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RESEARCH PAPER

Domoic Acid Variations in Response to Environmental Conditions in an Eutrophic Estuary, Golden Horn (Turkey)

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Abstract

Particulate domoic acid (pDA) concentrations, *Pseudo-nitzschia* abundances and their relationship with environmental variables were investigated from August 2011 to July 2012 in the Golden Horn Estuary (GHE), Turkey. To determine the distribution of pDA concentrations, the high performance liquid chromatography - fluoresence detection (HPLC-FLD) method was used (detection limit 0.2 µg ml^{-1}). pDA concentrations ranged from 0.24 to 21.03 µg ml^{-1} and the highest value was measured at FD3 station, located in the middle estuary, in May 2012. pDA levels were undetectable between Aug. 2011 and March 2012 period in all stations, except in Nov. 2011 (0.83 µg mL^{-1}, at FD3 station). pDA values reached the maximum value when *Pseudo-nitzschia* abundance was counted at the highest level (196×10^3 cells L^{-1}, at FD3 station).The relationship between pDA and environmental parameters was investigated by Spearman correlation analysis and the influencing factors of the pDA distribution were evaluated with Principal Component Analysis (PCA). According to the results, pDA production is mostly controlled by temperature, SiO_2 and NO_3+NO_2. This study reports the first results of DA in the GHE and these results may be used to consider the probability of finding similar conditions in the Turkish seas.

Keywords: Domoic acid, *Pseudo-nitzschia,* HPLC, Golden Horn Estuary.

Introduction

Pseudo-nitzschia is one of the most common, year-round members of marine phytoplankton communities worldwide (Hasle, 2002). The genus *Pseudo-nitzschia* comprises 46 species, 19 of which are known to produce domoic acid (Moestrup *et al*., 2009). Domoic acid (DA), a neurotoxic amino acid responsible for Amnesic Shellfish Poisoning (ASP), was first recorded in 1987 in Prince Edward Island, Canada (Bates, Garrison, & Horner, 1998). Over 100 people became ill and several died from consumption of blue mussels (*Mytulis edulis*) contaminated with DA (Bates *et al*., 1989). Since this incident, DA has become a potential threat in many regions worldwide (Hallegraeff, 2003; Thessen & Stoecker, 2008; Klein, Claquin, Bouchart, Le Roy, & Veron, 2010; Maric *et al*., 2011; Sahraoui *et al*., 2012; Parsons, Dortch, & Doucette, 2013).

Current knowledge of DA production is based on regional ASP events and cultured *Pseudo-nitzschia* species (Klein *et al*., 2010). On the other hand, there is a lot of variability between the regions in which toxic events occur, plus the species found also differ. Hence, it is difficult to understand which factors stimulate DA production. Moreover, all *Pseudo-nitzschia* species do not have the ability to produce DA besides toxic ones do not synthesize DA everytime (Dursun, Yurdun, & Ünlü, 2016). Therefore, it is really important to consider regions in isolation.

Recent studies have shown the importance of a variety of factors such as temperature (Lewis, Bates, McLachlan, & Smith, 1993), salinity (Thessen, Dortch, Parsons, & Morrison, 2005), pH (Lundholm, Hansen, & Kotaki, 2004) and nutrients (Bates *et al*., 1991). Thus, it is essential to understand the factors that stimulate *Pseudo-nitzschia* growth and DA production is essential. Some species of *Pseudo-nitzschia* have been reported in the Golden Horn Estuary (GHE) (Uysal, 1996; Tas & Okus, 2003; Tas, Yilmaz, & Okus, 2009; Tas &Lundholm, 2015). The GHE region's large human population, recreational activities and multiple fisheries make it potentially vulnerable to harmful algal blooms (HABs). So, it is important to determine the occurence and spatio-temporal distribution of *Pseudo-nitzschia* and domoic acid to assess the potential for ASP in the region.

The present study aims to describe the seasonal variations of *Pseudo-nitzschia* spp. abundance and pDA production in relation to several environmental factors. The results of this study may be used for a

better understanding of the ecology of *Pseudo-nitzschia* species and to assess the potential for a recurrent and more toxic event in the GHE.

