Toxicon 122 (2016) 54–66

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

# Algal toxins and producers in the marine waters of Qatar, Arabian Gulf

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ARTICLE INFO

*Article history:*
Received 25 June 2016
Received in revised form
18 September 2016
Accepted 20 September 2016
Available online 21 September 2016

*Keywords:*
Arabian Gulf
Amnesic shellfish toxin
Cyclic imine
Diarrhetic shellfish toxin
Paralytic shellfish toxin
Pinnatoxin
Portimine
*Vulcanodinium rugosum*

ABSTRACT

Harmful Algal Bloom species are ubiquitous and their blooms occur in the Arabian Gulf. In this study, two cruises were performed in 2012 and 2013 to collect phytoplankton samples from 4 sites in the Arabian Gulf. Toxin analyses of phytoplankton samples for 32 algal toxins from 5 different toxin groups were conducted on the samples using both enzyme linked immunosorbent assay (ELISA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Results demonstrated, for the first time, the presence of paralytic shellfish toxins (PSTs), diarrhetic shellfish toxin (DST), amnesic shellfish toxin (AST), cyclic imines (CIs) and polyether-lactone toxins in freeze-dried phytoplankton samples. Four *Vulcanodinium rugosum* cultures were established from field samples and these proved to contain between 603 and 981 ng pinnatoxin (PnTx) H per mg dry weight in addition to being positive for portimine. These strains from Qatar clustered with strains from Japan and Florida based on large subunit rRNA and rRNA internal transcribed spacer gene sequences.



## 1. Introduction

The Arabian (Persian) Gulf (hereafter the Gulf) is a partially-enclosed shallow sea at the northwest part of the Indian Ocean and connects to the ocean through the narrow Strait of Hormuz (Reynolds, 1993). The Gulf is a major, and for some countries, the only drinking/municipal water and seafood source in the region. In addition to naturally high temperatures and salinity levels, the Gulf has been under serious anthropogenic environmental disturbances, including coastal dredging, harmful effluents, overfishing, reduced freshwater input, and intense maritime traffic resulting in the transfer of exotic organisms from ballast water (Sheppard et al., 2010). Some of the aforementioned environmental disturbances have been linked to increased occurrences of dinoflagellates and Harmful Algal Blooms (HABs) in the Gulf (Richlen et al., 2010; Sheppard et al., 2010).

HABs have the potential to produce a wide variety of toxins in marine waters, including, paralytic shellfish toxins (PSTs), diarrhetic shellfish toxins (DSTs), polyether-lactone toxins, amnesic shellfish toxin (AST) and cyclic imines (CIs). PSTs are a class of neurotoxic alkaloids (57 analogs) that can be trophically transferred in the food web through vector organisms, such as bivalves and crustaceans (Wiese et al., 2010). Intoxication in humans can cause respiratory paralysis and death in severe cases (Cusick and Sayler, 2013; Wiese et al., 2010). To date, PSTs have been detected in some freshwater cyanobacteria (Foss et al., 2012) in addition to some marine dinoflagellate genera, including *Alexandrium*, *Pyrodinium* and *Gymnodinium* (Wiese et al., 2010).

DSTs are acidic polyether toxins including okadaic acid (OA) and dinophysistoxins (DTX 1, DTX 2 and their derivatives), presenting gastrointestinal symptoms including diarrhea and vomiting when consumed (Van Dolah, 2000). These toxins have been identified in marine dinoflagellate genera including *Dinophysis* and *Prorocentrum* (Fux et al., 2011; Reguera et al., 2014; Vale et al., 2009). *Dinophysis* species are also the only known producer of the pectenotoxins (PTXs), a group of poly-ether lactone toxins. PTXs exhibit

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http://dx.doi.org/10.1016/j.toxicon.2016.09.016
0041-0101/

hepatotoxicity in mice after intraperitoneal injection and are not considered diarrhetic, but are commonly grouped with the OA-group of toxins (Miles et al., 2004).

Amnesic shellfish poisoning is caused by the neurotoxin domoic acid (DA) through vector organisms such as shellfish or fish. DA is a tricarboxylic amino acid and glutamate receptor agonist (Van Dolah, 2000), causing calcium toxicity in neurons (Bejarano et al., 2008). Intoxication symptoms in humans include gastrointestinal problems and neurological disorders, such as memory loss, hence the name "amnesic shellfish poisoning" (Bejarano et al., 2008). DA is produced by several *Pseudo-nitzschia* species and *Nitzschia navis-varingica* (Kotaki et al., 2000).

CIs are a group of marine shellfish toxins with a common macrocycle structure and a cyclic imine moiety. CIs include the pinnatoxins (PnTxs), portimine, pteriatoxins, gymnodimines (GYMs), prorocentrolides, spiro-prorocentrimine, and spirolides (SPX) (Selwood et al., 2010). Since the compounds have similar chemical structures and toxicity, they have been grouped together. Generally, the CIs have the ability to inhibit acetylcholine receptors resulting in a wide range of neurotoxic symptoms (Bourne et al., 2010; Hellyer et al., 2015; Kharrat et al., 2008; MacKenzie et al., 2005; Munday et al., 2012, 2004; Selwood et al., 2013; Wandscheer et al., 2010). CIs are produced either by dinoflagellates or through shellfish metabolism. GYM has been isolated from the dinoflagellate *Karenia selliformis* (Miles et al., 2003, 2000). Interestingly, in 1999, a bloom of this species in the Kuwait bay was implicated in kills of wild and aquacultured fish, but toxins were not measured during that study (Heil et al., 2001). PnTxs, to date, have only been detected in the dinoflagellate *Vulcanodonium rugosum* (Hess et al., 2013; Rhodes et al., 2011, 2010; Selwood et al., 2014; Smith et al., 2011; Zeng et al., 2012). Portimine has also only been detected in *V. rugosum* cultures (Selwood et al., 2013).

Although routine monitoring of phytoplankton and HAB species does not currently exist in most of the Gulf, scattered short term studies provide some information on the spatial and temporal distribution of phytoplankton. Phytoplankton productivity is highest in the northern Gulf due to nutrients supplied by river discharges (Sheppard et al., 2010). Productivity decreases, but species diversity increases towards south (Subba Rao and Al-Yamani, 1998). Tropical diatoms and dinoflagellates dominate the reported phytoplankton species in the Gulf, with a few additional cyanobacteria species (e.g. *Trichodesmium* [Al-Muftah, 1991; Polikarpov et al., 2016; Subba Rao and Al-Yamani, 1998];). Primary production in the marine waters surrounding the Qatari Peninsula is low, but higher on the eastern coast and in the winter (Nour El-Din and Al-Khayat, 2005; Quigg et al., 2013). Although Dorgham and Al-Muftah (1986), and Al-Muftah (1991) identified over 250 species of phytoplankton along the Qatari coastline, Nour El-Din and Al-Khayat (2005) and Quigg et al. (2013) identified around 100 species; all dominated by diatoms.

The first HAB leading to fish mortalities in the Gulf was reported in 1999 from Kuwaiti coastal waters, where *Karenia selliformis* numbers reached $6 \times 10^6$ cells $L^{-1}$. This bloom resulted in kills of wild and aquacultured fish (Heil et al., 2001). Two years later a *Ceratium furca* bloom was blamed for Sea Bream mortalities in net pens, in the Kuwait bay (Glibert et al., 2002). Investigations of harmful algal species in the area during and after fish kills revealed the presence of high numbers of *Gymnodinium catenatum*, *Gyrodinium impudicum* and *Pyrodinium bahamense* var. *bahamense*, along with lower numbers of *Alexandrium* spp. and *Karenia* sp. Fish and shellfish were tested for saxitoxins and brevetoxins. Only the clam tissues were positive for saxitoxins, measuring 1.26 μg STX eq 100 $g^{-1}$ via a receptor binding assay (Glibert et al., 2002). Blooms of other potentially harmful species were also reported from Kuwait marine waters in 2000, including *Trichodesmium erythraeum* ($10^8$ cells $L^{-1}$), *Pseudo-nitzschia seriata* ($10^9$ cells $L^{-1}$), *Prorocentrum* spp. ($4.3 \times 10^6$ cells $L^{-1}$) and *Gymnodinium* spp. ($2.9 \times 10^6$ cells $L^{-1}$) (Al-Yamani et al., 2012). A cruise covering 43 sites in the Arabian Gulf between February and March 2006 illustrated the dominance of gymnodinoid dinoflagellates followed by cryptophytes, diatoms and other dinoflagellate groups. Cell numbers exceeding $10^6$ cells $mL^{-1}$ were recorded at the central Gulf off Qatar (Polikarpov et al., 2016).

