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Algal toxin profiles in Nigerian coastal waters (Gulf of Guinea) using passive sampling and liquid chromatography coupled to mass spectrometry

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Abstract :

Algal toxins may accumulate in fish and shellfish and thus cause poisoning in consumers of seafood. Such toxins and the algae producing them are regularly surveyed in many countries, including Europe, North America, Japan and others. However, very little is known regards the occurrence of such algae and their toxins in most African countries. This paper reports on a survey of phytoplankton and algal toxins in Nigerian coastal waters.

Seawater samples were obtained from four sites for phytoplankton identification, on three occasions between the middle of October 2014 and the end of February 2015 (Bar Beach and Lekki in Lagos State, Port Harcourt in Rivers State and Uyo in Akwa Ibom State). The phytoplankton community was generally dominated by diatoms and cyanobacteria; however several species of dinoflagellates were also identified: *Dinophysis caudata*, *Lingulodinium polyedrum* and two benthic species of *Prorocentrum*.

Passive samplers (containing Diaion® HP-20 resin) were deployed for several 1-week periods on the same four sites to obtain profiles of algal toxins present in the seawater. Quantifiable amounts of okadaic acid (OA) and pectenotoxin 2 (PTX2), as well as traces of dinophysistoxin 1 (DTX1) were detected at several sites. Highest concentrations (60 ng OA g^{-1} HP-20 resin) were found at Lekki and Bar Beach stations, which also had the highest salinities. Non-targeted analysis using full-scan high resolution mass spectrometry showed that algal metabolites differed from site to site and for different sampling occasions. Screening against a marine natural products database indicated the potential presence of cyanobacterial compounds in the water column, which was also consistent with phytoplankton analysis.

During this study, the occurrence of the marine dinoflagellate toxins OA and PTX2 has been demonstrated in coastal waters of Nigeria, despite unfavourable environmental conditions, with regards to the low salinities measured. Hence shellfish samples should be monitored in future to assess the risk for public health through accumulation of such toxins in seafood.

Keywords : Dinoflagellates, *Dinophysis, phycotoxins*, untargeted analysis, phytoplankton

1. INTRODUCTION

Toxins from marine micro-algae frequently accumulate in seafood, including fish and shellfish, and maximum concentrations for such toxins have therefore been regulated at global and regional levels (DeGrasse and Martinez-Diaz, 2012; Hess, 2012; Lawrence et al., 2011; Suzuki and Watanabe, 2012). As fisheries have only limited potential to increasingly contribute to the global food supply, it is expected that any growth in seafood supply will have to come from aquaculture. Therefore, it is important to investigate the potential of coastal areas for seafood production, and also the risks associated with such production. In terms of public health risks, those originating from harmful algal blooms are particularly common in many parts of the world and must therefore be assessed relatively early on in any survey for aquaculture feasibility.

To our knowledge, no algal toxins have been reported in coastal waters of central Western Africa, except one preliminary report on potentially toxic fish in Cameroon (Bienfang et al., 2008). The southernmost records of algal toxins in Northern Africa are from the Moroccan coastline where an official monitoring program is in place (Abouabdellah et al., 2008; Taleb et al., 2003). Lipophilic shellfish toxins were shown to accumulate in mussels, cockles, oysters and solen, causing poisoning in the Dakhla region, *i.e.* the South Atlantic Moroccan coast (Abouabdellah et al., 2011). Toxins of the okadaic acid (OA) group, *i.e*. OA and dinophysistoxins (DTXs) and their associated esters were the agents responsible for those shellfish poisoning events, attributable to the presence of several potentially toxic species of *Dinophysis*. Taleb et al. (2006) also were the first to report the presence of azaspiracids in mussels, in Morocco.

In southern parts of Africa, regular monitoring is in place in South Africa and Namibia. Production of saxitoxin (STX) off the west coast of South Africa has been attributed to *Alexandrium catenella* (Pitcher and Calder, 2000; Pitcher et al., 2001). Fawcett et al. (2006) have developed and deployed a bio-optical buoy for monitoring HABs in the southern Benguela Current region off South Africa. These buoys have proved their efficiency in providing both real-time and time-series data, giving interesting information on the occurrence of Prorocentrum triestinum in the region. The northernmost records of algal toxins in the southern African region are from Angola (Blanco et al., 2010; Vale et al., 2009).

Phytoplankton surveys in Nigeria by one of the authors have reported non-toxin producing as well as potentially toxic algae including *Prorocentrum micans*, *Protoperidinium depressum*, *Prorocentrum mite*, *Dinophysis caudata*, *Peridinium gatunense*, *P. cinctum*, *Gymnodinium fuscum* and an array of *Ceratium* species (Kadiri, 1999, 2001, 2002, 2006a, b, 2011). Previous studies by other authors also showed sporadic occurrences of *D. caudata*, *Protoperidinium depressum*, *P. diabolus*, *Prorocentrum micans*, *Noctiluca scintillans* in Lagos Lagoon (Nwankwo, 1991, 1997). A recent report additionally recorded *Lingulodinium polyedrum*, *Prorocentrum minimum*, *P. sigmoides* and *Scrippsiella trochoidea* in Lagos, Cross Rivers and Delta States (Ajuzie and Houvenaghel, 2009).

As potentially toxic algae have repeatedly been reported from Nigerian coastal waters this study attempted to verify whether algal toxins actually do occur in Nigerian waters. Since there was no algal culturing facility available on site, and as many dinoflagellates are difficult to bring into culture, in particular *Dinophysis*, we have opted for an indirect approach based on passive sampling of algal toxins in Nigerian coastal waters. This approach had been introduced for monitoring of toxins by MacKenzie et al. (2004). We have focussed on regulated lipophilic toxins known to cause problems in terms of public health but have also used in parallel an approach for untargeted analysis based on high-resolution mass spectrometry as previously described (Zendong et al., 2015).

2. MATERIALS AND METHODS

2.1. Chemicals, reagents and sorbent materials

Certified standard solutions of okadaic acid (OA), domoic acid (DA), dinophysistoxins (DTX1, DTX2), 13-desmethyl spirolide C (13-desmeSPX-C), pectenotoxin 2 (PTX2), gymnodimine A (GYM-A), azaspiracids (AZA1,-2 and -3), yessotoxin (YTX) and homo-yessotoxin (homo-YTX) were obtained from the National Research Council in Halifax, Canada. HPLC grade methanol and acetonitrile as well as ammonium formate and formic acid (98%) were acquired from AtlanticLabo (Bordeaux, France) and Sigma Aldrich (Steinheim, Germany). Deionized water was produced in-house to 18MΩ cm^{-1} quality, using a Milli-Q integral 3 system (Millipore). For analyses with the high resolution mass spectrometry instrument, acetonitrile and water of LC/MS grade were obtained from Fischer Scientific (Illkirch, France). For passive sampler devices, Diaon® HP-20 polymeric resin was purchased as bulk resin from Sigma-Aldrich and 12 mL capacity polypropylene 2 frits-Reservoirs were from Agilent Technologies.

Brucine-sulfanilic acid reagent was prepared by dissolving 1 g brucine sulfate [$(C_{23}H_{26}N_2O_4)_2$ H_2SO_4, $7H_2O$] and 0.1 g sulfanilic acid ($NH_2C_6H_4SO_3H$, H_2O) into 70 ml of hot distilled water. Concentrated hydrochloric acid (3 mL) was further added and this mixture was cooled, mixed and then

diluted to 100 mL with distilled water. The final mixture was stored in a dark bottle at 5 °C. For ascorbic acid, the ready-made PhosVer 3 Hach™ was used.

2.2. Study area

The study area (Figure 1), *i.e.* the Nigerian coastal area, is situated in the Guinea Current Large Marine Ecosystem, in the Gulf of Guinea. There are two main seasons in the deploying sites: the rainy (wet) season spanning from May to October and the dry season from November to April. The area is influenced by coastal upwelling which occurs seasonally along the northern and eastern coasts. There are two (major and minor) upwelling seasons. Those seasons occur annually with differing duration and intensities off Ghana and Cote d'Ivoire, in the central part of the large marine ecosystem. The major upwelling season occurs from June to September and transient upwelling events are from January to March (Ibe and Ajayi, 1985).

The coastline of Nigeria is approximately 853 km long between latitude 4°10′ to 6°20′ N and longitude 2°45′ to 8°35′ E. The Nigerian coastal area is low-lying of not more than 3.0 m above sea level, generally covered by fresh water swamp, mangrove swamp, lagoonal mashes, tidal channels, beach ridges and sand bars (Dublin-Green et al., 1997).

The Nigerian coast is composed of four distinct geomorphological units namely: the Barrier-Lagoon complex; the Mud coast; the Arcuate Niger delta; and the Strand coast (Ibe, 1988). The vegetation of the Nigerian coastal area is characterised by mangrove forests, brackish swamp forests and rain forests. The coastal zone is richly endowed with a variety of mineral resources, including oil and gas. The four selected sites are located in the Gulf of Guinea (Atlantic Ocean), two in the Bight of Bonny to the East (Arcuate Niger delta) and two in the Bight of Benin to the West (outside the Barrier-lagoon complex).

Seawater sampling for nutrients and for phytoplankton analysis, as well as passive sampling were carried out at sites and dates as listed in Table 1.

Table 1: Sampling sites and dates for water and toxin analysis (date format: dd/mm/yy)

Sampling site	Latitude	Longitude	Dates for water sampling	Dates for passive sampling
Bar Beach	N 6° 25.340’	E 3° 26.189’	18/10/14, 02/02/15, 21/02/15	18/10/14, 08/11/14, 07/02/15, 28/02/15
Lekki	N 6° 25.256’	E 3° 32.180’	21/02/15	08/11/14, 28/02/15
Port Harcourt	N 4° 41.828’	E 7° 10.706’	29/01/15, 22/02/15	04/02/15, 28/02/15
Uyo	N 4°33.203’	E 8° 00.202’	17/10/14, 28/01/15, 23/02/15	18/10/14, 07/11/14, 03/02/15, 25/02/15

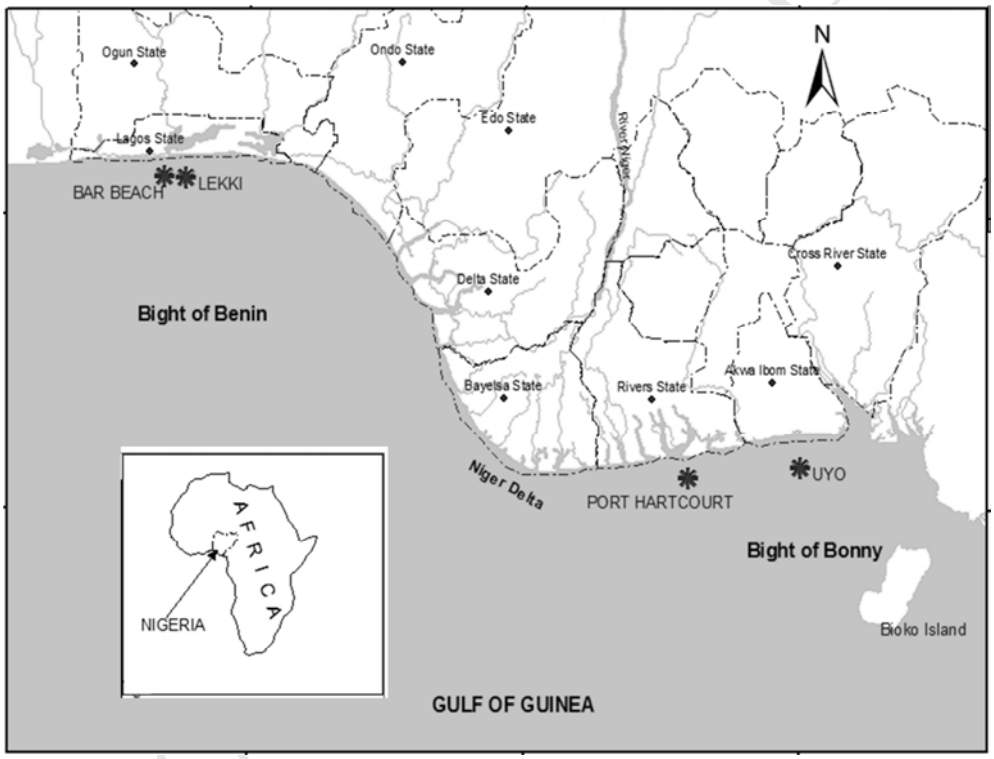

Figure 1: Location of sampling sites (stars): Bar Beach and Lekki are both in Lagos State off Lagos lagoon; Port Harcourt is in Rivers State, in the vicinity of the Niger delta, and Uyo is in Akwa Ibom State towards the Eastern Limit of Nigerian waters.