Materials and Methods

Study Area and Sampling Stations

The Golden Horn Estuary is located in the northeast of the Sea of Marmara, extending in northwest-southeast direction with 7.5 km long and 700 m wide (Figure 1). The study area was categorized in three sections; lower estuary (LE), middle estuary (ME) and upper estuary (UE) based on the hydrographic structure where six sampling stations were chosen along the estuary. The depth is 40 m at the section LE, it decreases rapidly to 14 m at the section ME and to 4 m at the section UE (Figure 1). Station FD1 is situated near the Galata Bridge at the section LE and interacts strongly with Strait of Istanbul (Bosphorus). The stations FD2 and FD3 are situated at the section ME, where there is an intermediate marine influence. The stations FD4, FD5 and FD6 are located at the section UE, and influenced by two streams (Alibey and Kagıthane). The section LE has a two layered structure with a less saline (~18‰) Black Sea water above and highly saline (~38‰) Mediterranean water below. A well developed pycnocline, which controls the transport of heat, salt and nutrients through the estuary, is placed at 18 to 28 m below the sea surface, depending on the seasons and water exchange along the Bosphorus Strait (Alpar, Burak, & Doğan, 2005).

Samplings were carried out monthly (between August 2011 and February 2012, June and July 2012) and biweekly (from March to May 2012) intervals at six stations located along the study area. A total of 180 water samples were collected by using 5 L Niskin bottles and analysed throughout the study period. Water samples were taken from three depths (0.5 m, 5 m and 10 m) at stations FD1, FD2 and FD3, and only 0.5 m at stations FD4, FD5 and FD6 depending on the water depth.

Seawater Analysis

Temperature, salinity, pH and dissolved oxygen (DO) were measured using a YSI Professional Plus multiparameter probe, and for light transparency Secchi disc was used. Samples for nutrient analysis were pre-filtered through 5 µm syringe filters and kept frozen at -20°C until analysed. Dissolved inorganic nutrient concentrations (NO_3+NO_2-N, PO_4-P, SiO_2-Si) were determined bystandart methods (APHA, 1999) using a Bran+Luebbe AA3 auto analyser. All environmental data were used as the average of 0.5 m, 5 m and 10 m depths.

For phytoplankton enumeration, 250 ml seawater was taken and fixed in 2% acidic Lugol's iodine solution (Throndsen, 1978) and 10, 25 or 50 mL sub samples were left to settle in Utermöhl sedimentation chambers (Utermöhl, 1958). *Pseudo-nitzschia* cells were counted at suitable magnifications under a Leica DM IL LED inverted light microscope. The abundance data of *Pseudo-nitzschia* were used as the average of 0.5 m, 5 m and 10 m depths.

Particulate Domoic Acid (pDA) Analysis

Duplicate portions of seawater (usually 1 L) were filtered onto a 47 mm Whatmann GF/C glass fiber filter in order todetermine pDAconcentrations in plankton samples. The filter was rolled, placed in a test tube and kept frozen until analysis. pDA concentrations were measured by pre-column derivatization with fluorenylmethoxycarbonyl (FMOC) and high performance liquid chromatography with fluorescence detection (HPLC-FLD) according to the procedure described by Pocklington *et al*. (1990). Samples were extracted as described in Quilliam, Greig, Archibald, and McInnes (1989) with slight modifications. According to this method, toxins in filters were extracted with 5 mL 10% aqueous methanol, vortexed for 2 min and centrifuged at 4.000 rpm for 10 min. The supernatant was passed through Millex-GS 0.22 µm filters (if necessary). After the extraction, FMOC was used for derivatization and samples were washed three times with ethyl-acetate, organic layer was discarded, and bottom layer was used for HPLC analysis.

Chromatographic experiments were performed by using an Agilent 1100 series HPLC system, including a quaternary pump with an inline degasser and fluorescence detector with a manual injection (50 µL) loop. DA was seperated on a Supelcosil LC-18, 5µm, 25 cm×4.6 mm column and protected by a suitable guard column. The mobile phases were, Milli-Q water plus 0.1% trifluoroacetic acid (TFA) and acetonitrile (MeCN) plus 0.1% TFA. Detection wavelengths were 264 and 313 nm for excitation and emission, respectively. The gradient elution was 40% MeCN maintained for 5 min, followed by an increase to 60% MeCN over 15 min, which was maintained for 2 min. The elution was then followed by an increase to 100% MeCN over 2 min before programming back to initial conditions over 2 min. Initial conditions were maintained for a further 8 min, resulting a total analysis time of 34 min with a flow rate of 0.8 mL min^{-1} (Dursun, 2015). DA has a retention time of 16.4 min. Six points calibration curve was linear for the range 0.02-1 µg ml^{-1} (r^2=0.99, n=3) (Figure 2). Limit of detection (LOD), based on signal to noise ratio of 3.3, was 0.2 µg ml^{-1} and limit of quantification (LOQ) was 0.6 µg ml^{-1}.