The most notable HAB event occurred between 2008 and 2009, when an 8-month bloom of the toxic dinoflagellate species *Cochlodinium polykrikoides* persisted in the coastal waters of Qatar, United Arab Emirates (UAE) and Oman resulting in massive die-offs of fish, marine mammals and coral reefs. The event also led to closures of desalination plants (Al-Azri et al., 2014; Richlen et al., 2010; Zhao and Ghedira, 2014).

Although potentially toxic dinoflagellate and diatom species and their blooms have been reported in the Gulf, to the authors' knowledge, Glibert et al. (2002) was the only study to measure algal toxins. Therefore the focus of this study was to investigate the presence of a variety of algal toxins in the Gulf and in dinoflagellate cultures isolated from the Qatari marine waters. Thirty-two toxins from 5 algal toxin groups were analyzed and included PSTs, DSTs, polyether toxins, AST and CIs. Qualitative algal identification and culture isolations were also conducted in order to help elucidate the source of the toxins detected in the Gulf.

## 2. Materials and methods

### 2.1. Study area and sampling

The Gulf is 1000 km long, 338 km wide (Fig. 1, inset), with an average depth of about 36 m (Reynolds, 1993). The State of Qatar is a peninsula located in the southwestern Gulf covering an area of approximately 11,500 $km^2$ with over 560 km of contiguous coastline (Fig. 1). In this present study two cruises were carried out on R/V Janan of Qatar University on October 18, 2012 and November 13, 2013 for the sampling stations shown in Fig. 1. These stations were chosen to sample both near-shore and off-shore marine waters of Qatar. Water depths of stations 1 to 4 were 10 m, 24 m, 28 m and 34 m, respectively. Vertical profiles of temperature, conductivity, pH and dissolved $O_2$ (DO) were obtained with YSI multiparameter probes connected to a YSI datalogger. Horizontal phytoplankton tows were performed at the surface with 20-μm and 50-μm phytoplankton nets for 10 min at each site, with a total of 7 samples collected over the study period. Samples for microscopic analyses were preserved with the addition of 5% (w/v) formalin or Lugol's solution. These were utilized for qualitative phytoplankton analyses as presence, or absence, at different sites on different sampling dates using a Leica upright microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Scanning Electron Microscopy (SEM) analyses were used to identify some dinoflagellates. For SEM, sample was washed with distilled water (5–7 times) and dehydrated through an ethanol series (50%, 70%, 90%, 100%), dried and coated with gold-palladium to examine on a Nova NanoSEM 450 (FEI, Hillsboro, Oregon, USA). Background in SEM photos of dinoflagellates was removed using Adobe Photoshop CC (Adobe Systems Incorporated, CA, USA). Taxonomic identifications were conducted according to various methods (Hallegraeff et al., 2010; Taylor, 1976; Tomas, 1997; Tregouboff and Rose, 1957; Wood, 1968). Phytoplankton tow samples (pooled 20-μm and 50-μm fractions) for toxin analyses were carried to the laboratory on ice, centrifuged in a Thermo Scientific SL40R (Thermo Scientific, Waltham, MA, USA) at 2400×*g* for 10 min, frozen and freeze-dried in a FreeZone 12 L Console Freeze Dry System (Labconco, Kansas City,

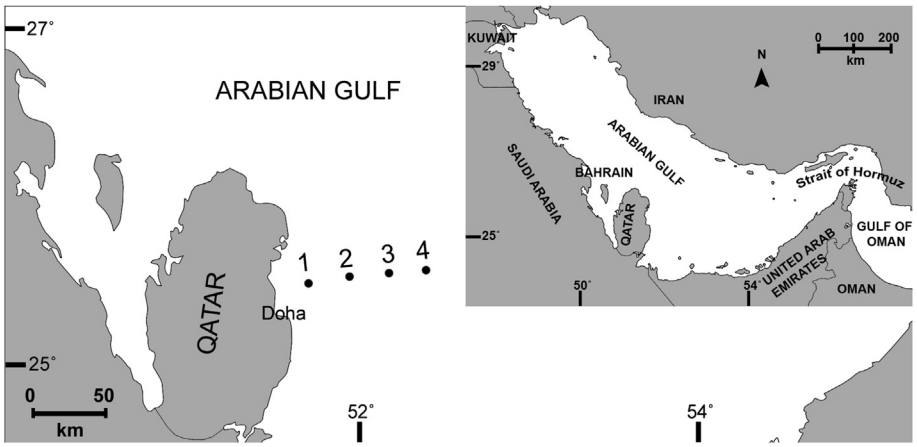


**Fig. 1.** Map of Qatar with sampling sites east of the Qatar Peninsula. Figure inset shows Qatar within the Gulf region. Latitudes and longitudes of sampling sites are: Site 1 (25.481667 N, 51.715533 E), Site 2 (25.521783 N, 51.957700 E), Site 3 (25.542333 N, 52.190866 E), Site 4 (25.559733 N, 52.409449 E).

MO, USA).

### 2.2. Standards and reagents

Standards of saxitoxin (STX), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), gonyautoxin 1&4 (GTX 1&4), gonyautoxin 2&3 (GTX 2&3), gonyautoxin 5 (GTX 5), decarbamoylgonyautoxin 2&3 (dcGTX 2&3) and N-sulfocarbamoyl-gonyautoxin 2 and 3 (C1&C2), domoic acid (DA), gymnodimine (GYM), desmethyl spirolide C (SPX DMC), desmethyl spirolide D (SPX DMD), pectenotoxin 1 (PTX 1), pectenotoxin 11 (PTX 11), pectenotoxin 2 (PTX 2), pectenotoxin 2 seco acid (PTX 2SA), okadaic acid (OA), dinophysis toxin 1 (DTX 1) and dinophysis toxin 2 (DTX 2), yessotoxin (YTX), homoyessotoxin (hYTX), azaspiracid 1 (AZA 1), azaspiracid 2 (AZA 2), and azaspiracid 3 (AZA 3) were certified reference materials from the National Research Council, Canada (NRC,Halifax, Nova, Scotia, Canada). Pinnatoxins (PnTxs) E, F, G and H were isolated at the Cawthron Institute. Standard reference material saxitoxin dihydrochloride (RM 8642 FDA) was from the National Institute of Standards and Technology (Gaithersberg, MD). If necessary, standards were diluted with either 0.003 M hydrochloric acid or 0.01 M acetic acid. HPLC grade acetonitrile, glacial acetic acid, hydrochloric acid (certified ACS) (>99%), formic acid, ammonium formate and methanol were utilized. All HPLC mobile phases were filtered through 0.45 μm PVDF Millipore filters (Thermo Fisher Scientific, Waltham, MA, USA) before use. Deionized water (18 MΩ-cm) was provided by a Pure Lab Ultra Filtration System (Siemens Water Technologies Corp. Warrendale, PA, USA) or by a Milli-Q system (Millipore, Nepean, ON, Canada).

### 2.3. Algal toxin analyses

#### 2.3.1. Enzyme linked immunosorbent assay for paralytic shellfish toxins (PSTs), diarrhetic shellfish toxins (DSTs) and amnesic shellfish toxin (AST)

Enzyme linked immunosorbent assays (ELISAs) for PSTs, DSTs (Abraxis LLC, PA, USA) and AST (Biosense Laboratories AS, Norway) were performed on freeze-dried phytoplankton tow samples (n = 7) and wet algal cultures (n = 16). Five to 10 mg of dried phytoplankton sample from each site and 10–15 mg wet culture were extracted in 500 μL of 80% methanol with glass beads for PST and DST ELISAs; extractions for AST were performed similarly but in milli-Q water. Extracts were centrifuged in a Mikro 120 centrifuge (Andreas Hettich GmbH & Co.KG Tuttlingen, Germany) at 15,000×*g* for 10 min. Supernatants were diluted with the kit provided diluents. Each sample was analyzed in triplicate, with standard deviations calculated. Plates were read on a Biotek Synergy H4 microplate reader (Bio Tek, Winooski, VT, USA). Concentration calculations were made according to instructions provided with each kit. After initial ELISA analyses, samples with enough remaining material were used for confirmation analyses using LC-MS/MS.