2.3. Physico-chemical parameters and water sampling for analysis of nutrients and phytoplankton identification

Water samples (1 L) were obtained for analysis of nutrients at an integrated depth of 10 m to the surface of the ocean, with a lund tube of 2.5 cm diameter. Temperature was measured with a mercury-in-glass thermometer. Dissolved oxygen was measured using a Milwaukee NW 600 probe and salinity was measured with a Hach™ Salinity/Conductivity probe probe (Hach Company, USA).

Nutrients were analysed according to ASTM (1980). For the determination of nitrate, brucine sulphanilic acid reagent (1 mL) was added to standard solutions as well as to samples (10 mL). The resultant mixtures were mixed thoroughly and allowed to stand for 15 min. Then 10 mL of H_2SO_4 solution were carefully added to 10 mL of distilled water and the resulting solution was added to each of the beakers containing the nitrate standard solutions and the water samples, respectively. This was allowed to stand for 20 min in the dark. Similar treatment was performed on the blank solution, using the same protocol except that no brucine sulphanilic reagent was added to it. The absorbance of standards and samples was determined at 410 nm wavelength using a UV/Visible spectrophotometer. Phosphate was determined using the ascorbic acid method. The programmed method of Hach was used using the Hach spectrophotometer DR2000™ (Hach Company, USA).

Phytoplankton samples were collected by horizontal and vertical tows using a plankton net made from fine bolting silk (10 µm mesh, length: 107 cm and Diameter: 29 cm). Samples were drained into the plankton bucket and preserved with Lugol's iodine in sample bottles. Light microscopy (LM) observations were carried out from 50 µL of fixed net samples deposited on a glass slide, using an Olympus IX70 inverted light microscope equipped with a digital camera DP72 (Olympus, Tokyo, Japan). Cells were photographed, either directly or after isolation with a micropipette, depending on concentration of organisms and particles.

2.4. Passive sampler design, handling and extraction

Passive sampling devices (Solid Phase Adsorption Toxin Tracking = SPATT) were prepared using a 68 mm embroidery frame (Singer, Nantes, France). Three grams (3 g) of Diaion® HP-20 polymericresin were placed between two layers of a 30 µm nylon mesh (Mougel, France), and clamped in the embroidery frame to form a thin layer of resin. To activate the HP-20 resin, the passive samplers were soaked for 3 h in methanol, rinsed twice with deionized water to remove methanol residues (Rundberget et al., 2009; Zendong et al., 2014) and directly deployed. Three SPATTs were put in three separate compartments cylinders made of steel, to firmly secure them, and deployed in the sea at 1 m depth for 7 days at each site. After deployment, the SPATTs were retrieved, rinsed with seawater to remove residual biofilm and transported in frozen ice packs to the laboratory. The SPATTs were shipped to the analytical laboratory in France on ice and arrived in good condition. They were then stored in a freezer (-20 °C) until analysis. The HP-20 resin was extracted according to previously

published methods, with slight changes (Fux et al., 2008; Zendong et al., 2014). Briefly, after deployment, the SPATTs were rinsed twice in 500 mL deionized water, transferred into empty polypropylene reservoirs placed on a manifold and eluted dropwise with 24 ml of methanol. The extracts were then evaporated at 45 °C under a gentle nitrogen stream. The dry residue was further reconstituted in 500 µL of 50% methanol, filtered on Nanosep MF centrifugal filters 0.2 µM (Pall) and transferred into HPLC vials for analysis.

2.5. Liquid chromatography - mass spectrometry analyses

Three different analytical systems were used: (1) for quantitative targeted analysis of toxins; (2) for untargeted screening of unknowns as well as known toxins; (3) for characterisation and confirmation of toxins. For all three systems, chromatographic separation was achieved after injection of a 3 µL sample volume onto a Phenomenex Kinetex XB-C18 (100 x 2.6 mm; 2.6 µm) column maintained at 40 °C, with a flow rate of 400 µl/min. The binary mobile phase consisted of water (A) and 95% acetonitrile/water (B), both containing 2 mM ammonium formate and 50 mM formic acid. The elution gradient rose from 5% to 50% of B in 3.6 min, then 100% B was reached by 8.5 min. After 1.5 min of hold time at 100% B, 5% B was reached within 10 s, followed by 5 min re-equilibration of the column at 5% B. The total chromatographic run time was 15 min. To avoid cross contamination of samples, the needle was washed for 10 s in the flush port with 90% MeOH before each injection. On all analytical systems, mass spectrometric acquisitions were carried out separately in positive (ESI^+) and negative (ESI^-) ionization modes.

2.5.1. System 1: LC-MS/MS for quantitative analysis

A UFLC-XR Shimadzu liquid chromatography system (Champs-sur-Marne, France) was connected to a hybrid triple quadrupole/linear ion-trap mass spectrometer (API4000-Q-Trap™; AB Sciex) equipped with a TurboIonSprayTM ionization source. For quantitation, the mass spectrometer was operated in MRM mode, scanning two transitions for each toxin. Q1 and Q3 resolutions of the instrument were set at Unit (arbitrary terms). Data were acquired in MRM, in separate chromatographic runs, using positive (ESI^+) and negative (ESI^-) ionization modes, respectively with a scan time of 1 s. In ESI^+, the following source parameters were used: curtain gas set at 30 psi, ion spray at 5500 V, a turbogas temperature of 450°C, gas 1 and 2 both set at 50 psi, and an entrance potential of 10 V. In ESI^-, the curtain gas was set at 20 psi, the ion spray at -4500 V, the turbogas temperature at 550°C, gas 1 and 2 at 40 and 50 psi, respectively, and finally the entrance potential at -13 V. MRM transitions used for each toxin are displayed in Table 2. Data acquisition was carried out with Analyst 1.6 Software (AB Sciex).

Table 2: Multiple Reaction Monitoring (MRM) transitions used for quantitative analysis on System 1 (30 msec dwell in ESI^+ and 80 msec dwell in ESI^-).

Toxin	DP	Q1	Q3 quantifier	CE	Q3 qualifier	CE
DA	61	312.1	266.1	23	161.1	35
GYM-A	86	508.4	490.2	33	392.3	49
13-desmeSPX-C	121	692.5	164.2	69	444.3	53
PnTX-G	141	694.5	164.1	75	458.3	75
AZA1	116	842.5	672.4	69	654.4	69
AZA2	116	856.5	672.4	69	654.4	69
AZA3	116	828.5	658.4	69	640.4	69
PTX2	91	876.5	823.5	31	805.6	37
PTX2sa	91	894.6	823.5	31	805.6	37
OA, DTX2	-170	803.5	255.1	-62	113.1	-92
DTX1	-170	817.5	254.9	-68	112.9	-92
YTX	-120	1141.4	1061.6	-48	855.5	-98
homo-YTX	-120	1155.6	1075.6	-48	869.4	-98

2.5.2. System 2: LC-HRMS for untargeted and targeted screening of toxins and unknowns

A UHPLC system (1290 Infinity, Agilent Technologies) was coupled to a 6540 UHD Accurate-Mass QToF (Agilent Technologies) equipped with a dual ESI source. Full-scan analyses were performed over the range *m/z* 65 to 1700 with an acquisition rate of 2 spectra s^{-1}. In ESI^+ the temperature of the Jet Stream Technologies™ source was set at 205°C with the drying gas flow-rate at 5 L min^{-1}. The sheath gas temperature was 355°C. Other parameters were as follows: capillary voltage, 2000 V; fragmentor voltage, 200 V. The parameters of the Jet Stream Technologies™ source in ESI^- were: gas temperature 305°C, drying gas flow 5 L min^{-1}, nebulizer pressure 50 psi, sheath gas temperature 355 °C, sheath 12 L/min, capillary voltage 3500 V, fragmentor voltage, 180 V.

All experiments were done with reference mass correction using purine (m/z 121.0509 $[M+H]^+$; *m/z* 119.03632 $[M-H]^-$) and HP-921 = hexakis(1H,1H,3H-tetrafluoropropoxy) phosphazine (m/z 922.0099 $[M+H]^+$; *m/z* 966.00072 $[M+HCOO]^-$). The reference ions were infused constantly with an isocratic pump to a separate ESI sprayer in the dual spray source.

2.5.3. System 3: LC-HRMS for toxins confirmation

Analyses were carried out using a UHPLC system (1290 Infinity II, Agilent Technologies) coupled to a 6550 iFunnel QToF (Agilent Technologies) equipped with a dual ESI source. This instrument was operated with a dual electrospray ion source with Agilent Jet Stream Technology™ in positive and

negative ionization modes. Analyses were performed over the range *m/z* 100 to 1200 with an acquisition rate of 2 spectra s^{-1}. The parameters of the Jet Stream Technologies™ source in ESI^+ were: gas temperature 205°C, drying gas flow 16 L/min, nebulizer pressure 50 psi, sheath gas temperature 355°C, sheath 12 L/min, capillary voltage 2000 V, fragmentor voltage, 200 V. In ESI^- the parameters were as follows: gas temperature 290°C, drying gas flow 12 L/min, nebulizer pressure 50 psi, sheath gas temperature 355°C, sheath 12 L/min, capillary voltage 3500 V, fragmentor voltage, 180 V. Three collision energies (20, 40 and 60 eV) were applied to the precursor ions to generate fragmentation spectrum. All experiments were done with reference mass correction as described above for System 2. MassHunter Acquisition B05.01 software was used to control the instrument and data were processed with MassHunter B07.00 service pack.