Seawater samples, spiked with DA at concentration of 5 µg ml^{-1} were extracted from pDA using the method described by Quilliam *et al*. (1989). Samples were derivatized with FMOC to determine pDA by HPLC-FLD; the recoveries of pDA in the extraction method ranged between 95% and 102% (Table 1).

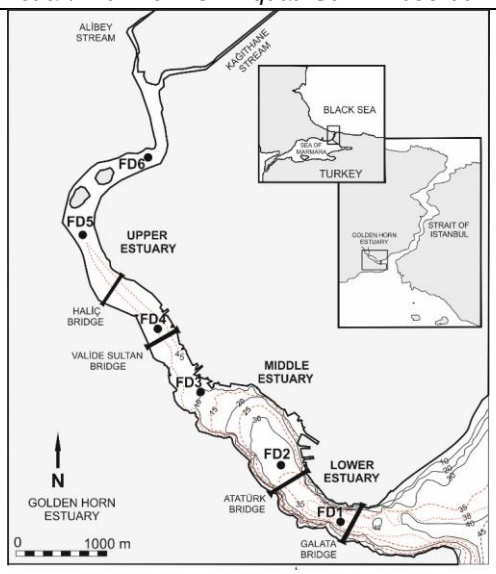

Figure 1. Study area and sampling stations.

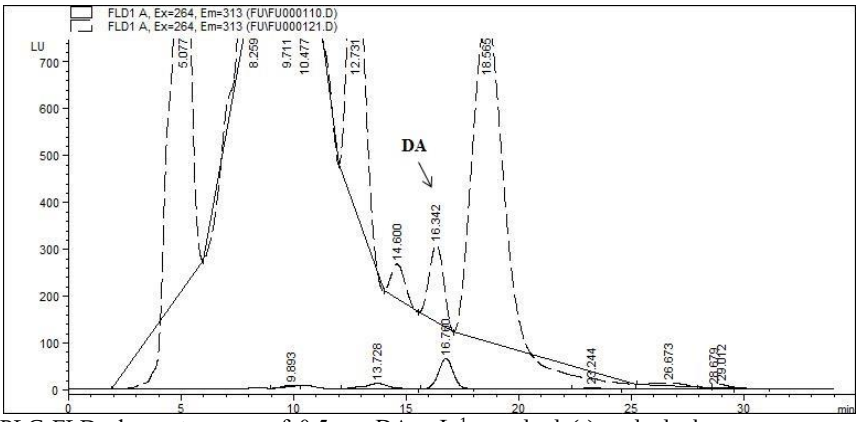

Figure 2. The HPLC-FLD chromatograms of 0.5 µg DA mL^{-1} standard (-) and plankton extract, at FD3 station, in November 2011 (- - -).

Statistical Analysis

Statistica 6.0 was used for Principal Component Analyses (PCA) to explore the temporal variations in environmental parameters, *Pseudo-nitzschia* spp. abundance and pDA concentrations. Logarithmic transformation (log(x+1)) was used on the data set to obtain the normal distribution. Standart Spearman correlation was used to quantify direct correlations between all parameters.

Results

Hydrography

The physical and chemical parameters as well as the biological variables measured in the GHE along the study period are shown in Figure 3. Temperature showed a seasonal distribution, varied between 4.1°C (at station FD1 in Feb. 2012) and 26.6°C (at station FD6 in July 2012) and increased generally from station FD1 to FD6 during the sampling period (Figure 2). Salinity ranged from 6.38 (at station FD6 in March 2012) to 20.18 (at station FD3 in Dec. 2011) and remarkably decreased from station FD1 to FD6 (Figure 3) particularly in rainy months owing considerable freshwater input by the Alibey and Kagıthane streams. Secchi depths decreased significantly from station FD1 to FD6 due to high amount of suspended materials carried by these two streams. The maximum Secchi depth was measured as 10 m at station FD1 (Dec. 2011), with a minimum of 0.3 m at station FD6 (Oct. 2011) (Figure 3).