#### 2.3.2. Paralytic shellfish toxin (PST) analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS)

All the samples collected in tow events from each station (n = 7) were analyzed for PSTs by LC-MS/MS. Each dried sample was subjected to 0.1 M acetic acid at a sample concentration of 100 mg $mL^{-1}$. Samples were placed in a boiling water bath (105 °C) for 5 min and then allowed to cool. After 1 min of continuous vortexing, samples were centrifuged at 1500×*g* for 10 min. The supernatant fractions were filtered (0.45-μm PVDF) and diluted further to a sample concentration of 10 mg $mL^{-1}$ and 1 mg $mL^{-1}$ with 0.003 M HCl for analysis. A Thermo Electron Corporation TSQ Quantum Access MAX mass spectrometer with a Surveyor MS Pump Plus and Auto-sampler were utilized for LC-MS/MS of PSTs. Separations were achieved using a Tosoh Bioscience TSKgel Amide-80 column (5 μm; 2 × 250 mm) (Tosoh Bioscience LLC, Grove City, OH). The following gradient using mobile phase A (2 mM formic acid and 3.6 mM ammonium formate in deionized water) and B (95% acetonitrile (v/v) in 2 mM formic acid and 3.6 mM ammonium formate) was used: 35% A held for 8 min, to 70% A over 8 min, back to 35% A over 6 min and held at 35% A for 4 min. Toxins used to calibrate this method were STX, NEO, dcSTX, GTX 1, GTX 2, GTX 3, GTX 4, GTX 5, dcGTX 2, dcGTX 3, C1 and C2. Post extraction spikes were prepared using STX, dcSTX, NEO, GTX 1–5, dcGTX 2&3 and C1&C2 at a range of concentrations from 0.3 ng $mg^{-1}$ to 10 ng $mg^{-1}$. Method detection limits (MDLs) were estimated from the instrument detection limits and a 2 point standard addition of the sample matrix. Table 1 shows MS/MS transitions monitored and MDLs.

#### 2.3.3. Analysis of domoic acid (DA), cyclic imines (CI) and lipophilic toxins by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS)

Samples collected in 2013 from Stations 3 & 4 (n = 2) and cultures (n = 4) were analyzed using UPLC-MS/MS for the lipophilic toxins, CIs and DA. The lyophilized samples were extracted with 1 mL methanol–water (4:1). Samples were placed in an ultrasonic

**Table 1**
Transitions monitored for paralytic shellfish toxins (PSTs). Toxins analyzed were saxitoxin (STX), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), gonyautoxins 1(GTX 1), 2 (GTX 2), 3 (GTX 3), 4 (GTX 4) and 5 (GTX 5), decarbamoylgonyautoxins 2 (dcGTX 2) and 3 (dcGTX 3), N-sulfocarbamoyl-gonyautoxin 2 and 3 (C1/C2). MDL stands for method detection limit and *m/z* for mass to charge ratio.

| Toxin | Molecular ion (*m/z*) | Fragment ions (*m/z*) | MDLs (ng $mg^{-1}$ dw) |
|---|---|---|---|
| STX | 300 | 282, 221, 204 | 0.05 |
| NEO | 316 | 282, 220, 138, 237 | 0.20 |
| dcSTX | 257 | 239, 222, 138 | 0.05 |
| GTX 1 | 412 | 332, 314, 253 | 0.05 |
| GTX 2 | 396 | 316, 298, 220 | 0.05 |
| GTX 3 | 396 | 316, 298, 220 | 0.08 |
| GTX 4 | 412 | 332, 314, 253 | 0.05 |
| GTX 5 | 380 | 300, 282, 204 | 0.05 |
| dcGTX 2 | 353 | 273, 255, 196 | 0.04 |
| dcGTX 3 | 353 | 273, 255, 196 | 0.01 |
| C1/C2 | 396 | 316, 298 | 0.15 |

bath for 10 min, centrifuged at 17,000×*g* for 5 min, and the supernatants were transferred to autosampler vials. To determine the concentration of esterified forms of OA and DTXs, a 200-μL subsample was hydrolyzed by adding 25 μL of 2.5 M NaOH and heating for 40 min at 76 °C. The hydrolyzed sample was cooled on ice, and then neutralized with 25 μL of 2.5 M acetic acid. Analyses were performed using a Waters Acquity ultra performance liquid chromatograph i-class interfaced to a Waters Xevo TQ-S triple-quadrupole mass spectrometer with a Z-Spray electrospray ionization (ESI) source (Waters Corporation, Milford, MA, USA). A Waters Acquity Shield RP C18 1.7 μm 50 × 2 mm column (column temperature 40 °C, injection volume 1 μL, flow rate 0.6 mL $min^{-1}$) was eluted using a gradient with eluent A; water/acetonitrile (95:5 v/v) and eluent B; acetonitrile/water (95:5 v/v), both containing 50 mM formic acid/33 mM ammonia (Table 2). Detection parameters were: ESI source at 100 °C with $N_2$ for nebulization (7 bar), desolvation (1000 L $h^{-1}$ at 500 °C), cone gas at 150 L $h^{-1}$, capillary 2.0 kV, cone 40 V, and collision gas argon (0.15 mL $min^{-1}$), with data acquired on multiple reaction monitoring (MRM) channels listed in Table 3.

### 2.4. Dinoflagellate isolation and culture

Water samples collected from the cruise or from coastal areas in Doha (within the vicinity of 25.298372 N, 51.520272 E) were inoculated in dilution series in 1/20 diluted L1 (Guillard and Hargraves, 1993) or Aquil (Price et al., 1989) medium in 96-well plates (Andersen and Kawachi, 2005). From the environmental samples, single cell dinoflagellate isolations were also performed with a micropipette and inoculated in 1/20 L1 or Aquil medium in 96-well plates. Dinoflagellate species growing in dilution series plates were further isolated with a micropipette into full strength media, if necessary. All monoalgal cultures were deposited at Qatar University Culture Collection of Microalgae and Cyanobacteria (QUCCCM) and stocks were kept in full strength 10 mL medium in screw-cap culture tubes. Plates and culture tubes were incubated in a Panasonic MLR-352H-PE Plant Growth Chamber (Panasonic Biomedical Sales Europe B.V., BTW nr. NL) at 25 °C, 50 μmol photons $m^2$ $s^{-1}$ light intensity and with a 12:12 h light–dark photoperiod. Tube cultures were used in molecular work and ELISA analyses. *Vulcanodinium rugosum* cultures were further scaled-up in 250 mL media at 25 °C, 100 μmol photons $m^2$ $s^{-1}$ light intensity and with a 12:12 h light–dark photoperiod. Cells were harvested by centrifugation in a Thermo Scientific SL40R (Thermo Scientific, Waltham, MA, USA) at 2400 × *g* for 10 min, washed with sterile distilled water and re-centrifuged. Pellets were frozen and freeze-dried (FreeZone, Labconco, Kansas City, MO, USA) for LC-MS/MS analysis.

**Table 2**
The Ultra Performance Liquid Chromatography (UPLC) gradient used for the domoic acid, cyclic imine and lipophilic toxin method.

| Time (min) | Eluent A% | Eluent B% | Flow (mL $min^{-1}$) | Curve |
|---|---|---|---|---|
| 0.00 | 100 | 0 | 0.500 | Initial |
| 0.20 | 100 | 0 | 0.500 | 6 |
| 0.50 | 85 | 15 | 0.500 | 6 |
| 1.00 | 70 | 30 | 0.500 | 6 |
| 5.00 | 20 | 80 | 0.500 | 6 |
| 7.50 | 0 | 100 | 0.500 | 1 |
| 8.00 | 100 | 0 | 0.500 | 1 |

### 2.5. DNA isolation, polymerase chain reaction (PCR) and phylogenetic analysis

Culture tubes were centrifuged in a Thermo Scientific SL40R (Thermo Scientific, Waltham, MA, USA) at 2400 × *g* for 10 min. Pellets were washed with sterile distilled water and centrifuged again. DNA isolation from pellets was performed with the GenElute Plant Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA). For the amplification of the dinoflagellate large subunit rRNA (LSU rRNA) gene and internal transcribed spacer (ITS) region covering ITS1-5.8S-ITS2 rRNA genes, primer pairs D1R-D3B (Nunn et al., 1996; Scholin et al., 1994) and ITSfor- ITSrev (Murray et al., 2012a) were used, respectively. Each PCR reaction contained 10 ng of genomic DNA, 20 pmol of each primer (IDT Inc., Coralville, IA, USA), 200 μM of each deoxynucleoside triphosphate (GeneAmp, Applied Biosystems, CA, USA), 1.5 mM $MgCl_2$, 10 μL of 5x green buffer and 1.25 units of GoTaq DNA polymerase (Promega, WI, USA) in a total volume of 50 μL. Polymerase chain reaction was performed on a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) and included an initial denaturation step of 2 min at 95 °C followed by 33 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. Final extension was performed for 5 min at 72 °C. Amplified products were run on a 1.5% agarose gel containing EnviroSafe DNA/RNA stain (Helixx Technologies Inc., Ontario, Canada) and visualized with UV transillumination. Bands were excised from gels and purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany). DNA quantification of purified PCR fragments was performed using Nanodrop (Thermo Scientific, Wilmington, DE, USA). Purified PCR products (20–30 ng for each reaction) were sequenced on both strands with primers used in PCR reactions on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences obtained were manually corrected using Mega, version 5.2 (Tamura et al., 2011). Homologous sequences were obtained by Basic Local Alignment Search Tool (BLAST) implemented at the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and downloaded into Mega, version 5.2 alignment explorer (Tamura et al., 2011). Sequence alignment was performed with Clustal W using default settings. While LSU rRNA sequence alignment contained 102 sequences and 944 nucleotide positions with gaps, ITS sequence alignment contained 147 sequences and 669 nucleotide positions including gaps. Best model that fits the alignment was estimated and maximum-likelihood (ML) trees were constructed using the Tamura-Nei model (Tamura and Nei, 1993) of nucleotide substitution with gamma-distributed rate variation in Mega 5.2 (Tamura et al., 2011). Robustness of the tree was estimated by bootstrapping with 1000 replicates. New LSU rRNA and ITS region gene sequences obtained in this work were deposited in GenBank under accession numbers KX853174–KX853189, and KX853190–KX853203, respectively.