2.6. Data processing and statistical analyses

Raw data files obtained on System 2 (section 2.5.2) were processed using the Agilent *Molecular Feature Extractor* (MFE) algorithm in MassHunter Qual software (B.07). This algorithm was used to obtain the *Total Compound Chromatogram* of samples as previously described (Zendong et al., 2015). This algorithm designed for use with full scan data treats the mass spectral data as a three-dimensional array of retention time, *m/z* and abundance values. Any point corresponding to persistent or slowly-changing background is removed from that array of values. Then the algorithm searches for ion traces that elute at very nearly the same retention times. Those ion traces are then grouped into entities called *Compounds* regrouping all ion traces that are related, *i.e.* those that correspond to mass peaks in the same isotope cluster, or can be explained as being different adducts or charge states of the same entity. The results for each detected *Compound* are a mass spectrum containing the ions with the same elution time and explainable relationships, and an extracted compound chromatogram (ECC) computed using all of these related ion traces in the compound spectrum (and only those traces). The results from the MFE analysis were then uploaded to the Agilent Mass Profiler Professional (MPP) software (B.13.00) as compound exchange format file (.cef) for further statistical analyses (PCA: Principal Component Analysis). In MPP, feature profiles were aligned with 15 ppm and 0.2 min bins of mass and retention time windows, respectively. Data were log2 transformed, centered and normalized to give features equal weight in classification. Groups/conditions were composed of SPATT samples from the same location and/or the same deployment date. Data were analyzed by univariate and multivariate analysis to detect features of interest. For the multivariate data analysis (MVDA) comparing all samples, all features present in less than 20% of all samples from the data set were discarded. For univariate data analysis comparing only samples from a given site, only entities with p-values > 0.05 and fold-change > 2 were retained. PCA was carried out on conditions *i.e.* to allow for the detection of similarities between samples. Features that were considered characteristic were tentatively identified based on

mass and spectral accuracy using the Dictionary of Marine Natural Products (DMNP) library (Blunt and Munro, 2008) (Wolfender et al., 2015).

3. RESULTS AND DISCUSSION

3.1. Physico-chemical measurements

Water temperatures, salinity and nutrient levels in the study area confirm a strong correlation with seasonality (Table 3). Salinity ranged from 2 to 20, all areas and periods confounded, which is comparatively low for marine dinoflagellates. The two stations in the North-west of the study area (Lekki and Bar Beach) displayed the highest salinities, ranging from 17.2 to 18.2 during the end of the wet season (October 2014), and from 18.1 to 19.3 during the dry season (January / February 2015). The stations closer to the Niger delta (off the cities of Port Harcourt and Uyo) showed much lower salinities, with the Port Harcourt station (directly outside the main delta in River States) showing the lowest overall salinity of 2 in wet season (October 2014) but still reaching a salinity of 9 during dry season (February 2015).

Table 3: Surface water temperature, dissolved oxygen (DO), salinity and nutrient concentrations at sampling stations in Nigerian coastal waters 2014-15.

Parameter	**Bar Beach**		**Lekki**		**Port Harcourt**		**Uyo**	
	18/10/14	02/02/15	17/10/14	30/01/15	19/10/14	04/02/15	18/10/14	03/02/15
Water Temp (˚C)	26	27	27	26	32	30	25.5	31
DO (mg/L)	7.8	9.8	7.8	7.9	7.8	6.5	7.4	6.5
Salinity	18.2	19.3	17.2	18.1	2.0	8.8	6.6	7.8
PO_4^{3-} (mg/L)	0.07	0.51	0.03	0.2	0.07	0.22	0.03	0.51
NO_3^- (mg/L)	1.48	1.61	1.44	1.59	0.37	1.26	1.48	1.61

3.2. Identification of phytoplankton species

Phytoplankton samples were generally dominated by diatoms and cyanobacteria, especially filamentous cyanobacteria. However, several species of marine dinoflagellates were also observed (Figure 2). In particular, a few cells of *Dinophysis caudata* were observed in a sample from Bar Beach (21 February 2015). *D. caudata* had previously been reported as a producer of OA and PTX2 in different areas and should thus be considered as a potentially toxic species (Fernández et al., 2006; Holmes et al., 1999; Li et al., 2015; Marasigan et al., 2001). Interestingly, different regions reported

different profiles of toxins in picked cells of *D. caudata*. In Northwestern Spain and China, the toxin profile was dominated by PTX2 (Fernández et al., 2006; Li et al., 2015), while OA was shown to be present in picked cells of *D. caudata* from both Japanese and Singapore waters (Holmes et al., 1999; Marasigan et al., 2001).

Another potentially toxic dinoflagellate was observed in the sample from Bar Beach: *Lingulodinium polyedrum*. This species is characterized by its polyhedral shape with a flat antapex lacking any projections, thick thecal plates with ridges along the sutures and circular depressions over the surface of the plates (Dodge, 1989), see also Figure S2 (supplementary information). The same organism had also been detected at a concentration of several thousand cells L^{-1} in coastal waters of Atlantic Morocco (Bennouna et al., 2002), and cultures of Spanish strains of *L. polyedrum* were shown to produce yessotoxin (Paz et al., 2004).

Three cells of two unidentified benthic *Prorocentrum* species have also been observed (Figure 2c and d). A number of benthic *Prorocentrum* species have been associated with the production of toxins of the okadaic acid, the prorocentrolide and the hoffmaniolide groups: *P. lima, P. belizeanum, P. maculosum, P. rhathymum* and *P. hoffmanianum* (An et al., 2010; Hu et al., 1996; Jackson et al., 1993; Morton et al., 1998), but even a pelagic species of *Prorocentrum* (*P. texanum*) has recently been associated with the production of okadaic acid (Henrichs et al., 2013). Therefore, this observation should be verified to determine the exact species of *Prorocentrum*.

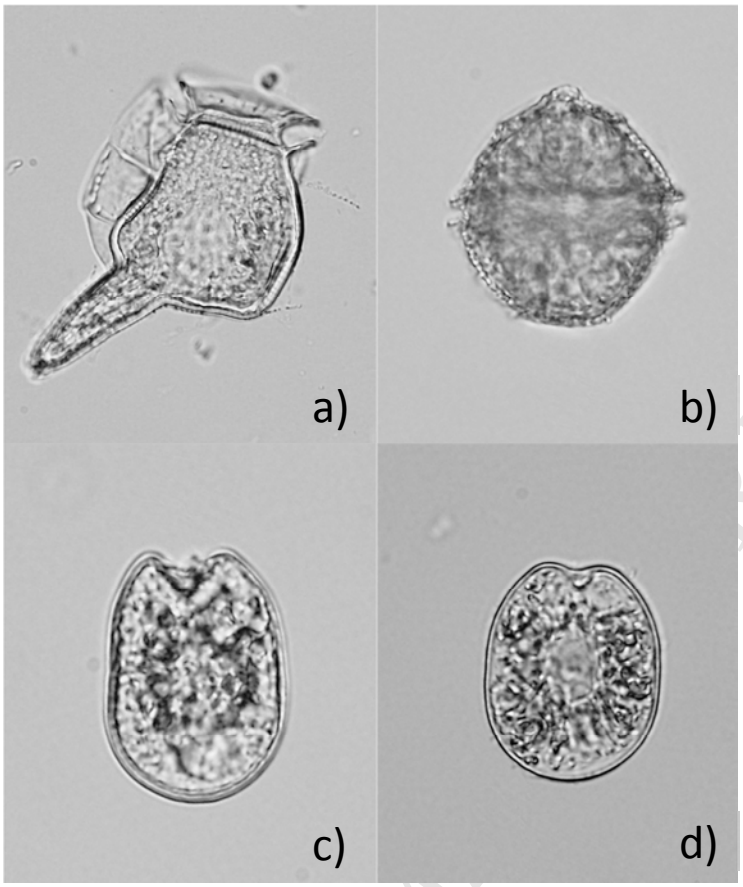

Figure 2: Marine dinoflagellates identified on Bar Beach (Lagos State, Nigeria, 21 February 2015): a) *Dinophysis caudata* (L = 100 µm), b) *Lingulodinium polyedrum* (L x W: 40 x 38 µm), c) *Prorocentrum* sp1 (L x W : 37 x 27µm) and d) *Prorocentrum* sp2 (L x W: 36.3 x 28.8 µm).

3.3. Quantitative analysis of SPATT samples and toxin confirmation

Passive samplers were deployed on different dates in November 2014 and February 2015. Analyses of SPATT carried out on System 1 revealed the presence of OA and PTX2 at different concentrations (Figure 3a). Concentrations of OA and PTX2 were significantly higher at Lekki and Bar Beach compared to Port Harcourt and Uyo. This overall pattern seems consistent with the higher potential for *Dinophysis* to survive in areas of higher salinity (Delmas et al., 1992). *Dinophysis caudata* had also been previously found at Bar Beach and Lekki, sites which at that time had almost oceanic salinity (Ajuzie and Houvenaghel, 2009). As abovementioned, *D. caudata* had previously been associated with the production of OA and PTX2, and hence the occurrence of these toxins in Nigerian waters can most likely be attributed to this species. The levels of okadaic acid found (ca. 60 ng OA g^{-1} HP-20 resin) were of a similar order of magnitude than those found by MacKenzie et al. (2004) in the initial study introducing passive sampling for algal toxins, but comparatively low compared to those reported in a previous study in Ireland (Fux et al., 2009). However, the concentrations in mussels (*M. edulis*) in the

latter study also exceeded the regulatory level ca. 6-fold, and hence the actual contamination levels in shellfish in Nigeria should be verified to evaluate the risk for public health or before establishing commercial aquaculture sites. Interestingly, the levels of PTX2 observed in the present study were similar to those observed in the Irish study (Fux et al., 2009), which may be attributed to the different causative species in both areas: *D. acuminata* and *D. acuta* in Ireland, as compared to *D. caudata* in Nigeria. Rundberget et al. (2009) had used passive samplers of the same geometry in Norway, and they also found levels of a similar height of order as those in the present study. They also established that SPATTs contained typically three times as much toxin as mussels in a given location, yet occasionally levels in mussels were higher than those in the passive samplers. Since the Irish study did not have the same ratios as those established in the Norwegian study, we anticipate that any correlation between the concentrations observed in passive samplers and a given shellfish species would have to be established locally and verified over time.

The ratio of OA to PTX2 was examined to look for major changes in phytoplankton community structure of OA-producing organisms (Figure 3b). As *Prorocentrum* species have not been found to produce PTX2 but DTX1, a relative increase of OA over PTX2 could be indicative of their increasing importance. The ratio remained relatively constant over the study period indicating that there was either not much change in the population of micro-algae or similar ratios were produced by the organisms present. This is also consistent with the fact that DTX1 was found only in trace amounts at Lekki and Bar Beach, but not found at all in the two other locations. DTX1 has been reported from *P. lima* (Pan et al., 1999) and the low concentrations in passive samplers deployed at 1 m below the surface could be related to the dilution effect for these toxins if they had been produced by low density benthic species. However, it has been shown that even toxins from *P. lima* can accumulate to significant levels in shellfish locally (Lawrence et al., 2000), and hence care should be taken before discarding benthic organisms as a risk to public health.

At Bar Beach, it appeared that toxin concentrations were higher in November and in February which also coincides with a slight increase in salinity and the dry season, for which upwelling had been previously indicated (Ibe and Ajayi, 1985). At Port Harcourt and Uyo, concentrations of OA and PTX2 in the passive samplers were *ca*. 10-fold lower than the maxima observed at Bar Beach and Lekki. This significant difference is understandable from the very low salinities observed at Port Harcourt and Uyo (Table 3), which are detrimental for most marine dinoflagellates, in particular *Dinophysis* (Delmas et al., 1992). The differences in concentrations found in passive samplers extracts from Port Harcourt and Uyo on one hand and Lekki and Bar Beach on the other are much larger than what could be expected from the simple differences in adsorption due to different salinities. A recent study has shown that kinetics of adsorption may be affected (Fan et al., 2014), however, this should be negligible for the 1-week deployment periods in the present study. Port Harcourt and Uyo are

considered to be brackish water zones and are consequently significantly different from Bar Beach and Lekki (see also section on untargeted analysis).

