took place during periods of upwelling and highest wind intensity (August–November). Given the potential importance of nitrogen nutrition in regulating *Pseudo-nitzschia* blooms in various regions, including the sBUS (Seeyave *et al.*, 2009), the relationship between *Pseudo-nitzschia* concentration and the four nutrients measured during this study was examined. However, significant correlations were observed only between *Pseudo-nitzschia* concentration and $SiO_2$ ($r = -0.549$, $P = 0.018$), and $PO_4^{3-}$ concentrations ($r = 0.471$, $P = 0.036$), and both of these showed a negative or inverse relationship. More targeted studies (e.g. nutrient uptake experiments) will be required in order to properly evaluate the nitrogen nutritional status and nitrogen preference (i.e. $NO_3^-$ vs $NH_4^+$ vs urea) for *Pseudo-nitzschia* populations, and thus the potential influence of nitrogen on bloom dynamics in this region.

Louw *et al.* (2016) reported that $NO_3^-$ and $SiO_2$ levels in surface waters showed seasonality—i.e. low during summer and high during upwelling. Strong seasonality also existed for $PO_4^{3-}$ and $SiO_2$ at the 10 NM station on the 23°S transect (Figs. 9c and a). In the surface

layers, $PO_4^{3-}$ remained at about <1.6 µmol $L^{-1}$ during the summer months (December up to April), but these low levels persisted through May/June. From March to May, major phytoplankton blooms were expected, coinciding with breakdown of the thermocline and upward mixing of $NO_3^-$ (Louw *et al.*, 2016) that occurred after the first wind peak (Fig. 9b). Between August and November, $PO_4^{3-}$ levels remained relatively high, between 1.6 and 2.4 µmol $L^{-1}$ (Fig. 9c).

Silicate concentrations showed more or less the same pattern as $PO_4^{3-}$ levels and remained high during the upwelling period with values above 10 µmol $L^{-1}$. Silicate declined to 10–20 µmol $L^{-1}$ before summer (December) and fell to <10 µmol $L^{-1}$ in the upper water column. Levels remained low until June or July when $SiO_2$ concentrations increased again to above 10 µmol $L^{-1}$ (Fig. 9a). Ammonium concentrations were generally low with elevated levels apparent in March and July to September, with a peak in December (Fig. 9d).

Our data suggested that surface nutrients can drop below the threshold values required for phytoplankton growth (Wasmund *et al.*, 2014) and therefore can become limiting during summer and extending as late as June. However, the continual increase in wind speed did appear to be one of the primary factors mediating

termination of *Pseudo-nitzschia* blooms prior to the main upwelling season. During the upwelling period from August to October, major nutrient concentrations were in excess, except for $SiO_2$, which declined from 20 to $10 \, \mu mol \, L^{-1}$ and was reduced further by the general phytoplankton community to below $10 \, \mu mol \, L^{-1}$ during the summer months. Nonetheless, although nutrients remained low in the summer, *Pseudo-nitzschia* species concentrations above $200\,000$ cells $L^{-1}$ were recorded over the study period.

# CONCLUSION

*Pseudo-nitzschia* species are considered cosmopolitan, occurring over a wide range of temperature and salinity regimes. Evaluation of the data set obtained for the nBUS showed that increases in *Pseudo-nitzschia* species abundance were associated with moderate temperature conditions (13–16°C) and moderate nutrient conditions as also reported for other regions, e.g. US west coast (Trainer *et al.*, 2002). *Pseudo-nitzschia* species in the nBUS dominated during the autumn period (May–July) and reached values $>10^6$ cells $L^{-1}$ and occasionally between $2 \times 10^6$ and $8 \times 10^6$ cells $L^{-1}$. These blooms seem to initiate following periods of high total phytoplankton concentrations (i.e. high Chl-a blooms). *Pseudo-nitzschia* species can dominate the phytoplankton assemblage at different times of the year, consistent with findings in other marine ecosystems (Bresnan *et al.*, 2015).

The wind seems to be a primary factor controlling *Pseudo-nitzschia* blooms, with such events increasing during relaxation and utilization of nutrients remaining after the ambient phytoplankton bloom. However, as the wind increases to an unknown threshold speed, *Pseudo-nitzschia* blooms can be dispersed. Fryxell *et al.* (1997) noted that larger scale weather patterns, such as during non-El Niño Southern Oscillation (ENSO) years when upwelling is high, can lead to high *Pseudo-nitzschia* abundance as has been observed along certain parts of the US west coast. However, this pattern does not seem to describe the dynamics of *Pseudo-nitzschia* species in the nBUS. By comparison, Seeyave *et al.* (2009) reported that *Pseudo-nitzschia* blooms in the sBUS were, in fact, favoured by upwelling and the accompanying increase in $NO_3^-$ concentration. It should be noted that the nBUS is out of phase with the sBUS, with the main upwelling period occurring in the former region during January/February. By the end of the main upwelling period in the sBUS, *Pseudo-nitzschia* species concentrations increase as the wind subsides. It is also the time when nitrogen supply for *Pseudo-nitzschia* species switches to $NH_4^+$, as $NO_3^-$ becomes depleted. Seeyave *et al.* (2009) concluded that although both forms of nitrogen become depleted in the sBUS, *Pseudo-nitzschia* species are still able to thrive under these conditions due to their small size and high nutrient uptake affinity compared to other phytoplankton, which seems to be the same for the nBUS.

Since the nBUS is as productive as any other of the Eastern boundary upwelling systems, it is expected that nutrients should not be limiting for phytoplankton growth. However, controlling mechanisms exist (i.e. wind, strength of thermocline), which regulate nutrient availability to and utilization by phytoplankton in the upper water column. The nBUS time series showed that *Pseudo-nitzschia* blooms are not associated with the main coastal upwelling period in August–September, as also reported by Lange *et al.* (1994) and Trainer *et al.* (2000, 2001). Rather, major blooms occur during periods of weak or relaxed upwelling associated with low wind conditions, as well as low macronutrient concentrations. This scenario is similar to that described for central California, USA, where outbreaks typically follow upwelling events as nutrients become less available to a well-developed multi-species phytoplankton bloom during relaxation periods (Kudela *et al.*, 2004; Lewitus *et al.*, 2012). For the nBUS, the duration of relaxation periods between wind cycles in summer seems to be important for enhancing stratification and reducing upwelling activity. These low wind conditions, accompanied by removal of nutrients by the opportunistic general phytoplankton assemblage (i.e. species other than *Pseudo-nitzschia*) appear to be a prerequisite for *Pseudo-nitzschia* blooms in the nBUS and reinforce the argument that wind is a major factor controlling these events.

Finally, it is known that certain *Pseudo-nitzschia* species in the nBUS (onshore and offshore) are toxic (NatMIRC, unpublished data), as in the case of the sBUS (Fawcett *et al.*, 2007). These taxa are capable of producing the potent neurotoxin domoic acid, causative agent of amnesic shellfish poisoning. Therefore, more detailed study of the morphology and toxicity of *Pseudo-nitzschia* species for the nBUS is a gap that is currently being addressed by our group. This information, along with the development of taxon-specific molecular probes, will contribute to a more effective and efficient monitoring program. This is a critical need for Namibian coastal waters given our data describing the frequency and intensity of *Pseudo-nitzschia* blooms in the nBUS along with the detection of low DA levels in molluscs at some mariculture sites in this region (NatMIRC, unpublished data).

# SUPPLEMENTARY DATA

Supplementary data can be found online at *Journal of Plankton Research* online

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