**Table 3**
Multiple reaction monitoring (MRM) channels monitored for the domoic acid, cyclic imine and lipophilic toxin method. Analyzed toxins were domoic acid (DA), gymnodimine (GYM), desmethyl spirolide C (SPX DMC) and D (SPX DMD), pinnatoxins (PnTX) E,F,G and H, azaspiracids (AZA) 1, 2, and 3, pectenotoxins (PTX) 1, 2, and 11, pectenotoxin 2 seco acid (PTX 2SA), okadaic acid (OA), dinophysis toxins (DTX) 1 and 2, yessotoxin (YTX), and homoyessotoxin (hYTX). MDL stands for method detection limit. The transitions in italics are secondary channels. They were not used for quantitation but for confirmation.

| Toxin | Mode | Transition | Cone voltage | Collision energy (eV) | MDLs (ng mg dw$^{-1}$) |
|---|---|---|---|---|---|
| DA | ES+ | 312.1 > 266.1 | 30 | 16 | 0.02 |
| | *ES+* | *312.1 > 161.0* | *30* | *25* | |
| GYM | ES+ | 508.4 > 490.3 | 40 | 25 | 0.002 |
| SPX DMC | ES+ | 692.5 > 164.1 | 50 | 50 | 0.002 |
| SPX DMD | ES+ | 694.5 > 164.1 | 50 | 50 | 0.002 |
| PnTX E | ES+ | 784.5 > 164.1 | 50 | 50 | 0.002 |
| PnTX F | ES+ | 766.5 > 164.1 | 50 | 50 | 0.002 |
| PnTX G | ES+ | 694.5 > 164.1 | 50 | 50 | 0.002 |
| PnTX H | ES+ | 708.5 > 164.5 | 50 | 50 | 0.002 |
| AZA 1 | ES+ | 842.5 > 654.5 | 50 | 55 | 0.002 |
| AZA 2 | ES+ | 856.5 > 645.5 | 50 | 45 | 0.002 |
| AZA 3 | ES+ | 828.5 > 640.5 | 50 | 55 | 0.002 |
| PTX 2 | ES+ | 876.6 > 823.5 | 40 | 25 | 0.005 |
| PTX 1 | ES+ | 892.5 > 839.5 | 40 | 25 | 0.010 |
| PTX 11 | ES+ | 892.5 > 839.5 | 40 | 25 | 0.010 |
| PTX 2SA | ES+ | 894.5 > 823.5 | 40 | 25 | 0.010 |
| OA | ES+ | 827.5 > 723.4 | 70 | 50 | 0.004 |
| | *ES−* | *803.5 > 255.2* | *80* | *45* | |
| DTX 2 | ES+ | 827.5 > 723.4 | 70 | 50 | 0.004 |
| | *ES−* | *803.5 > 255.2* | *80* | *45* | |
| DTX 1 | ES+ | 841.5 > 737.4 | 70 | 50 | 0.004 |
| YTX | ES − | 1141.5 > 1061.5 | 50 | 33 | 0.004 |
| hYTX | ES − | 1155.5 > 1075.5 | 50 | 33 | 0.004 |

## 3. Results and discussion

### 3.1. Physical parameters at collection sites

The depth of the Gulf in the vicinity of Qatar in this study increased from station 1 to station 4 (10m–34 m). In general, the vertical profiles of temperature, salinity, DO, and pH were similar for all the stations over the study period (Table S1). There was a slight increase in salinity (≤1 ppt) from 10 to 20 m or 27 m depths for some stations (e.g. Station 2 of 2012), concomitant with a slight decrease of DO (<1 mg L$^{-1}$). Salinity (~46 ppt) and DO values (~5.5 mg L$^{-1}$) were similar for both years. Temperature and pH generally showed a uniform distribution at different depths. The temperatures at the 2012 stations (30 °C-33 °C) were higher than of those sampled in 2013 (27 °C-28 °C). Stations sampled in 2013 had higher pH values than stations sampled in 2012 (~7.9 vs. ~7.5, respectively). Field data suggested vertical mixing of the whole water column, or at least to 20 m depths for some stations (e.g. Station 3 of 2013). In a study conducted by Quigg et al. (2013) mixing of the whole water column was also observed for stations sampled east of the Qatar peninsula (February, May and July of 2010 and 2011).

### 3.2. Paralytic shellfish toxins

Paralytic shellfish toxins (PSTs) detected and quantified in field samples using LC-MS/MS were STX, NEO, GTX 5 and dcSTX (Table 4). The remaining PSTs (GTX 1–4, dcGTX 2–3 and C1/C2) were not detected above method detection limits in this study. Station 1 from the 2013 cruise did not have any detectable PSTs, both with LC-MS/MS and ELISA. All stations but one (station 1, 2013) had measurable STX. Neosaxitoxin (NEO) was observed at stations 1 and 2 of 2012 and station 3 of 2013. Gonyautoxin 5 (GTX 5) was present in all stations but one (station 1, 2013). Decarbamoylsaxitoxin (dcSTX) was detected only in Station 1 and 2 of 2012. Gonyautoxin 5 had the highest percent contribution among detected PSTs from ~52% to ~90% in different stations. The PST ELISA used in this study reports varying cross-reactivity with the different analogs of PST toxins, with STX = 100%, NEO = 1.3%, GTX 5 = 23% and dcSTX = 29% cross-reactive. Based on the reported cross-reactivity by the manufacturer, expected ELISA responses from LC-MS/MS results were generally in good agreement with the obtained ELISA values (Table 4).

Neither LC-MS/MS nor ELISA analysis techniques account for the actual toxicity of PSTs detected in samples, as different analogs have varying toxicity. Therefore, the European Food Safety Authority has attempted to address this issue through the use of toxicity equivalency factors (TEFs), which help express the detected PST analogs as saxitoxin equivalents (Alexander et al., 2009). The TEFs were derived using acute *i.p.* toxicity with mice, with STX = 1, NEO = 1, dcSTX = 1 and GTX 5 = 0.1. This approach was applied to the LC-MS/MS PST data and is illustrated in Table 4.

#### 3.2.1. Paralytic shellfish toxin producers

The potential PST producers observed in sample collections included *Alexandrium* sp. and *Pyrodinium bahamense* (Table 5, Figs. 2F and 3D). Both genera have been previously observed in the Gulf (Al-Kandari et al., 2009; Dorgham and Al-Muftah, 1989; Glibert et al., 2002; Steidinger et al., 1980).