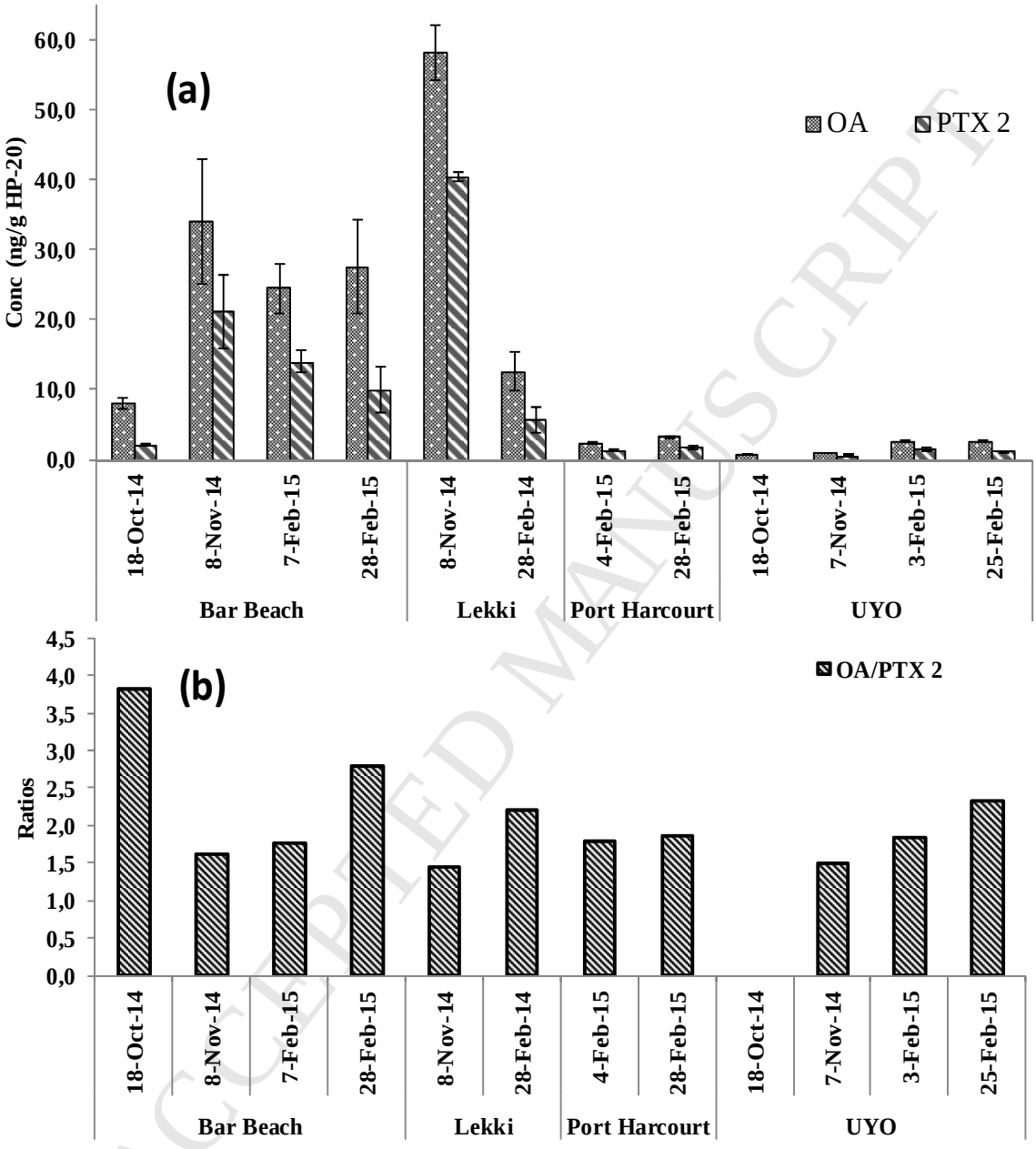

Figure 3: Average concentrations (a) and ratios (b) of okadaic acid (OA) and pectenotoxin 2 (PTX2) detected at each deployment site (ng/g of HP-20 resin ± RSD%, n=3).

3.4. Confirmation of okadaic acid and pectenotoxin 2 by high resolution mass spectrometry coupled to liquid chromatography

For confirmatory purposes, System 3 was used to obtain high resolution spectra from toxins quantified using System 1. For instance, the spectra for OA in negative ionisation mode obtained from a standard solution and a sample from Bar Beach were compared, and showed the same major ions characteristic for OA (Figure 4).

Accurate mass measurements for OA for the sample from Bar Beach were also verified and compared well with those of the certified standard of OA: the molecular ion $[M-H]^-$ of OA in the Bar Beach sample (*m/z* 803) showed 1.2 ppm mass error compared to the standard, while the two main fragments *m/z* 113.060 and 255.123 had a mass error of 0.88 and 0.39 ppm, respectively. Mass accuracy for PTX2 was slightly less good, but fragmentation pattern and fragment ion ratios matched very well that of the standard (see Figure S1, supplementary information). Therefore, the presence of OA and PTX2 can be considered unequivocal as demonstrated by both low and high resolution tandem mass spectrometry.

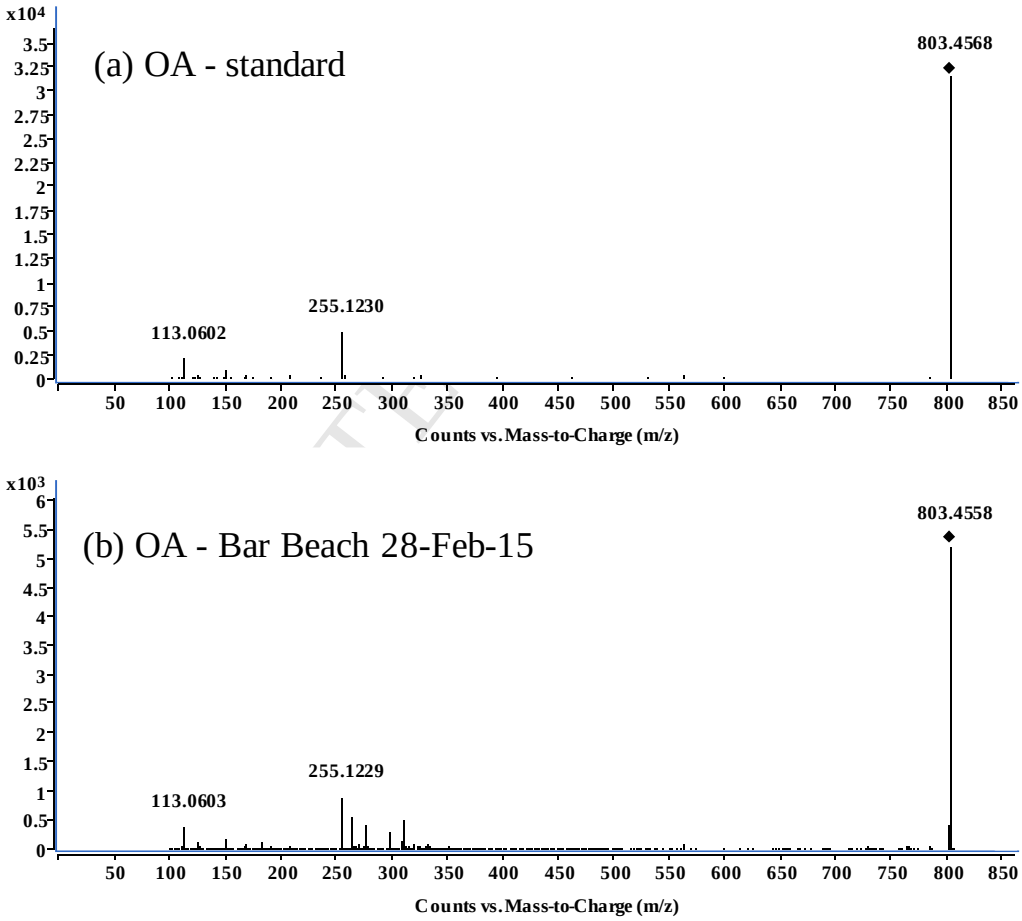

Figure 4: Average high resolution spectrum of (a) OA standard and (b) OA in a SPATT extract from Bar Beach. Spectra were obtained on System 3 (QToF 6550) in ESI^- using target MS/MS with collision energies of 20 V, 40 V and 60 eV.

3.5. Untargeted screening approach for passive samplers

Principal component analysis including all masses identified in extracts of the passive samplers clearly showed separation between samples taken at the end of the wet season and those taken during the dry season, irrespective of the sampling site (Figure 5).

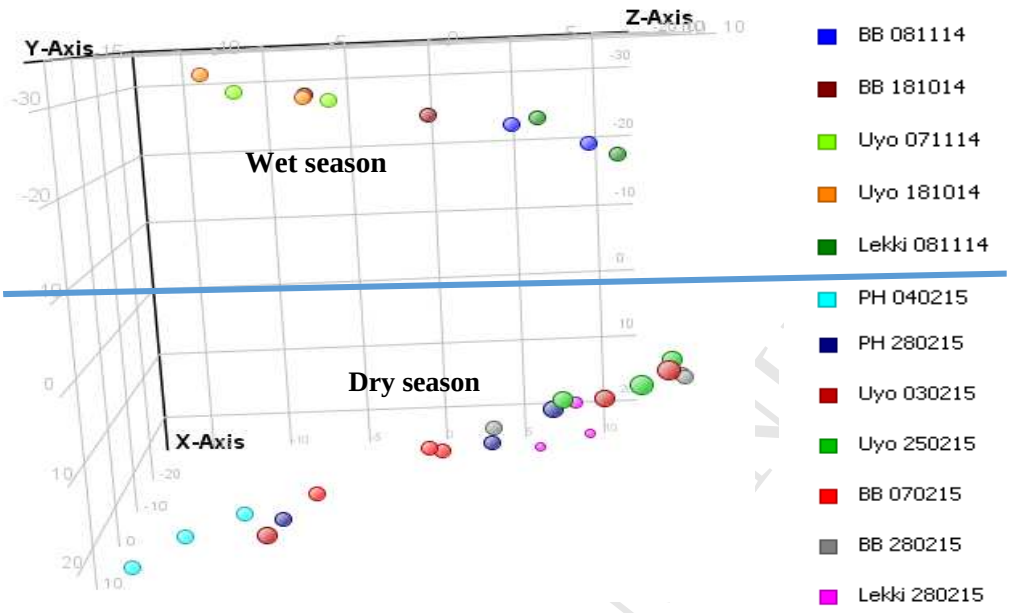

Figure 5: Score plot of the principal component analysis of all passive samplers (n=2 for 2014 and n=3 for 2015). Data were acquired by full scan HRMS on System 2. During Molecular Feature Extraction samples were blank-subtracted, ion traces extracted and combined into compounds. The three principal components plotted on the X, Y, and Z axes account for ca. 58% of the total variability in the data set (40.94% for X; 11.18% for Y and 7.06% for Z). *Note:* BB=Bar Beach and PH= Port Harcourt.

This separation of seasons in the passive sampler extracts was not as distinct as in the targeted analysis of toxins (Figure 3a) but is consistent with changes expected in the phytoplankton community structures in different seasons. When analysing the trend on a single site, Bar Beach (BB), it was also apparent that each sampling occasion gave a different chemical profile (Figure 6).