Nutrient concentrations showed clear seasonal patterns, generally increasing over the winter months and decreasing to trace concentrations during the late spring and summer months. Nutrients increased remarkably from station FD1 to FD6 along the study area (Figure 3). NO_3+NO_2 values were detected between 0.02 µM (at station FD6, late May 2012) and 21.59 µM (at station FD4 in Apr. 2012). PO_4 concentrations ranged from 0.09 µM in Aug. 2011 (at

Table 1. Spike-recovery experiments of DA in plankton samples (n=10)

Samples	μg ml^{-1} of DA added	μg ml^{-1} of DA found	Percent recovery (%)
FD1(August)	5.00	4.96	99.2
FD2 (September)	5.00	4.91	98.2
FD1 (October)	5.00	5.10	102.0
FD1 (November)	5.00	4.93	98.6
FD3 (December)	5.00	4.90	98.0
FD4 (March)	5.00	4.85	97.0
FD1 (March)	5.00	4.82	96.4
FD6 (March)	5.00	4.75	95.0
FD3 (April)	5.00	4.86	97.2
FD5 (April)	5.00	5.06	101.2

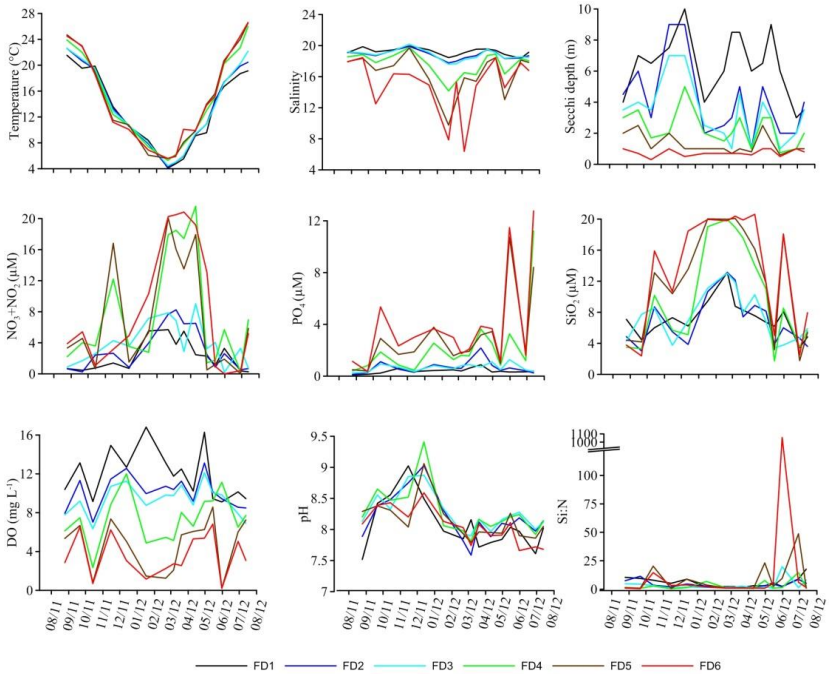

Figure 3. The spatio-temporal fluctuations in environmental parameters measured in the study area.

station FD1) and 12.76 μM in July 2012 (at station FD6). SiO_2 values differed between 1.74 μM at station FD4 (May 2012) and 20.62 μM at station FD6 (Apr. 2012). Despite low concentrations of nutrients between May and July 2012, insignificant increases were observed. Si:N ratios were recorded between 0.45 (Sept. 2011) and 1056 (late May 2012) at station FD6 and it was generally >1.0 over the study period (Figure 3). The station FD6 often had higher Si:N ratios than the other stations. Si:N ratio was remarkably higher (1056) in late May 2012 at station FD6, than the other months.

DO concentrations showed the highest values at station FD1 in Jan. 2012 and Nov. 2011 (16.8 mg L^{-1} and 16.3 mg L^{-1}, respectively) and the lowest (0.2 mg L^{-1}) at station FD6 in late May 2012 (Figure 3). DO values were generally high at station FD1 due to the strong interaction with Strait of Istanbul and it decreased from the section LE to the UE. pH values were relatively high between Sept. 2011 and Jan. 2012 and ranged from 7.5 (at station FD1 in Aug. 2011) to 9.4 (at station FD4 in Dec. 2011), showing no trend over the sampling period.