*Alexandrium* species, including *A. catenella, A. tamarense*, *A. fundyense*, *A. minutum*, and *A. ostenfeldii* have been known to produce various combinations of PST analogs (Wiese et al., 2010). One species, *A. tamarense* has been shown to be non-toxic, as well as producing a variety of analogs, including STX, NEO, GTX 1–4, and C1-2 (Ichimi et al., 2002; Parkhill and Cembella, 1999; Wiese et al., 2010), as well as dcSTX and GTX 5 (Borkman et al., 2014; Murray et al., 2012b). This underlines the strain specific differences in toxin production. Many PST producing *Alexandrium* species share overlapping morphological characteristics, making identification to species level challenging, as within this study. Therefore, it is currently unknown if the *Alexandrium* observed in this study was a known toxin producer and inferences cannot be made about which PSTs it may have contributed to in samples. Previous studies have shown that isolates of *P. bahamense* var. *compressum* from Palau and Malaysia produce the analogs STX, NEO, GTX 5–6, and dcSTX (Harada et al., 1983, 1982; Oshima, 1989; Usup et al., 1994). A

**Table 4**
Concentrations of paralytic shellfish toxins (PSTs), diarrhetic shellfish toxins (DSTs) and amnesic shellfish toxin (AST) in freeze-dried phytoplankton samples as determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) and/or enzyme linked immunosorbent assay (ELISA). Analyzed toxins were saxitoxin (STX), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), gonyautoxin (GTX) 1 to 5, decarbamoylgonyautoxin (dcGTX) 2 and 3, N-sulfocarbamoyl-gonyautoxin 2 and 3 (C1/C2), okadaic acid (OA), dinophysis toxins (DTX) 1 and 2, domoic acid (DA). For DSTs and AST an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was used. All concentrations are in ng mg $dw^{-1}$ except for DA which is in pg mg $dw^{-1}$. BD denotes values below detection limit and – denotes samples not tested for the particular toxin. SD stands for standard deviation.

| Algal toxins (ng mg $dw^{-1}$) | 18 October 2012 cruise | | | 13 November 2013 cruise | | | |
|---|---|---|---|---|---|---|---|
| | Station 1 | Station 2 | Station 3 | Station 1 | Station 2 | Station 3 | Station 4 |
| **PSTs** | | | | | | | |
| STX | 2.4 | 4.0 | 3.1 | BD | 0.3 | 1.1 | 0.2 |
| NEO | 0.4 | 1.5 | BD | BD | BD | 0.4 | BD |
| GTX 5 | 15.1 | 55.7 | 26.2 | BD | 0.3 | 9.2 | 1.5 |
| dcSTX | 0.4 | 4.2 | BD | BD | BD | BD | BD |
| GTX 1 | BD | BD | BD | BD | BD | BD | BD |
| GTX 2 | BD | BD | BD | BD | BD | BD | BD |
| GTX 3 | BD | BD | BD | BD | BD | BD | BD |
| GTX 4 | BD | BD | BD | BD | BD | BD | BD |
| dcGTX 2 | BD | BD | BD | BD | BD | BD | BD |
| dcGTX 3 | BD | BD | BD | BD | BD | BD | BD |
| C1/C2 | BD | BD | BD | BD | BD | BD | BD |
| PST ELISA (±SD) | 6.23 ± 0.71 | 21.20 ± 2.07 | 15.92 ± 1.05 | BD | 0.31 ± 0.05 | 4.76 ± 0.43 | 0.89 ± 0.24 |
| Expected ELISA response from LC-MS/MS results (using reported kit cross-reactivity) | 5.99 | 18.05 | 9.13 | BD | 0.37 | 3.22 | 0.55 |
| Saxitoxin Toxicity Equivalents (STX eq.) | 4.7 | 15.3 | 5.7 | | 0.3 | 2.4 | 0.5 |
| **DSTs** | | | | | | | |
| OA | – | – | – | – | – | BD | 0.006 |
| DTX 1 | – | – | – | – | – | BD | BD |
| DTX 2 | – | – | – | – | – | BD | BD |
| Total OA[a] | | | | | | BD | 0.008 |
| DST ELISA (±SD) | 1.37 ± 0.23 | 2.04 ± 0.49 | 0.99 ± 0.27 | 0.94 ± 0.11 | 0.99 ± 0.11 | 1.00 ± 0.21 | 0.81 ± 0.18 |
| **AST** | | | | | | | |
| DA (pg mg $dw^{-1}$) | – | – | – | – | – | BD | 198 |
| DA ELISA (pg mg $dw^{-1}$) (±SD) | 16.69 ± 1.57 | 31.80 ± 4.48 | 37.11 ± 1.11 | BD | BD | BD | 110.14 ± 12.11 |

[a] Total OA is the concentration of OA and OA released by alkaline hydrolysis.

**Table 5**
Qualitative list of potentially harmful phytoplankton species observed during 2012 and 2013 cruises. – denotes "not observed", + denotes "observed". Multiple + signs reflect the abundance observed based on the empirical judgement of the taxonomist.

| Species name | 18 October 2012 cruise | | | 13 November 2013 cruise | | | |
|---|---|---|---|---|---|---|---|
| | Station 1 | Station 2 | Station 3 | Station 1 | Station 2 | Station 3 | Station 4 |
| **Dinoflagellates** | | | | | | | |
| *Alexandrium* sp. | – | + | + | – | – | – | – |
| *Ceratium* sp. | + | +++ | ++ | + | ++ | ++ | ++ |
| *Dinophysis caudata* | – | ++ | + | + | ++ | + | ++ |
| *D. miles* | – | + | +++ | + | + | + | + |
| *D. rotundata* | – | + | + | – | + | + | + |
| *Gonyaulax* sp. 1 | – | – | + | + | + | + | + |
| *Gonyaulax* sp. 2 | + | + | + | – | + | + | + |
| *Prorocentrum gracile* | + | + | + | + | ++ | + | ++ |
| *P. micans* | + | + | ++ | + | + | + | + |
| *P. minimum* | – | + | + | – | + | + | + |
| *P. sigmoides* | – | + | + | + | + | + | + |
| *Protoceratium* sp. | + | + | + | + | + | + | + |
| *Pyrodinium bahamense* | + | + | ++ | + | ++ | ++ | ++ |
| *Scrippsiella* sp. | – | + | + | + | + | + | + |
| *Lingulodinium* sp. | – | – | + | – | + | + | + |
| *Vulcanodinium rugosum* | – | – | – | – | + | + | + |
| **Diatoms** | | | | | | | |
| *Pseudonitzschia* sp. (*P. seriata* complex) | + | ++ | ++ | – | ++ | + | + |
| *Pseudonitzschia* sp. (*P. delicatissima* complex) | + | + | + | – | + | + | + |
| **Cyanobacteria** | | | | | | | |
| *Trichodesmium* sp. | – | – | +++ | + | + | + | + |

Philippine isolate of *P. bahamense* var. *compressum* produced a high ratio of STX (~90% during exponential growth), with dcSTX and GTX 5 in culture (Gedaria et al., 2007). In contrast, *Pyrodinium* in Florida have been shown to produce a higher ratio of GTX 5 (73–90%), with less STX and dcSTX (Landsberg et al., 2006).

The PST analogs detected in Qatari marine waters were STX, NEO, GTX 5 and dcSTX. Although GTX 6 was not measured in this study, PST composition found in Stations 1 and 2 of 2012 cruise is similar to what was found for the Palau and Malaysian isolates of *P. bahamense* var. *compressum*. The absence of NEO and dcSTX in some of the other stations might be due to below detection limit concentrations, which showed low percentage contributions in

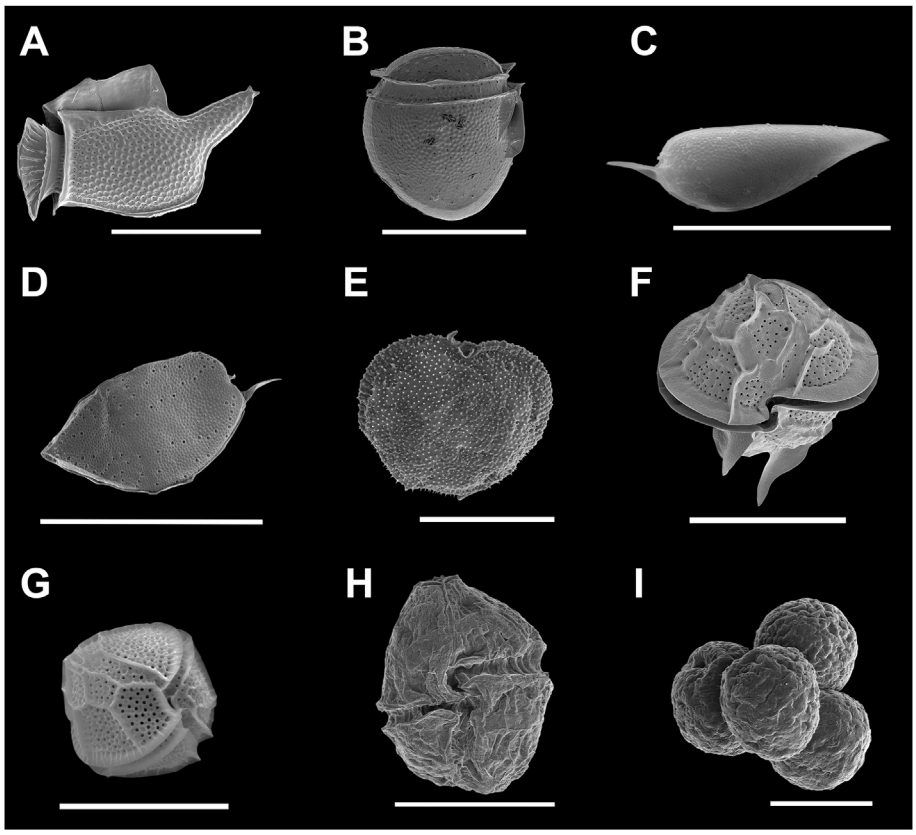


**Fig. 2.** SEM photographs of some of the observed dinoflagellate species in the Gulf. A) *Dinophysis caudata* (scale bar 50 μm), B) *Dinophysis rotundata* (=*Phalocrama rotundatum*) (scale bar 40 μm), C) *Prorocentrum gracile* (scale bar 50 μm), D) *Prorocentrum micans* (scale bar 40 μm), E) *Prorocentrum minimum* (scale bar 10 μm), F) *Pyrodinium bahamense* (scale bar 50 μm), G) *Lingulodinium* sp. (scale bar 40 μm), H) *Vulcanodinium rugosum* culture QUCCCM 92 (scale bar 20 μm), I) *V. rugosum* culture QUCCCM 92 non-motile cells (scale bar 20 μm).