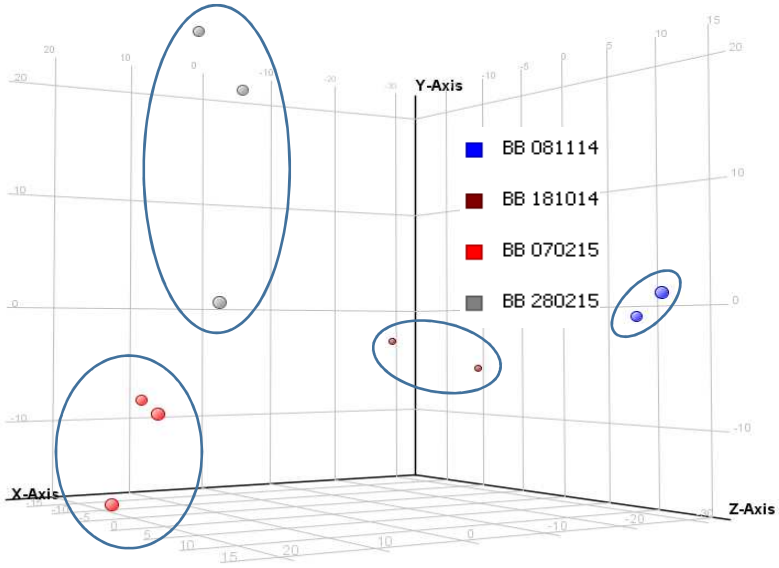

Figure 6: Score plot of the principal component analysis of passive samplers from Bar Beach taken on four separate occasions (n=2 for 2014 and n=3 for 2015). Data were acquired by full scan HRMS on system 2. The three principal components plotted on the X, Y, and Z axes account for ca. 83% of the total variability in the data set (69.72% for X; 7.78% for Y and 5.78% for Z)

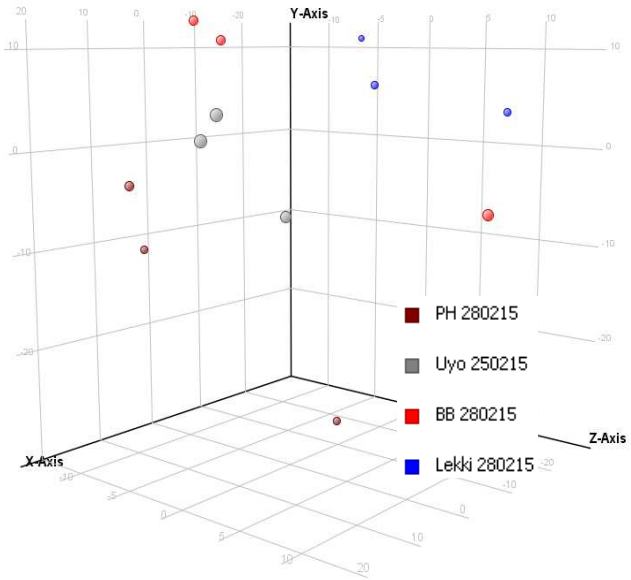

Figure 7: Score plot of the principal component analysis of passive samples from all four sites (BB=Bar Beach, PH=Port Harcourt), all taken during week 9 of 2015 (n=3). The three principal components plotted on the X, Y, and Z axes account for ca. 66% of the total variability in the data set (39.04% for X; 18.79% for Y and 9.04% for Z).

Interestingly, all four sites gave also different chemical profiles on a single sampling occasion (Figure 7). In this initial untargeted analysis, no identification of compounds was necessary to obtain this trend.

Still, the separation of the sites by PCA is not surprising when considering that the complete set of data for these four sites on a single occasion consisted of 2394 compounds. Amongst those compounds, 1828 occurred only at the two sites of high salinity (Lekki and Bar Beach) and 245 were unique to the sites with low salinity (Uyo and Port Harcourt). This also means that only 321 compounds were common to all four sites during that particular week. This observation also led us to tentatively identify what compounds may occur on the different sites. For this purpose, several samples were also screened against a database derived from the Dictionary of Marine Natural Products (Blunt and Munro, 2008). When applying stringent criteria (1 ppm mass accuracy, 5000 count abundance threshold) for matching compounds identified in the Nigerian data set by full scan HRMS, several hundred compounds gave tentative hits.

In particular, we examined what compounds were responsible for distinguishing weeks at Bar Beach station. In the PCA analysis for Bar Beach samples from October/November 2014 were grossly separated from samples taken during February (Figure 6). When examining compounds with extreme loadings in the PCA analysis (< -0.03 or > 0.03 normalised loading values, arbitrary choice, see Figure S3), 20 compounds of 196 distinctive entities were tentatively identified for the earlier period (October and November 2014, end of wet season), while 20 compounds of 424 distinctive entities were tentatively identified for the later period (February 2015, dry season, Table 4). In summary, among the database propositions were many compounds that had initially been identified either in tropical sponges, nudibranches or marine or freshwater cyanobacteria (Table 4). The fact that cyanobacterial compounds were identified appears coherent with previous identification of cyanobacteria as a problem in Nigerian waters (Odokuma and Isirima, 2007). These findings also suggest that additional efforts in Nigerian coastal waters should focus on identifying cyanobacterial toxins and source organisms.

Without any pre-selection of compounds on a given site for one sampling occasion (contrarily to the comparative PCA described above), many compounds can be tentatively identified, however, not all are distinctive features of that site – occasion combination. For instance Bar Beach was analysed for identifiable compounds on 08/11/2014 and 170 compounds gave a hit in the Dictionary of Marine Natural Products (Blunt and Munro, 2008). Interestingly, these compounds tentatively identified in the non-targeted analysis also included for instance okadaic acid already identified in the targeted analysis (Table S1).

Table 4. Compounds tentatively identified in non-targeted analysis using high-resolution mass spectrometry (system 2)

No.	Compound	Month	Freq.	T_R	Mass	Identification Marine Natural Products Dictionary
1	6-Tridecylamine	Feb	6	4.01	199.2296	Isolated from the cyanobacterium *Microcoleus lyngbyaceus*
2	Hedaol B; A5-Isomer(Z-)	Feb	6	7.08	261.1977	Constituent of a *Sargassum sp.*
3	8,11,14-Heptadecatrienal; (all-Z) -form, 14,15-Dihydro	Feb	6	5.27	250.2293	Constituent of cucumber, tobacco and wheat. Also found in the algae *Enteromorpha sp., Scytosiphon lomentaria* and *Ulva pertusa*
4	Glycerol 1-alkyl ethers; Glycerol 1-pentadecyl ether	Feb	6	8.61	302.2818	Constituent of D*esmapsamma anchorata* and *Tethya aurantiaca*
5	10-Aromadendranol; (1ct,4a,5 13,6a,7a,10a)-form, O -(2-O -	Feb	6	7.21	405.3120	Constituent of *Eucalyptus globulus* (Tasmanian blue gum) and *Thryptomene kochii*
6	Petroformyne 1; 3- or 44- Ketone	Feb	6	3.22	666.5018	Constituent of *Petrosia ficiformis*
7	Dideacetylraspacionin; 10,28- Dihydro, 103-hydroxy, 4,10, 15,21-tetra-Ac	Feb	6	8.16	661.4090	Constituent of sponge *Raspaciona aculeata*
8	Dideacetylraspacionin; 10,28- Dihydro, 103-hydroxy, 21- ketone, 4,10,15-tri-Ac	Feb	6	8.21	634.4068	Constituent of sponge *Raspaciona aculeata*
9	Cholestane-3,5,6,7-tetrol; (3i, 5ct,613,713)-form, 3,7-Di-Ac	Feb	6	8.25	503.3500	Constituent of the gorgonian *Plexaurella grisea*
10	6-Pentadecyl-1,2,4-benzenetriol; 1-Ac	Feb	6	7.59	400.2594	Constituent of the sponge *Axinella polycapella*
11	Etzionin; N,O -Di-Ac	Feb	6	7.41	558.3422	cytotoxic & antifungal; isolated from *Didemnum rodriguesi*
12	Fumiquinazoline F; 4-Epimer	Feb	6	7.47	358.1420	Cytotoxin prod. by a marine-derived *Aspergillus fumigatus*
13	Picrotoxinin	Feb	6	3.77	314.0767	Ichthyotoxin isolated from desmosponge *Spirastrella inconstans*
14	Louludinium(1+)	Feb	6	4.82	294.2211	Isolated from marine cyanobacterium *Lyngbya gracilis*
15	Aeruginosamide	Feb	6	2.66	560.3399	Isolated from *Microcystis aeruginosa* (cyanobacteria)
16	Bengamide Z; 6-Deoxy	Feb	6	2.30	372.2264	Isolated from sponge *Jaspis cf. coriacea*
17	Ulithiacyclamide F	Feb	6	9.26	814.2046	Isolated from the ascidian *Lissoclinum patella*

No.	Compound	Month	Freq.	T_R	Mass	Identification Marine Natural Products Dictionary
18	4-Cadinen-10-ol; (1ct,63,7i3,10 13)-form	Feb	6	4.46	222.1987	Isolated from the sponge *Acanthella cavernosa*.
19	Drechslerine G	Feb	6	3.85	270.1823	Metabolite of the algicolous fungus *Drechslera dematioidea*
20	Acremonin A; (+)-form	Feb	6	5.25	176.0836	Prod. by a marine-derived micromycte *Acremonium sp.*
21	10-O -(3,4- Dihydroxy-E -cinnamoyl) geniposidic acid	Oct/Nov	4	2.87	536.1535	Constituent of *Genipa americana* (genipap) and *Premna barbata* (higher terrestrial plants)
22	6- Sulfate Cholestane-3,6,8,15,24-pentol; (313,5ct,6a,15ct,24S)-form,	Oct/Nov	4	2.1	532.3077	Constituent of *Oreaster reticulatus* (tropical sea star)
23	3-Propanoyl, 12-Ac-3,12-Dihydroxy-20,24-dimethyl-17-scalaren-25,24-olide	Oct/Nov	4	6.36	545.371	Constituent of *Phyllospongia lamellosa*
24	1-Tricosene	Oct/Nov	4	9.23	339.3865	Constituent of the alga *Botryococcus braunii* and various plant spp. incl. *Gardenia tahitensis*
25	4,10-Dimethyldodecanoic acid	Oct/Nov	4	3.85	245.2352	Isolated from a halophilic *Bacillus sp.*
26	2-Amino-11-dodecen-3-ol	Oct/Nov	3	5.50	199.1939	Isolated from a marine sponge *Haliclona n. sp.*
27	N - Eicosanoyl 2-Aminobenzoic acid	Oct/Nov	4	5.65	453.3218	Isolated from aerial parts of *Ononis natrix* (African terrestrial plant)
28	Dysidazirine; (S ,E)-form	Oct/Nov	4	3.69	307.2516	Isolated from Fijian marine sponge, *Dysidea fragilis*
29	Malonganenone B	Oct/Nov	4	6.75	470.3256	Isolated from *Leptogorgia gilchristi* (gorgonian, soft coral)
30	Glanvillic acid A	Oct/Nov	4	6.29	306.2190	Isolated from *Plakortis halichondrioides*
31	13',14'-Dihydro-amphiasterin B2	Oct/Nov	3	6.72	401.3506	Isolated from *Plakortis quasiamphiaster* (marine sponge)
32	Enterocin	Oct/Nov	4	3.75	444.1060	Isolated from a marine ascidian *Didemnum sp.*
33	Phloeodictyne A; Phloeodictyne 4,6i	Oct/Nov	4	8.59	407.3622	Isolated from the New Caledonian deep water sponge *Phloeodictyon sp.* and shallow-water sponge *Oceanapia fistulosa* (*Phloeodictyon fistulosa*)
34	2-Amino-18-methyl-4- nonadecene-1,3-diol	Oct/Nov	3	8.7	327.3141	Isolated from the sponge *Discodermia calyx*
35	2-Amino-9-hexadecen-3-ol; (2 S ,3R ,9Z)-form	Oct/Nov	4	7.87	255.2563	Isolated from the tunicate *Pseudodistoma obscurum*