Pseudo-Nitzschia Abundance

The genus *Pseudo-nitzschia* was present throughout the year, altough as not a dense, monospecific bloom, with abundances ranging between 0.5×10^3 and 196×10^3 cells L^{-1} (Figure 4). *Pseudo-nitzschia* cells were detected in 101 of the 180 water samples (56%) analysed throughoutthis study. In general, *Pseudo-nitzschia* abundance was highest in spring, corresponding to the annual diatom bloom (Tas &Lundholm, 2015), while low abundance has occurred between Aug. 2011 and Apr. 2012. The maximum abundance of *Pseudo-nitzschia* cells were 196×10^3 cells L^{-1} at station FD3 and 194×10^3 cells L^{-1} at station FD1 in late May 2012 (Figure 3). However, in Nov. 2011, *Pseudo-nitzschia* abundances showed significant differences from seasonal pattern, 22×10^3 and 115×10^3 cells L^{-1} were counted at stations FD4

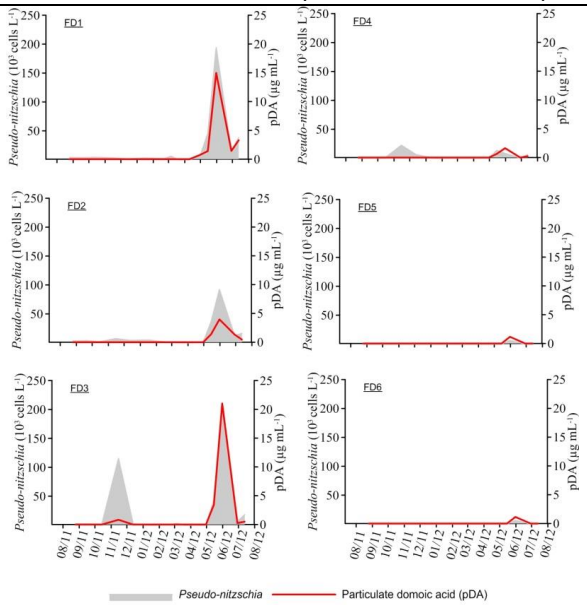

Figure 4. The spatio-temporal distributions of *Pseudo-nitzschia* and particulate domoic acid (pDA).

and FD3. Also, *Pseudo-nitzschia* species were most commonly found at the stations FD1, FD2 and FD3, and very low abundance at the station FD4, no cell was observed at stations FD5 and FD6 except in late May 2012 (6×10^3 cells L^{-1}) over the sampling period.

pDA Monitoring

Despite many of our samples contained *Pseudo-nitzschia*, pDA mostly was not a detectable and quantifiable level. pDA concentrations in seawater samples that tested positive ranged from 0.24 µg mL^{-1} at station FD4 in July 2012 to 21.03 µg mL^{-1} at station FD3 in late May 2012 (Figure 4). pDA levels were undetectable between Aug. 2011 and March 2012 period in all stations except in Nov. 2011 (0.83 µg mL^{-1}, at station FD3). However, the highest pDA concentrations were observed in late May 2012 at stations FD1, FD2 and FD3 (15, 3.97 and 21.03 µg mL^{-1}, respectively) when the *Pseudo-nitzschia* cells reached the highest abundance (194×10^3, 92×10^3 and 196×10^3 cells L^{-1}, respectively). pDA levels were generally higher at station FD3 along the study area. pDA was not observed at stations FD5 and FD6 over the sampling period except late May 2012 (1.16 µg mL^{-1} and 1.14 µg ml^{-1}, respectively) (Figure 4).

Relationship between Pda, Pseudo-Nitzschia Spp. and Environmental Variables

Pseudo-nitzschia species were observed at a wide range of temperature from 4.1°C (Feb. 2012) and 22.6°C (Aug. 2011), but high abundances occurred at a narrow temperature range (from 13.8°C to 17.4°C) in May 2012, while their abundance decreased remarkably between June and July 2012 with temperature >19°C. *Pseudo-nitzschia* species were observed at a salinity range of 13.05 (May 2012) to 20.18 (Dec. 2011) and at the highest abundance (196×10^3 cells L^{-1}, at station FD3) was 18.4 in late May 2012. Nutrient availability was highly variable (NO_3+NO_2, PO_4 and SiO_2 varied between 0.19-4.05, 0.32-1.27 and 3.42-8.09 µM, respectively) in May 2012. High inorganic nutrient concentrations in the early spring provided an increase in abundance of *Pseudo-nitzschia*. *Pseudo-nitzschia* cell density decreased significantly from station FD3 to FD6; correlating with a decreasing Secchi depth and it almost disappeared at stations FD5 and FD6 due to low light availability. According to the Spearman correlation coefficients (r), *Pseudo-nitzschia* abundance was positively correlated ($P<0.01$, $n=90$) with temperature, salinity and dissolved oxygen during the study period. However, there were significant negative correlations between *Pseudo-nitzschia* abundance and inorganic nutrient concentrations ($P<0.01$) for all stations, also there was a significant negative correlation between *Pseudo-nitzschia* abundance and SiO_2 concentrations ($P\leq0.001$) (Table 2).