Stations 1 and 2 of 2012 (i.e. 2%–6%). *Pyrodinium bahamanse* was observed at all stations for both years, while *Alexandrium* sp. was present only at stations 2 and 3 of 2012 cruise (Table 5). Isolates would be required to elucidate specific PST analog contributions from the observed potential PST producers.

### 3.3. Diarrhetic shellfish toxins (DSTs) and pectenotoxins (PTXs)

All stations had detectable levels of OA-equivalents using ELISA, from 0.8 to 2 ng mg $dw^{-1}$ (Table 4). Confirmatory UPLC-MS/MS analysis in this study was performed only for stations 3 and 4 of 2013, due to insufficient phytoplankton biomass from other stations. The DTXs (DTX 1 & 2) tested by UPLC-MS/MS were below detection limits for both station 3 and 4. Okadaic acid (OA) was below detection for station 3, but was confirmed in station 4 (0.006 ng mg $dw^{-1}$). Based on the reported cross-reactivities by the manufacturer and UPLC-MS/MS data, the expected ELISA responses for station 3 and 4 would have been BD and 0.006 ng mg $dw^{-1}$, respectively. However, ELISA resulted in levels of DSTs at around 1 ng mg $dw^{-1}$ for all samples.

The observed discrepancy between methods for DSTs could be due to a number of reasons. One possibility is the presence of a C8 Diol metabolite reported to react with the ELISA at 52%, but was not tested by UPLC-MS/MS. Another possibility is that additional unknown DTX related compounds may have reacted with the ELISA, such as DTX 5b. Finally, the responses may also be due to non-specific interactions from non-related compounds resulting in false positive data.

Stations 3 & 4 (2013) were tested for the pectenotoxins PTX1, 2, 2SA and 11. While PTX 1 and PTX 11 were below detection limits, PTX 2 and PTX 2SA were detected and quantified at stations 3 and 4 of 2013 cruise (Table 6). Pectenotoxin 2 (PTX 2) has been suggested to be converted enzymatically to PTX 2SA in net haul materials due to damaged cells or in shellfish tissues. However, there have been reports of PTX 2SA presence in picked cells of live *Dinophysis acuta* (Puente et al., 2004). It is unknown if the presence of PTX 2SA in this work is due to enzymatic transformation or original production by the organism.

#### 3.3.1. Diarrhetic shellfish toxin and PTX producers

Both *Dinophysis* and *Prorocentrum* have common occurrences in plankton surveys in the Gulf, including the species reported in Table 5 (Al-Kandari et al., 2009; Al-Yamani et al., 2012; Dorgham and Al-Muftah, 1989; Heil et al., 2001; Quigg et al., 2013). All the *Dinophysis* species in Table 5 (Figs. 2A,B and 3A) have been implicated in toxin production from different parts of the world. *Dinophysis caudata* has a distribution in tropical/subtropical seas or during warm seasons in temperate seas (Luisa Fernández et al., 2006). Individually picked cells of *D. caudata* from Singapore (Holmes et al., 1999), the Philippines (Marasigan et al., 2001), and Spain (Luisa Fernández et al., 2006) revealed that strains of this species may produce OA, DTX 1 and/or PTX 2. *Dinophysis miles* has a common occurrence in the Arabian Sea area, but also have been observed from the Mediterranean to different regions of the Indian and West Pacific Oceans (Reguera et al., 2014). Picked cells of *D. miles* from the Philippines contained OA and DTX 1 (Marasigan et al., 2001). *Dinophysis rotundata* (= *Phalacroma rotundatum*) is a more cosmopolitan species and some strains have been reported to

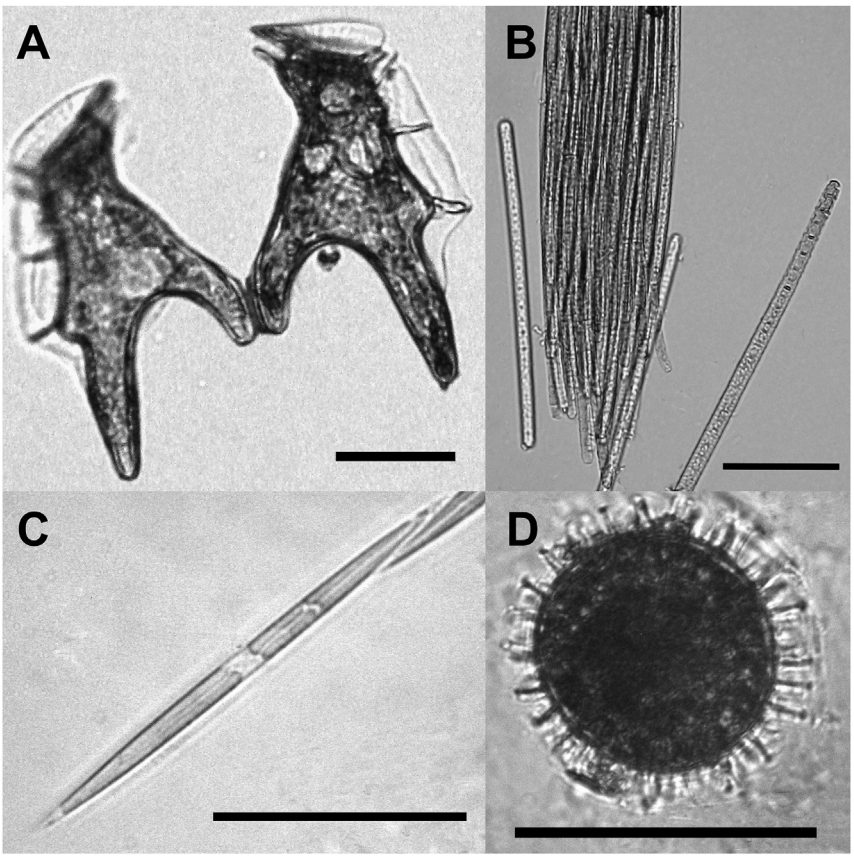


**Fig. 3.** Light microscopy photographs of some of the observed phytoplankton species in the Gulf. A) *Dinophysis miles* (scale bar 40 μm), B) *Trichodesmium* sp. (scale bar 100 μm) C) *Pseudonitzschia* sp. (*P. seriata* complex) (scale bar 50 μm), D) *Pyrodinium bahamense* cyst (scale bar 50 μm).

**Table 6**
Concentrations of various other algal toxins in plankton tow samples and *V. rugosum* cultures as determined by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Analyzed toxins were pinnatoxins (PnTX) E,F,G and H, portimine, gymnodimine (GYM), desmethyl spirolide C (SPX DMC) and D (SPX DMD), azaspiracids (AZA) 1, 2 and 3, pectenotoxins (PTX) 1, 2 and 11, pectenotoxin 2 seco acid (PTX 2SA), yessotoxin (YTX), and homoyessotoxin (hYTX). Portimine was present at both stations and *V. rugosum* cultures, but could not be quantified due to a lack of a standard. BD denotes values below detection limit, and – denotes samples not tested for the particular toxin.

| Algal toxins (ng mg dw$^{-1}$) | 13 November 2013 cruise | | *V. rugosum* cultures | | | |
|---|---|---|---|---|---|---|
| | Station 3 | Station 4 | QUCCCM91 | QUCCCM92 | QUCCCM93 | QUCCCM94 |
| **PnTxs** | | | | | | |
| PnTx E | BD | BD | BD | BD | BD | BD |
| PnTx F | BD | BD | BD | BD | BD | BD |
| PnTx G | 0.006 | 0.006 | BD | BD | BD | BD |
| PnTx H | 0.043 | 0.047 | 772 | 603 | 842 | 981 |
| **Other toxins** | | | | | | |
| Portimine | Present | Present | Present | Present | Present | Present |
| GYM | 0.117 | 0.078 | – | – | – | – |
| SPX DMC | BD | BD | – | – | – | – |
| SPX DMD | BD | BD | – | – | – | – |
| AZA 1 | BD | BD | – | – | – | – |
| AZA 2 | BD | BD | – | – | – | – |
| AZA 3 | BD | BD | – | – | – | – |
| PTX 1 | BD | BD | – | – | – | – |
| PTX 2 | 0.277 | 0.367 | – | – | – | – |
| PTX 2SA | 0.013 | 0.041 | – | – | – | – |
| PTX 11 | BD | BD | – | – | – | – |
| YTX | BD | BD | – | – | – | – |
| hYTX | BD | BD | – | – | – | – |

produce DTX 1 and PTX 2 (Suzuki et al., 2009).