No.	Compound	Month	Freq.	T_R	Mass	Identification Marine Natural Products Dictionary
36	6-Octadecenoic acid; (E)- form	Oct/Nov	4	7.72	282.2559	Minor constituent of plant oils
37	Choline; O -(2-Methyl-2- propenoyl)	Oct/Nov	4	8.08	194.1157	Monomer. Polymers are used as coagulants in sewage treatment
38	2-Dodecenoic acid; (E)-form, Et ester	Oct/Nov	4	7.12	226.1909	Occurs in pears
39	Hexadecanoic acid; Dimethylamide	Oct/Nov	4	5.74	283.2876	Widely distributed in plants
40	2-Methylpropanoic acid	Oct/Nov	4	7.69	106.0627	The free acid and its esters occur in many plants

4. CONCLUSION

The survey in Nigerian coastal waters confirmed the presence of toxic algae in this area, in particular *Dinophysis caudata*. For the first time, lipophilic toxins were identified in Nigerian coastal waters. Okadaic acid and pectenotoxin 2 have been quantified in passive samplers deployed for 1-week periods and can most likely be attributed to *Dinophysis* species, although a partial contribution by *Prorocentrum* species cannot be excluded. Untargeted analysis using high resolution mass spectrometry also pointed towards the possible accumulation of cyanobacterial metabolites in the passive samplers. Therefore, any further studies investigating the risks for public health from shellfish consumption should examine concentrations of algal as well as cyanobacterial toxins.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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Supplementary material

Algal toxin profiles in Nigerian coastal waters (Gulf of Guinea) using passive sampling and liquid chromatography coupled to mass spectrometry

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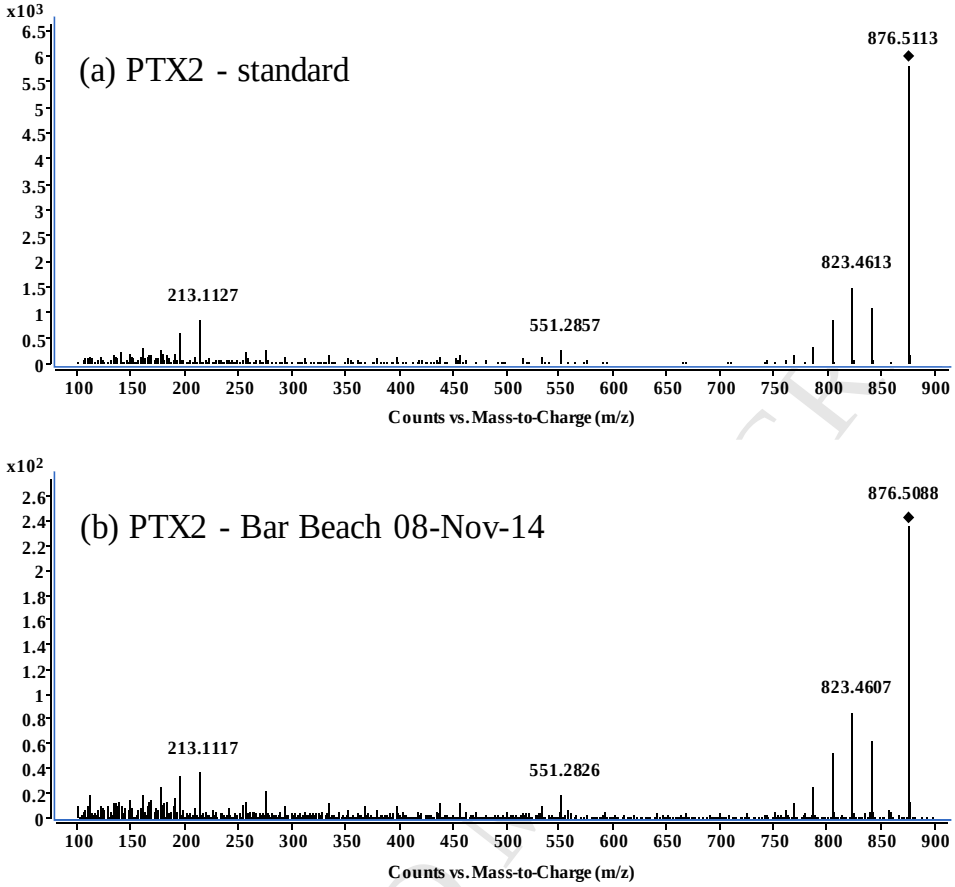
Figure S1: Comparison of the high-resolution mass spectrum of PTX2 in a sample from Bar Beach to the spectrum of a certified standard of PTX2.

Figure S2: Surface focus of a *Lingulodinium polyedrum* cell in ventro-antapical view, in ventro-apical view and, in dorso-apical view showing ornamentation of plates (ridges along the sutures and circular depressions).



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179 **Figure S1:** Comparison of the high-resolution mass spectrum of PTX2 in a sample from Bar Beach to the
180 spectrum of a certified standard of PTX2.

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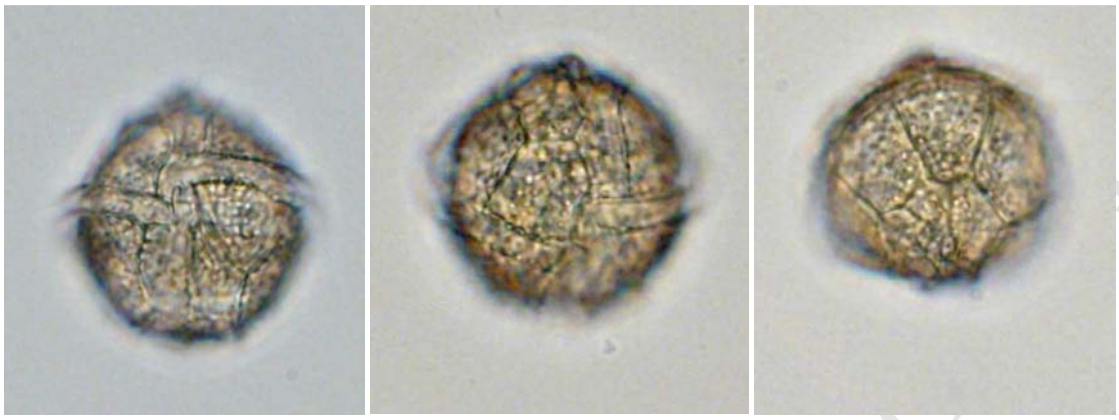

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184 **Figure S2:** Surface focus of a *Lingulodinium polyedrum* cell in ventro-antapical view, in ventro-apical
185 view and, in dorso-apical view showing ornamentation of plates (ridges along the sutures and circular
186 depressions).

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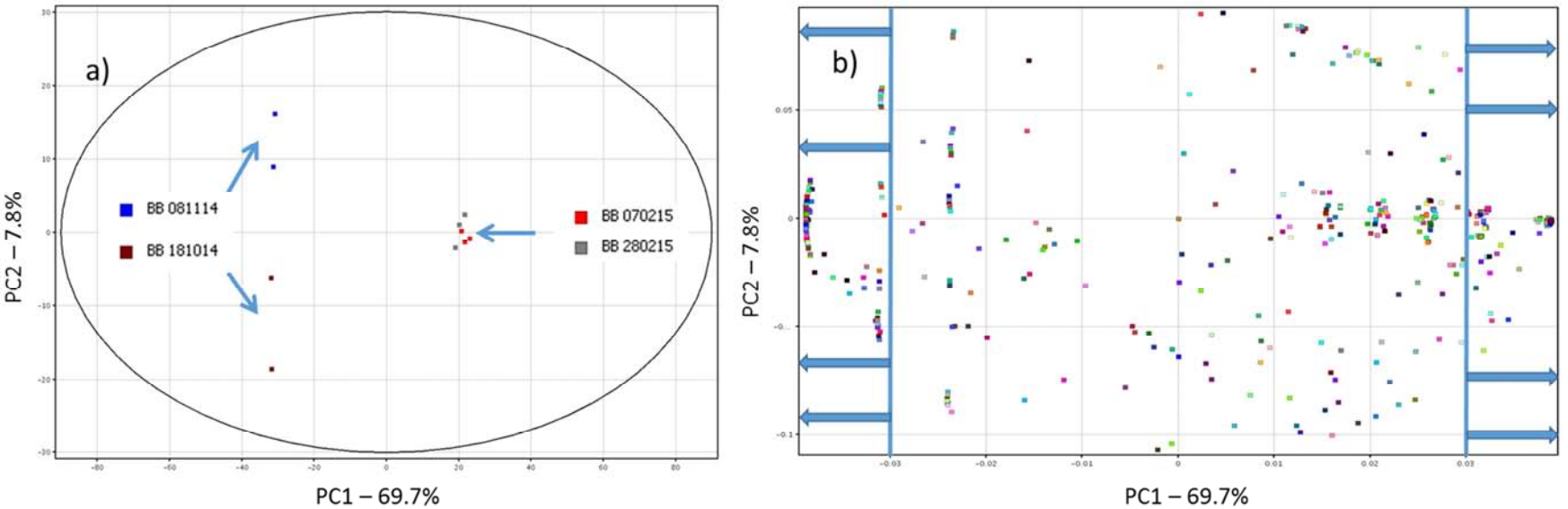

Figure S3. Principal Component Analysis (PCA) of passive samples taken at Bar Beach, Nigeria, in 2014/2015. The score plot (a) shows good separation of samples from October/November (left-hand side of graph) from those taken in February (right-hand side of graph). As this separation was almost exclusively on principal component 1 (accounting for almost 70% variability in the dataset), compounds most responsible for this separation are those that appear on the left- and right-hand side of the loadings plot (b). An arbitrary cut-off of 0.03 was chosen to select the most "separative" compounds. These compounds were subsequently screened against the Marine Natural Products Dictionary and results are given in Table 4 of the manuscript.