Spearman correlation coefficients showed that there were significant correlations between pDA and environmental factors such as temperature, NO_3+NO_2 and SiO_2 during the study period (Table 2). The levels of SiO_2 and NO_3+NO_2 were negatively correlated with pDA concentrations as was the abundance of *Pseudo-nitzschia* but there was not any significant correlation between PO_4 and pDA. No correlation was also found between pDA concentrations and salinity, DO. These results show that pDA concentration is mostly controlled by temperature, SiO_2 and NO_3+NO_2.

There was not always a relation between *Pseudo-nitzschia* abundance and pDA concentrations during the study period. Although *Pseudo-nitzschia* abundance was high (115×10^3 cells L^{-1}) in Nov. 2011

at station FD3, DA value was low (0.83 µg ml^{-1}) when compared with the mean abundances (Figure 4). However, the concentrations of pDA started to increase in early May 2012 following the increase in abundance of *Pseudo-nitzschia.* Moreover, the highest pDA concentration (21.03 µg ml^{-1}) was detected at station FD3 when *Pseudo-nitzschia* abundance reached the highest level (196×10^3 cells L^{-1}) in late May 2012.

Data Analysis

Time based Principal Component Analysis (PCA) results showed that the first two components (PC1 and PC2) explained 48% of the variance of the data set during the study period in months which detected pDA (Figure 5). The first principal component (PC1) accounted for 30.8% of the total variance and strongly related to salinity, PO_4, DO, SiO_2 and temperature while NO_3+NO_2 and pH acted as primary factors on the second principal (PC2) component which explained 16.7% of the total variance. The decrease in PO_4, SiO_2 and temperature had a negative effect on *Pseudo-nitzschia* abundance and pDA concentrations over the course of the study period when compared to pH and NO_3+NO_2 (Figure 5a). Furthermore, the increase in salinity and DO had a positive effect on pDA concentrations. The PCA ordination diagram showed monthly separation and it is indicated that the highest variations were in Nov. 2011, April and May 2012, while June and July 2012 showed similar environmental features (Figure 5b).

Discussion

In recent years, environmental factors which stimulate HAB events, such as eutrophication, chemical pollutants, coastal development, fisheries activities, climate change etc. have been investigated around the world. Besides, it is known that every species has different adaptation strategies and responses to the environmental changes in the marine ecosystem. Thus, regional monitoring programs for detecting biotoxin concentrations, to protect ecosystem and public health, have a great importance to identify relationships between marine biotoxins and physico-chemical parameters (Lundholm *et al*., 2004; Thessen *et al*., 2005; Klein *et al*., 2010; Maric *et al*., 2011; Sahraoui *et al*., 2012).

Pseudo-nitzschia was known as a member of diatom community with low abundances in the GHE in the previous years (Uysal &Unsal, 1996; Tas & Okus, 2003).However, recent studies showed that *Pseudo-nitzschia* abundance has increased compared to the past. *P. delicatissima* and *P. pungens* were present in almost every year between June 1998 and May 2002 with the highest abundance (1.6×10^6 cells L^{-1}) in May and June (Tas *et al*., 2009). In another study carried out at the GHE, two blooms of *Pseudo-nitzschia* were reported in Jan. and May 2010 (Tas & Lundholm, 2015); however no study was performed on domoic acid occurence in the area.

Liefer, MacIntyre, Novoveska, Smith and Dorsey (2009) has stated that *Pseudo-nitzschia* species were observed over a wide range of temperature (4.1 to 22.6°C), which is also supported by our results, but mainly higher abundances occured between 13.8 and 17.4°C in late May 2012, while it started to decrease at 19°C in July 2012. During the sampling period, *Pseudo-nitzschia* species were found at a wider range of salinity (13.05 to 20.18) than in the previous study (Tas &Lundholm, 2015), but they reached highest cell density at 18.4 in late May 2012 along similar lines with the current study. The high *Pseudo-nitzschia* abundances with low temperatures and high salinity is similar to the pattern for *P.calliantha* in the Adriatic Sea (Caroppo, Congresti, Bracchini, & Albertano, 2005) and *Pseudo-nitzschia* spp. on the West Coast of the United States (Trainer *et al*., 2000). In our study area, *Pseudo-nitzschia* spp. wasnot found at salinity below 10 and temperature above 23°C. This is consistent with laboratory growth experiments showing a lower salinity limit at 7 (Thessen *et al*., 2005).