The observed *Prorocentrum* species in Table 5 (Fig. 2C–E) are all planktonic and, to the authors' knowledge, none of them have been implicated in DST production. Several benthic species of *Prorocentrum* (*P. belizeanum, P. faustiae, P. maculosum, P. lima*, and *P. rhathymum*) have been shown to produce OA, DTX 1, DTX 2, and/or their derivatives (Cruz et al., 2006; Fernández et al., 2003; Macpherson et al., 2003; Morton et al., 1998; Vale et al., 2009).

Although not observed in this study, the latter two species have been reported in the Gulf (Al-Kandari et al., 2009; Quigg et al., 2013). *Prorocentrum minimum* was observed in this study and has been shown to produce an uncharacterized water soluble neurotoxic compound in Mediterranean isolates (Denardou-Queneherve et al., 1999; Grzebyk et al., 1997). Four *Prorocentrum* sp. isolated from coastal samples during this study (Figs. S1 and S2) were negative for DST production using the DST ELISA (data not shown). Based on the observed dinoflagellates and above information, sources of both OA and PTX 2/PTX 2SA seem to be the *Dinophysis* species listed in Table 5.

### 3.4. Domoic acid (DA/AST)

Enzyme linked immunosorbent assay (ELISA) analyses indicated DA presence in all 2012 collections (~16—~37 pg DA mg $dw^{-1}$) and in one 2013 collection (Station 4), as reported in Table 4. Only stations 3 & 4 of 2013 were analyzed using UPLC-MS/MS due to limited sample biomass. UPLC-MS/MS confirmed the presence of DA (198 pg DA mg $dw^{-1}$) at station 4, which was measured at 110.14 ± 12.11 pg DA mg $dw^{-1}$using ELISA.

#### 3.4.1. Domoic acid producers

Domoic acid (DA) production is mainly attributed to several *Pseudo-nitzschia* species (Kotaki et al., 2000). The taxonomic identification to the species level was not possible for the observed *Pseudo-nitzschia* sp. (Fig. 3C) in this study, and taxonomic groupings were based on the transapical axis lengths (Table 5) (Hasle and Syvertsen, 1996). Several members of observed *P. seriata* and *P. delicatissima* complexes are known producers of DA (Bates et al., 1998, 1989). Both *Pseudo-nitzschia* complexes were observed at all stations, but one (station 1, 2013) (Table 5). Although *Pseudo-nitzschia* sp. was present in Stations 2 & 3 (2013), the ELISA was below detection for DA. This observation may suggest the presence of toxic and non-toxic *Pseudo-nitzschia* sp. in the sampled waters. However, cell counts with more precise taxonomic identifications and cultures/picked cells would be needed to confirm this. The potential DA producers *P. pungens*, *P. delicatissima* and *P. seriata* have all been previously reported in the Gulf (Al-Kandari et al., 2009; Al-Yamani et al., 2012; Dorgham and Al-Muftah, 1989).

### 3.5. Cyclic imines (CIs)

During this study stations 3 & 4 (2013) were analyzed for the CIs pinnatoxins (PnTxs) E, F, G, H, portimine, gymnodimine (GYM), and spirolides (SPX DMC/DMD) with UPLC-MS/MS (Table 6). The compound GYM was detected at both stations, while SPX DMC/DMD were below detection limits. Stations 3—4 analysis also revealed the presence of PnTx-H, PnTx-G, and portimine at both stations, with PnTx E and F not detected in the dry net haul material.

#### 3.5.1. Cyclic imine producers

*Karenia selliformis* was not observed during the phytoplankton survey (Table 5) and the source of GYM in the marine waters of Qatar is currently unknown. Desmethyl spirolide C and D (SPX DMC and DMD) were also not detected at these stations, which is in agreement with the absence of the *Alexandrium ostenfeldii/peruvianum* complex dinoflagellates, which were reported to produce these compounds (Borkman et al., 2012; Cembella et al., 1999).

*Vulcanodinium rugosum* is the only known producer of PnTxs and portimine (Selwood et al., 2013). New Zealand isolates have been reported to produce PnTx E and F (Rhodes et al., 2010); South Australian isolates PnTX A, E, F, and G (Rhodes et al., 2011); Japanese and French isolates PnTx G (Hess et al., 2013; Smith et al., 2011) and South China Sea isolates produce PnTx H (Selwood et al., 2014; Zeng et al., 2012). A strain isolated in Hawaii and another one from a ship's ballast sediment in Florida did not produce any PnTxs, illustrating that not all strains are toxin producers (Garrett et al., 2014). Non-motile cells of *Vulcanodinium rugosum* were observed at stations 2, 3 and 4 of the 2013 cruise (Table 5) and this is the first observation of this species in the Gulf.

*3.5.1.1. Vulcanodinium rugosum cultures.* Dilution cultures of samples collected from Stations 2, 3 & 4 (2013) yielded 4 mono-algal isolates, consisting mostly of non-motile cells with few vegetative cells (Fig. 2H and I), as reported previously in cultures of this species (Garrett et al., 2014; Rhodes et al., 2011, 2010). These isolates (QUCCCM91-94) produced PnTx H (603 ng mg $dw^{-1}$ to 908 ng mg $dw^{-1}$), and portimine as determined by UPLC-MS/MS (Table 6). Differences in the observed toxin content may be due to the different media used in this study and/or the metabolic state of the cultures (Abadie et al., 2015; Rhodes et al., 2010).

All LSU rRNA gene and ITS region sequences of *V. rugosum* cultures from Qatar (this study), Japan and Florida were identical for the regions analyzed. Therefore these strains clustered with high bootstrap support in both LSU rRNA and ITS ML trees (Figs. 4 and 5). The Florida strain of *V. rugosum* was isolated from the ballast sediment of a ship and authors postulated that the strain might have originated from Japan, where the ship had previously travelled (Garrett et al., 2014). Given the intense maritime traffic in the Gulf, this scenario is plausible for *V. rugosum* observed in this study. Although strains from Qatar, Japan and Florida share identical gene sequences, their toxin compositions are different. While Qatari strains produce PnTx H and portimine; strains from Japan produce only PnTx G (Smith et al., 2011). The Florida strain produces only portimine and does not produce any PnTxs (Garrett et al., 2014). The only other strain that produces PnTx H and portimine is from China (Selwood et al., 2014; Zeng et al., 2012). However LSU rRNA gene and ITS region sequences of this strain show only 97% and 86% identity, respectively, to sequences of strains from Qatar.

In both LSU rRNA and ITS ML trees, New Zealand and Australian strains formed a separate cluster, as reported previously (Garrett et al., 2014; Zeng et al., 2012). *Vulcanodinium* strain G65 from China was not included in the LSU rRNA ML tree (Fig. 4) since the available sequence was only 596 bp long. Based on LSU rRNA gene sequences, strain G65 from China was most closely allied to sequences of New Zealand/Australian strains (99% identity). On the other hand LSU rRNA gene sequence identities of strain G65 to those of strains from Qatar/Japan/Florida and France were lower (97% identity). Similarly, for the ITS region, sequence of strain G65 was more similar to those of strains from New Zealand and Australia (98% identity). In both LSU and ITS ML trees, *V. rugosum* strain IFR10-017 from France was separated from other *V. rugosum* strains. Interestingly, while its LSU rRNA gene sequence was most closely allied to New Zealand/Australia strain sequences (98% identity), based on ITS region sequences strain from France was more closely allied to strains from Qatar/Japan/Florida and China (89% identity vs. 88% identity to New Zealand/Australia sequences).

The presence of PnTx G in the net haul material suggests that there may be other strains of *V. rugosum* that can produce this toxin in the Gulf. Another possibility is that, under the culture conditions used in this study, PnTx G was present, but below detection limits using UPLC-MS/MS.

### 3.6. Azaspiracids and yessotoxins

Stations 3 & 4 (2013) were tested for azaspiracids (AZA 1, 2, 3) and yessotoxins (YTX and hYTX). The polyether marine toxins, azaspiracids, are produced by *Azadinium* species (Krock et al., 2009). Neither the *Azadinium* genus nor the associated toxins

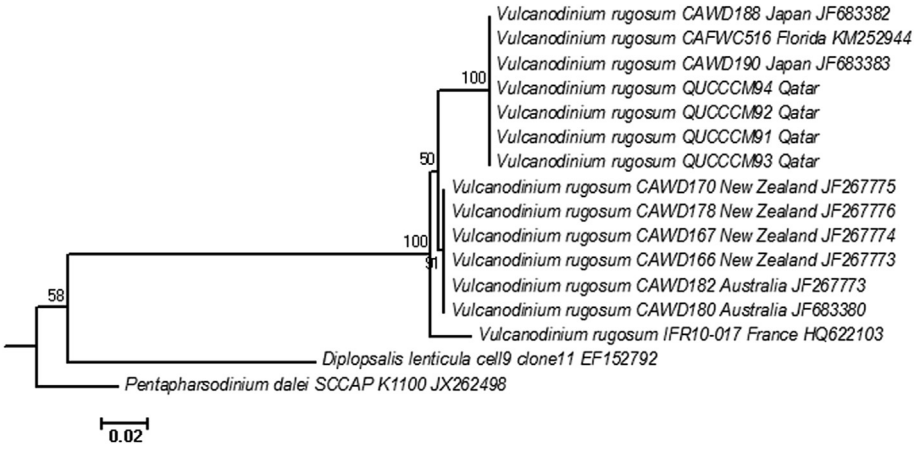


**Fig. 4.** Maximum likelihood tree of partial LSU rRNA gene sequences of *Vulcanodinium rugosum* and closely related species. This tree was extracted from a larger LSU rRNA gene sequence tree including other dinoflagellate species isolated in Qatar (Fig. S1) using Mega, version 5.2 (Tamura et al., 2011). Bootstrap values above 50% are shown next to the nodes. Branch lengths are proportional to the number of substitutions per site (see the scale bar).

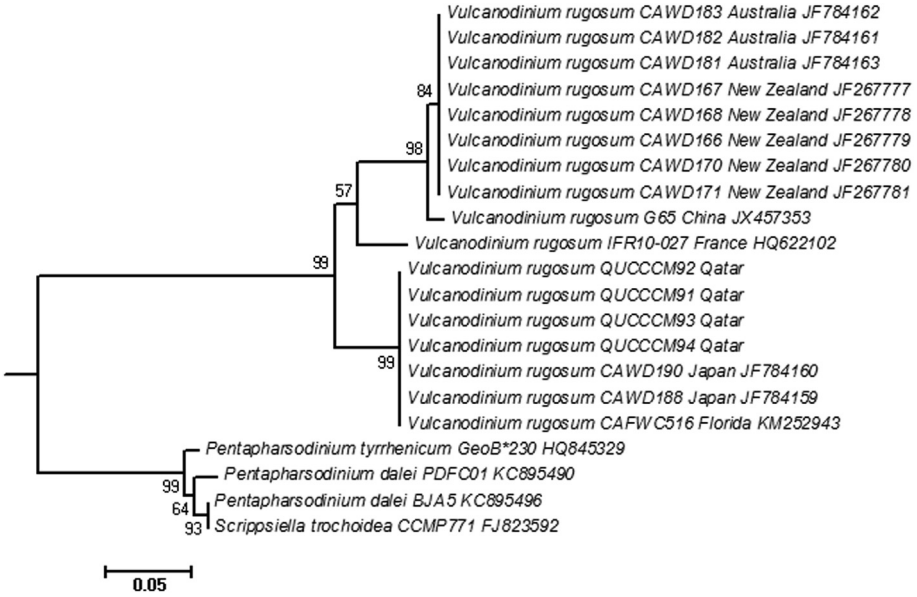


**Fig. 5.** Maximum likelihood tree of partial ITS region sequences of *Vulcanodinium rugosum* and closely related species. This tree was extracted from a larger ITS region sequence tree including other dinoflagellate species isolated in Qatar (Fig. S2) using Mega, version 5.2 (Tamura et al., 2011). Bootstrap values above 50% are shown next to the nodes. Branch lengths are proportional to the number of substitutions per site (see the scale bar).

were observed in this study. Although some of the potential yessotoxin producers were observed (Paz et al., 2008) (Table 5 - *Protoceratium* sp, *Lingulodinium* sp [Fig. 2G]. and *Gonyaulax* sp.), YTX and hYTX were not detected above method detection limits.

### 3.7. Other HAB species

During the phytoplankton surveys in 2012 and 2013, *Ceratium* sp. was observed at all stations (Table 5). *Ceratium* is not known to produce toxins, but it forms blooms in various regions of the world (Park et al., 2013), which might cause anoxia at night or during their decay. *Ceratium furca* was implicated in mortalities of sea bream in net cages in the Kuwait bay in 2001. This species was commonly reported in the Gulf and off Qatar (Quigg et al., 2013), sometimes as the dominant species in phytoplankton counts (Dorgham and Al-Muftah, 1989).

The dinoflagellate *Scrippsiella* sp. was observed at all stations but Station 1 of 2012. This cosmopolitan genus occurs in Kuwaiti (Al-Kandari et al., 2009) and Qatari (Al-Muftah, 2008) waters in the Gulf. Although generally considered non-toxic, blooms have been reported with adverse effects on fish and shellfish (Hallegraeff, 1992). Tang and Gobler (2012) also reported mortality of shellfish larvae in laboratory experiments conducted with cell extracts of *S. trochoidea* strains isolated in Virginia, USA.

The cyanobacterium *Trichodesmium* sp (Fig. 3B). was observed at all stations but 1 and 2 of the October 2012 cruise (Table 5). Blooms of this genus were previously reported to contain palytoxins and show ciguatoxin-like activity (Kerbrat et al., 2011, 2010). *Trichodesmium* has a common occurrence in the oligotrophic waters of the Gulf (Al-Kandari et al., 2009; Al-Yamani et al., 2012; Dorgham and Al-Muftah, 1989; Subba Rao and Al-Yamani, 1998) and is suggested to provide new nitrogen into the Gulf ecosystem (Quigg et al., 2013). In fact Zhao and Ghedira (2014), using satellite imagery, detected patches of *Trichodesmium* during the massive

*Cochlodinium* bloom between 2008 and 2009 and suggested newly fixed nitrogen to contribute to the continuation of the bloom.

Strains belonging to the genera *Heterocapsa*, *Karlodinium*, *Gymnodinium* and *Amphidinium* were isolated from coastal samples collected in Doha, Qatar. Although not observed in cruise samples, these genera were reported from the Gulf waters in previous studies (Al-Kandari et al., 2009). Their LSU rRNA and ITS region sequences were determined (Figs. S1 and S2), but these strains proved to be negative in PST, DST and AST using ELISA analyses (data not shown).

## 4. Conclusion

The Gulf is exhibiting elevated occurrences of HABs, either due to increased awareness and monitoring efforts in the region and/or a result of environmental deterioration. Due to high maritime traffic, it has been suggested that exotic algae has been introduced through ballast water discharges (Quigg et al., 2013; Subba Rao and Al-Yamani, 1998). The establishment of the toxic dinoflagellate *V. rugosum* in the Gulf, as reported in this study, provides an example. Given the opportune conditions (e.g. organic or inorganic nutrient enrichment) these new species might start blooming with devastating effects in the region, such as the *Cochlodinium* bloom observed between 2008 and 2009.

The presence of potentially toxic phytoplankton has long been known for the Gulf region, and for the first time, this study demonstrates the presence of PSTs, DST, AST, PTXs, PnTxs, portimine and GYM. Routine monitoring for phytoplankton biomass and toxins along with intensive remediation measures in general are warranted given that the Gulf is a major drinking water and sea food supply for the region.

## Acknowledgements

This paper is dedicated to the memory of Malcolm Potts. Authors thank laboratory technicians Abdul Ali Moghdasi and DM Estremadura for help during sample collections. They also thank the captain and crew of R/V Janan and the Environmental Studies Centre of Qatar University for access to the R/V. Support for this work was provided, in part, through a research and development initiative of Qatar Airways, Qatar National Research Fund and Qatar University (Grant number: QUEX/QFQAQU-10/11-3).

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.toxicon.2016.09.016.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2016.09.016.

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