These results argue a relatively broad tolerance of both salinity and temperature for *Pseudo-nitzschia* spp. in the study area. Spearman correlation analysis indicates that, high salinity and temperature may favor high *Pseudo-nitzschia* abundances, but generally low correlation coefficients ($P<0.01$) suggests a weak dependence on these physical parameters in the GHE. It is unclear if our results are caused by a direct relationship between *Pseudo-nitzschia* abundance and salinity, and temperature. Other environmental variables, such as nutrients are known to affect *Pseudo-nitzschia* abundance in the Gulf of Mexico (Dortch *et al*., 1997). For example, a significant but weak correlation was recorded for phosphate (-0.13) and nitrogen (-0.15) with *Pseudo-nitzschia* abundance in the Gulf of Mexico which is similar to the results of this study. However, silicate showed strong correlation with *Pseudo-nitzschia* abundance ($p\leq0.01$, -0.40) in our current study.

Previous studies from other estuarine environments reported that pDA concentrations have significant positive correlation with *Pseudo-nitzschia* abundances (Bargu, Goldstein, Roberts, Li, & Gulland, 2012; Parsons *et al*., 2013). In this research, analysis of pDA data using statistical analysis allowed us to say that pDA concentration was measured during late May 2012 wasmore likely to be associated with the occurence of *Pseudo-nitzschia* spp. However, during the first occurence of *Pseudo-nitzschia* spp. in November 2011, very low pDA levels were detected. This might be caused by several environmental factors such as nutrient limitation, precipitation, strong vertical mixing etc. It is known that domoic acid production is stimulated by nutrient stress, as a result of phosphate or silicate limitation (Fehling, Davidson, & Bates, 2004). Therefore, if we consider

Table 2. Spearman correlation coefficients (r) between *Pseudo-nitzschia*, particulate domoic acid (pDA) and environmental parameters (n=90

Environmental parameters		All stations (r)
Temperature	*Pseudo-nitzschia*	0.30*
	pDA	0.24*
Salinity	*Pseudo-nitzschia*	0.25*
	pDA	-
NO_3+NO_2	*Pseudo-nitzschia*	-0.32*
	pDA	-0.21*
PO_4	*Pseudo-nitzschia*	-0.32*
	pDA	-
SiO_2	*Pseudo-nitzschia*	-0.40**
	pDA	-0.28*
DO	*Pseudo-nitzschia*	-0.24*
	pDA	-

*Significant correlations are indicated by symbols: *P<0.01; **P≤0.001

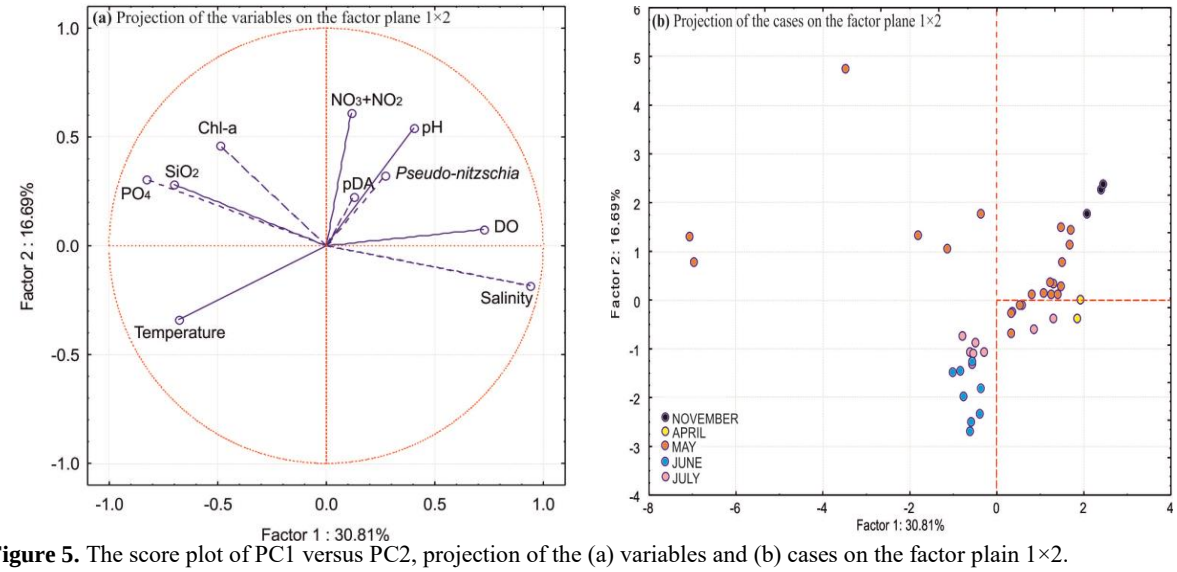

Figure 5. The score plot of PC1 versus PC2, projection of the (a) variables and (b) cases on the factor plain 1×2.

the environmental conditions at the time pDA was detected at high levels, lower Si:N ratio (19.86) at station FD3 than station FD6 (1056) during the late May 2012 was probably due to the consumption of high amounts of silica at station FD3 by *Pseudo-nitzschia* species which may stimulate the production of domoic acid at this sampling point.

It is reported that correlation between *Pseudo-nitzschia* abundance and pDA concentration is not significant when the study area does not have sufficient water circulations (Takahashi *et al*., 2007; Schnetzer *et al*., 2007; Bargu *et al*., 2012). In the sampling area, if we consider the water circulations at stations FD5 and FD6, it is possible to say that unsuitable environmental conditions at the section UE may limit the growth of *Pseudo-nitzschia* species and also their toxin production.

In the study area, pDA concentrations were detected similar to the other regions (Walz *et al*., 1994; Trainer *et al*., 2000; Busse *et al*., 2005; Parsons *et al*., 2013) which experienced DA toxicity events. However, previous studies have reported toxic blooms of *Pseudo-nitzschia* spp. in Monterrey Bay (Bargu, Powell, Wang, Doucette, & Silver, 2008), Gulf of Mexico (MacIntyre *et al*., 2011), Los Angeles harbour (Schnetzer *et al*., 2007) and coastal Washington state (Trainer *et al*., 2009) with maximal pDA values of 8.49, 8.0, 12.7 and 13.0 µg mL^{-1} respectively; while the highest pDA concentration was measured as 21.03 µg mL^{-1} in late May 2012 in the GHE. Altough a regulatory value for DA in seawater has not been defined yet and concentrations have been measured at moderate level in the GHE, it has never suffered any ASP events, in spite of the presence of toxic *Pseudo-nitzschia* species. However, the abundance of these species was never at a bloom level (>10^6 cells L^{-1}) during the study period. According to these conclusions, the question is "Why has no ASP event been observed in the Golden Horn Estuary?" One possibility is that *Pseudo-nitzschia* species might be diluted by other phytoplankton species to allow for significant amounts of pDA to move to higher trophic

levels. Another possibility is that dominant *Pseudo-nitzschia* species may produce insufficient domoic acid to yield a large toxic impact in higher trophic levels (Downes-Tetmar, Rowland, Widdicombe, Woodward, & Llewellyn, 2013).

In this research, the marine biotoxin, domoic acid (DA), was monitored in the GHE for one year. *Pseudo-nitzschia* spp. abundance at this area seemed to react differently to the environmental conditions. Although some *Pseudo-nitzschia* species produced generally low pDA concentrations, there is a potential for toxin production to occur in this area. pDA production mostly occured in May 2012 when water temperature increased and there was silicate limitation in seawater. These findings indicate that pDA may be produced in similar conditions to the study area. However, more investigation containing all environmental variables is needed to get a clear understanding of the *Pseudo-nitzschia* occurence and pDA production.

In conclusion, the present study shows the importance of the detailed and frequent monitoring of environmental variables to understand the pDA production process and *Pseudo-nitzschia* bloom dynamics. Our study did not include an assesment of pDA accumulation in fish and shellfish exposed to *Pseudo-nitzschia* blooms, but that would be a logical next step as humans and animals have not become ill from DA in water or algae, but are intoxicated through a shellfish or fish vector (Hallegraeff, 2003). In addition, these information is important for biotoxin and harmful algae monitoring programmes and can be used to build early warning systems for toxic algal blooms.

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