197 **Table S1.** 170 compounds tentatively identified at Bar Beach on 08/11/2014
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Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
1(10),5-Germacradien-4-ol; (1(10)E ,4a,5E)-form, O -(2-O - Acetyl-13-D-glucopyranoside)	444,296	14204	-0,87	6,33	99,29	426,2621
1,21-Heneicosanediol	328,3575	58944	-0,24	5,30	99,96	328,3342
1,2-Benzenedicarboxylic acid; Ditridecyl ester	535,4124	33691	-0,5	9,08	99,72	530,4338
1,7-Dihydroxy-2,5,10,14- phytatetraen-13-one; (2E ,5E ,7r, 10E)-form	303,2316	38682	0,73	5,32	99,63	320,2349
1,7-Dihydroxy-2,5,10,14- phytatetraen-13-one; (2E ,5E ,7r, 10E)-form	303,2318	32854	0,11	6,57	99,99	320,2351
10-Aromadendranol; (113,4a,5 13,6a,7ct,10ct)-form, O -[2-Methylpropanoyl-(ï¿½*2)-3-Dfucopyranoside]	438,3216	12346	-0,5	6,80	99,76	438,2984
11,14-Dihydroxy-12-spongien-16- one; (1113,14a)-form	317,2111	93172	0,12	4,84	99,99	334,2144
11,15-Dihydroxy-9-oxo-13- prostenoic acid; (8R ,11R ,12R , 13E ,15S)-form, Et ester	365,2687	26037	-0,07	6,61	100	382,272
11,15-Dihydroxy-9-oxo-5,13-prostadienoic acid; (5Z ,8R ,11R ,	366,2637	25639	0,49	2,22	99,8	366,2404
12,16,22-Trihydroxy-24-methyl- 24-oxo-25-scalaranoic acid; (12/3, 16/3)-form, 22-Ac, Me ester	506,3474	98047	0,39	4,59	99,83	506,3242
13,17-Epoxy-16-hydroxy-19- kauranoic acid; (ent -16ct)-form, Me ester	331,2268	64105	-0,03	5,08	100	348,2301
13-Docosenoic acid; (E)-form	338,3419	22888	-0,41	8,99	99,87	338,3186
14-Hydroxy-4,7,10,12,16,19-docosahexaenoic acid; (4Z ,7Z , 10Z ,12E ,14?,16Z ,19Z)-form	327,2321	37561	-0,73	6,48	99,59	344,2354
15,17-Epoxy-15,17-dihydroxy-16-isocopalanoic acid; (13aH ,14aH , 15a,1713)-form, Di-Ac, Me ester	468,296	49780	-0,97	4,82	99,07	450,2622
15-Anhydrothyrsiferol; 10- Epimer, A15(28)-isomer	586,33	18533	0,33	7,44	99,86	586,3067
16,24-Epoxy-21,24,25-trihydroxy- 17-cheilanthen-19,25-olide; (24?, 25?)-form	452,3005	99390	0,41	4,73	99,84	434,2667
16,24-Epoxy-21,24,25-trihydroxy- 17-cheilanthen-19,25-olide; (24?, 25?)-form	452,3007	35080	-0,1	4,82	99,99	434,2669
17-Methyl-1,17-tricosadiene-4,6,8,10,12,14,16-heptol	446,3472	26475	0,83	5,86	99,32	446,324
1-Amino-4,12-tridecadien-2-ol; (ï¿½)-(E)-form, N ,O -Di-Ac	278,2114	28107	0,11	3,70	99,99	295,2147
1-Bromo-4,6-eudesmanediol; (113,4a,6a,713H)-form, 4,6-Di- Ac	420,1945	201266	-0,35	2,60	99,89	402,1607
1H-Indole-5,6-diol; Di-Me ether, N-Me	191,118	45686	-0,45	1,99	99,92	191,0947
1-O -Alkylglycero-3- phosphocholines;	576,3786	34960	0,34	7,52	99,86	571,4000

Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
1- Hexadecylglycero-3-phosphocholine, 2-Benzyl ether						
2-(Aminomethyl)-2-propenoic acid; N -(2-Hydroxyhexadecanoyl), Me ester	370,2951	19604	0,35	7,35	99,90	369,2878
2-(Aminomethyl)-2-propenoic acid; N -(2-Oxohexadecanoyl), Me ester	350,2691	129147	-0,44	2,71	99,84	367,2724
2-(Aminomethyl)-2-propenoic acid; N -Hexadecanoyl	322,2739	88009	0,4	3,82	99,88	339,2772
2,3,4,9-Tetrahydro-1H -pyrido[3, 4-b]indol-1-one; 6-Methoxy	199,0868	148648	-0,98	1,73	99,57	216,0901
2,3,5,14,20,22,26- Heptahydroxyergost-7-en-6-one	510,3429	72813	-0,63	5,44	99,56	510,3196
2,3,5,14,20,22,26- Heptahydroxyergost-7-en-6-one; (213,313,513,14a,20R ,22R ,24?,25R)-form	510,3419	36288	-0,05	5,32	100	510,3193
2,3'-Iminobispropanoic acid; (ï¿½)-form, Di-Et ester, N -Ac	242,1386	39061	0,3	3,13	99,95	259,1419
2',4,6,6'-Tetrahydroxy-4'-methylbenzophenone-2-carboxylic acid; 2',6-Di-Me ether, Me ester	329,1021	34581	-0,34	3,66	99,91	346,1054
2,4,6-Tribromophenol; 4-Methylbenzenesulfonyl	486,8214	23862	-0,94	2,95	99,08	481,8427
2,6,10-Farnesatrien-1-oic acid; 6S,7S :10R ,11-Diepoxide, Me ester	300,2169	76280	0,13	5,21	99,99	282,1831
22,25-Epoxy-24-methylfurostane-2,3,11,20-tetrol; (2ct,3ct,5ct,1113, 1613,20R ,22S ,24S)-form, 3-Ac	543,3288	21999	0,73	5,29	99,4	520,3396
24-Methyl-16-pentacosene-2, 4-diyne-1,6-diol	393,313	39095	-0,66	8,80	99,62	388,3344
25-Methyl-1,25- hentriacontadiene-4,6,8,10, 12,14,16,18,20,22,24-undecol	640,4633	19787	-0,48	2,92	99,7	622,4295
2-Amino-1,3,4,5- octadecanetetrol	334,2953	70164	-0,45	4,64	99,85	333,2881
2-Amino-1,3,4-hexadecanetriol; (2S ,3R ,4S)- form	290,2689	70470	0,2	4,65	99,98	289,2616
2-Amino-1,3-octadecanediol;(2R ,3S)-form, N ,O ,O -Tri-Ac	410,3266	19374	-0,01	7,17	100	427,3298
2-Amino-14-methyl-1,3,4-pentadecanetriol	290,2691	142823	-0,42	2,96	99,89	289,2618
2-Amino-3-hydroxy-4- octadecene-1-sulfonic acid	708,502	22708	-0,94	2,23	99,29	363,2447
2-Amino-4-octadecene-1,3- diol; (2?,3?,4?)-form	282,2793	42655	-0,56	8,36	99,79	299,2826
2-Azetidinecarboxylic acid; (R) -form, N -Benzyl, Me ester	223,1441	45912	-0,14	2,16	99,99	205,1103
2-Ethyl-11-methoxy-3-methyl-3H -[1,6]naphthyridino[6,5,4-def] quinoxaline	279,1606	315814	-0,69	9,86	99,7	279,1374
2-Hydroxy-10-oxo-4,10-seco-4, 13(15),17-spatatrien-12-al	299,2008	64485	-0,69	6,37	99,66	316,2041
2-Methyl-1,16-dithiocyanato- 8-hexadecanol	370,2346	636724	-0,03	1,94	100	370,2113
2-Oxohexadecanoic acid; Me ester, (Z)-	282,2428	73942	-0,03	2,94	100	299,2461

Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
oxime						
3-(12-Nitrododecyl)pyridine	310,2488	164095	0,38	3,54	99,91	292,215
3(20)-Phytene-1,2-diol; (2?,7 ?,11?)(1)-form, Di-Ac	397,3311	52692	0,26	8,29	99,94	396,3239
3-(2-Hydroxyethyl)-6- prenylindole; Aldehyde, oxime	243,149	146928	0,67	3,72	99,77	242,1418
3-(3-Oxo-1-nonadecenyl) oxiranecarboxylic acid	367,2845	65781	-0,68	6,33	99,63	366,2773
3,11-Dihydroxy-15-cembren-6- one; (1R ,3R ,4R ,8S ,11R ,12R)- form	307,2634	43991	-0,68	9,02	99,67	324,2667
3,24-Dihydroxy-24-methyllanost- 8-en-30-oic acid; (313,24?)-form	511,3757	36745	0,19	8,68	99,96	488,3865
3,4,6,24-Tetrahydroxycholest- 8(14)-en-15-one; (3i ,43,5ct,6a, 25S)-form	448,3425	48064	-0,77	7,81	99,41	448,3192
3,5-Dihydroxy-6,7- megastigmadien-9-one; (3S ,5S , 7R ax)-form	224,1645	127638	0,16	3,61	99,99	224,1412
3,6-Epidioxy-6-methoxy-4,16,18-eicosatrienoic acid	349,2372	40176	0,25	6,35	99,95	366,2405
3,7,11-Cembratrien-15-ol; (1S ,3E ,7E ,11E)-form, O -(6-O - Acetyl-13-D-galactopyranoside)	494,348	28402	-0,86	8,11	99,21	494,3248
3-Hydroxy-11-oxo-12-oleanen-30- oic acid; (3i3,183)-form, 3-O-13-DGlucuronopyranoside	646,3948	29679	0,29	2,88	99,89	646,3715
3-Hydroxycholan-24-oic acid; (3ct, 513)-form, Glycine amide	416,3163	48077	-0,93	7,81	99,18	433,3196
3-Hydroxypregn-5-en-20- one; (313,13ct,17ct)-form, Ac	358,2738	80308	0,59	4,35	99,72	358,2506
3-Hydroxypregn-5-en-20- one; (313,17ct)-form, Ac	341,2477	16660	-0,41	6,58	99,87	358,2509
3-Hydroxyundecanoic acid; ( )-form, O -[ct-L- Rhamnopyranosyl-(1--*3)-3-hydroxyundecanoyl]	550,3946	19328	0,72	2,55	99,41	532,3608
4,15:6,7-Diepoxy-1,8- dihydroxy-5-hirsutanone; (1ct, 4?,63,7i3,83)-form, 1-(2- Hydroxyoctanoyl)	405,227	58107	0,34	2,10	99,89	422,2303
4,7-Epoxy-3-hydroxy-8,12(18)-dolabelladien-13-one	301,2164	142942	-0,49	6,04	99,83	318,2197
4,8-Dimethyl-3-nonen-1-ol	193,1563	13004	0	5,92	100	170,1671
4-Amino-3-hydroxybenzoic acid; 2,7-Tetradecadienyl ester (2E ,7?)	328,2271	80099	0,14	3,02	99,99	345,2303
4-Hydroxy-16-heptadecene-5,7- diyn-2-one	243,1742	17956	0,65	6,27	99,76	260,1775
4-Hydroxyphenylacetic acid; O -(3-Methyl-2-butenyl), nitrile	201,1383	34054	0,16	2,38	99,99	201,1153
4-Nitrophenol; Octadecanoyl	388,2848	206543	-0,48	2,83	99,79	405,2881
5,6-Epoxy-3,11-dihydroxyergost- 22-en-1-one; (3i3,5i3,63,11ct,22E , 24R)-form, Di-Ac	533,3241	28449	-0,7	3,67	99,44	528,3455
5,6-Epoxy-7,10-cyclofarnesadien- 9-ol; (513,63,7Z ,9?)-form	236,2008	30610	0,27	2,22	99,96	236,1776

Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
5,8-Epidioxyergosta-6,24(28)- dien-3-ol	411,3257	34172	0,06	9,12	100	428,329
5,8-Epidioxyergosta-6,9(11),22- trien-3-ol; (3i ,5ct,8a,22E ,24R)- form, O -3-D-Glucopyranoside	589,3741	301379	-0,81	3,40	99,19	588,3667
5-Cyclohexene-1,2,3,4-tetrol; (1RS ,2RS ,3SR ,4RS)-form, Tetra-Ac	297,0971	57968	-0,62	1,87	99,73	314,1004
6,13-Epoxy-4(18)-eunicellene-3,8, 9,12-tetrol; (3a,6ct,83,9i3,12i3,13ct)-form, 12-Butanoyl, 3-Ac	466,3167	132888	-0,9	5,07	99,18	466,2935
6,13-Epoxy-4(18)-eunicellene-7,8, 9,12-tetrol	354,2644	12350	0,62	6,63	99,7	354,2404
6,13-Epoxy-4(18)-eunicellene-8,9, 12-triol; (6ct,8ctOH ,9i,12ctOH ,13 a)-form	321,2427	114679	-0,94	6,63	99,34	338,246
6,13-Epoxy-4,8,9,12- eunicellanetetrol; (4I ,6a,8ct,913, 1213,13ct)-form, 9-Me ether, 12-Ac	825,5718	835123	0,23	2,91	99,95	412,2824
6,13-Epoxy-8(19)-eunicellene-3,4, 9,12-tetrol; (3ct,413,6a,913,123,13a)-form, 3,4,12-Tri-Ac	480,2953	39046	0,49	3,25	99,75	480,2721
7,11-Dihydroxy-13-spongien-16- one; (713,1113)-form	334,2379	44495	-0,83	7,81	99,48	334,2147
7,11-Dihydroxy-8-drimen-12,11- olide; (7ct,1113)-form, 11-Et ether	277,1797	72298	0,49	5,74	99,84	294,183
7,14-Dihydroxy-15-nor-16-isocopalanoic acid	307,227	56414	-0,67	5,64	99,67	324,2303
7,8-Didehydro-13,13-carotene-3, 3',4,4'-tetrol; (3S ,3'S ,4S ,4'S) -form	581,3992	17509	-0,53	8,49	99,65	598,4025
9,11,15-Trihydroxyprost-13-enoic acid; (8RS ,9SR ,11RS ,13E ,15 SR)-form, Me ester	353,2684	67371	0,76	5,85	99,52	370,2716
9-Hydroxy-5,7,11,14- eicosatetraenoic acid; (5Z ,7E ,9S ,11Z ,14Z)-form, Me ester	317,2476	62084	-0,15	5,78	99,98	334,2508
9-Hydroxy-7-hexadecenoic acid; (7E ,9S)-form, Ketone	251,2007	32112	-0,62	6,20	99,78	268,204
Ageline B	532,3523	52722	-0,6	7,48	99,59	531,3451
Amphiasterin C1; 3-Epimer	484,3034	112434	-0,11	2,49	99,99	479,3247
Amphidinolide T2	452,3373	24688	-0,53	6,64	99,72	452,314
Antillatoxin B	548,3478	73840	0,92	6,65	99	565,3511
Aplaminone; (R)-form, A9' - Isomer(E -), 11'-hydroxy	492,2308	22650	0,04	3,40	100	509,234
Aplysia MIP-related peptides; GAPRFI amide	641,3888	16326	-0,96	5,12	98,77	658,3921
Aspergillamide A	492,2967	10636	0,55	6,92	99,69	474,2628
Batzellaside A	314,2691	30268	-0,34	7,98	99,92	331,2724
Belamide A	622,3959	17749	0,71	7,48	99,36	604,362
Bengazole Z; O 6- Heneicosanoyl	629,4136	191826	-0,03	2,74	100	606,4244
Biliverdin IX6; Di-Me ester	593,2762	16779	-0,51	8,79	99,67	610,2794
Chaetoglobosin A; 19-Deoxy, 20-deoxo	481,2854	26963	-0,87	6,93	99,18	498,2887
Cholest-9(11)-ene-3,6,22-triol; (3i ,5ct,6a,22R)-form, 22-Ac, 3,6-di-O -	638,303	91823	-0,5	4,70	99,67	620,2692

Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
sulfate						
Cholestane-3,5,6,7-tetrol; (313, 5ct,6ct,7ct)-form, 3,6,7-Tri-Ac	545,3833	50889	0,73	4,18	99,37	562,3865
Clathrynamide A; N -(4- Hydroxy-1-methylpentyl)	584,2541	34317	0,94	4,39	98,96	561,2648
Cochlioquinone A; 17-Methoxy	545,311	19411	-0,1	4,28	99,99	562,3142
Cochlioquinone A; Hydroquinone, 11-ketone	532,327	43782	-0,28	5,14	99,91	532,3038
Conicamine	206,1178	56844	0,2	1,81	99,98	201,1391
Crambescidin 431	449,3123	36591	-0,21	2,46	99,96	431,2785
Criamide A; N 1-Me	682,4764	54752	-0,23	2,75	99,93	682,4532
Cyclo(tryptophylvalyl); (3S ,6S)-form, 3,6-Bis(methylthio)	377,1462	53953	0,68	1,97	99,61	377,1229
Dideacetylraspacionin; 10,28- Dihydro, 103-hydroxy, 10,15- di-Ac	594,437	38496	-0,96	9,56	98,87	594,4137
Didemniserinolipid C; O 10-Ac	602,4022	20574	0,88	3,33	99,03	597,4235
Dolabelide B	755,4944	23468	-0,47	2,57	99,68	754,4871
Ergosta-7,24(28)-diene-3,4,6,20, 22-pentol; (33,4f3,5a,6a,20?,22?) -form, 4,6-Di-Ac	546,379	48858	-0,11	6,36	99,98	546,3557
Ergostane-3,5,6,7,15-pentol	471,3449	15081	-0,79	4,62	99,36	466,3662
Exophilin A	576,4109	38199	-0,42	6,72	99,78	576,3876
Fasciospongide B	466,28	94817	-0,13	4,11	99,98	448,2462
Fellutamide A; 1-Deoxy	556,3703	19846	0,39	2,12	99,82	555,363
Fungichromin	671,4006	13344	-0,68	5,83	99,37	670,3933
Glutamic acid; (S)-form, N -(9Z , 12Z ,15Z -Octadecatrienoyl)	815,5419	51820	-0,34	3,50	99,89	407,2673
Glycerol 1,2-dialkanoates; Glycerol 1-hexadecanoate 2- tetradecanoate, 3-O -3-DGalactopyranoside	725,5178	40381	-0,51	2,99	99,63	702,5286
Glycerol 1,2-dialkanoates;Glycerol 1-(9Z-hexadecenoate) 2-tetradecanoate, 3-O -?-DGalactopyranoside	723,5021	32832	-0,46	3,03	99,7	700,5129
Glycerol 1-alkanoates; Glycerol 1- (8,9-methylenehexadecanoate)	365,2665	26024	-0,69	5,76	99,64	342,2772
Glycerol 1-alkanoates; Glycerol 1- (9Z -octadecenoate), 2-Ac	381,3001	40190	0,54	6,87	99,74	398,303
Glycerol 1-alkyl ethers; Glycerol 1-tetradecyl ether	288,2895	39536	0,62	5,17	99,76	288,2663
Halichomycin	557,3951	18682	-0,29	9,06	99,9	539,3612
Halimedalactone	314,2116	49951	-0,5	2,64	99,83	314,1884
Hemiasterlin	526,3747	469332	0,8	3,06	99,28	526,3515
Hennoxazole A; 4-Deoxy	498,3331	18685	-0,89	4,09	99,14	498,3098
Hurghaperoxide	405,3001	32507	-0,48	8,05	99,79	422,3034
Kahalalide D	613,3826	29507	-1	2,70	98,77	595,3488
Kailuin A	680,459	20500	0,35	3,18	99,83	697,4623
Kailuin B	726,5014	96294	-0,3	3,16	99,87	725,4941
Korormicin D	430,295	131313	0,32	4,36	99,9	447,2983
Labiatamide A	536,3218	15258	0,02	6,11	100	535,3145

Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
Lasonolide F; Et ester	632,3797	168668	-0,63	2,67	99,5	614,3459
Manzamine A; (+)-form, 6,31 13-Dihydroxy, 32,33-dihydro	600,3904	17080	0,66	3,27	99,46	582,3566
Manzamine A; (+)-form, 8- Hydroxy, 1R ,2,3,4-tetrahydro, N 2-Me	600,4272	27839	0	2,08	100	582,3934
Martiriol	522,3788	213917	0,21	4,32	99,95	522,3555
Melophlins; Melophlin K	306,2424	31428	0,97	3,26	99,32	323,2457
Montipyridine	274,1803	76954	-0,47	2,47	99,86	291,1836
Muqubilin; (13R ,16R ,17R)- form	392,3159	32409	-0,06	5,51	100	392,2927
Noroxopenlanfuran	201,1272	12797	0,73	6,27	99,76	218,1305
Okadaic acid	822,5002	10380	-0,62	6,02	99,42	804,4665
Oscillatoxin A	583,2873	20647	0,72	2,75	99,37	578,3087
Palinurine A	440,2795	89875	0,16	3,66	99,97	457,2828
Pectenotoxin 1; Dihydro (?)	876,5109	29177	-0,54	6,57	99,53	876,4876
Pectinoacetal C	535,3627	24008	0,45	7,13	99,76	534,3554
Phosphatidylcholine; Glycerol 1,2-didecanoate 3- phosphocholine	583,4079	169882	0,44	2,81	99,76	565,3741
Pinnaic acid	408,2299	79150	0,31	3,23	99,91	425,2332
Plakortic acid*; 3-Epimer, 9, 10-dihydro, Me ester	319,2246	48230	-0,76	7,17	99,6	314,2459
Pregnane-3,20-dione; 5ct- form	299,2371	62148	-0,65	5,78	99,7	316,2404
Rhopaladin A; Debromo, 6- deoxy	354,1347	44400	0,37	2,58	99,89	354,1115
Roserythrin	578,3628	13687	0,14	4,80	99,98	578,3395
Saframycin A; 5ct-Hydroxy	596,2345	31886	1	3,79	98,79	578,2007
Sarcotragin A; N -De-(2- phenylethyl), N - (carboxymethyl)	447,2857	80580	-0,85	2,99	99,29	447,2625
Scalusamide A; 6',7'- Didehydro(E -), 8',9'-dihydro	264,1960	37731	-0,74	2,41	99,66	281,1993
Secoasbestinin	392,2434	59078	-0,7	2,09	99,58	392,2202
Semiplenamide E; 2R *,3S *- Epoxide	434,3243	17707	-0,6	5,99	99,67	411,3351
Siphonarin A; 3R -Alcohol	491,3001	27544	0,38	6,53	99,84	508,3034
Solanapyrone B; 73-Hydroxy, 4'-demethoxy, 4'-[(2-hydroxyethyl)amino], 1- aldehyde	365,2073	42412	-0,71	2,49	99,61	347,1735
Sordaricin; O -[2-Methyl-2Z, 4E -hexadienoyl-(ï¿½*3)-6- deoxy-4-O -methyl-3-Daltropyranoside]	618,3632	12711	0,85	2,57	99,1	600,3293
Stigmast-5-en-3-ol; (313,24R)- form, O -[3-D-Glucopyranosyl-(1 ï¿½*4)-ct-L-arabinopyranoside]	709,4880	210398	0,8	2,52	99,11	708,4807
Stigmast-5-en-3-ol; (313,24R)- form, O -[ct-Rhamnopyranosyl-(1 ï¿½*5)-ct-L-arabinofuranoside]	692,5090	587162	0,8	2,77	99,12	692,4858
Stigmasta-7,22-diene-2,3,5,6,9, 11,19-heptol; (2a,3i3,5ct,63,11ct, 24S)-form, 11,19-Di-Ac	575,3574	40356	0,83	3,25	99,15	592,3606
Stigmastane-3,4,6,15,16,29- hexol; (313,4f3,5ct,6ct,1513,163,24R)-form	519,3661	68075	-0,96	2,97	99,01	496,3769
Stigmastane-3,5,6,15,29-pentol;	477,3578	24274	-0,7	8,94	99,48	494,3611

Name	*m/z*	Height	Diff (DB, ppm)	RT (min)	Score (DB) %	Mass
(3i3,5a,63,15a,24R)-form, 29-Carboxylic acid						
Stolonic acid A	434,3268	20806	-0,73	7,80	99,49	434,3035
Synechobactin A	578,3756	20813	0,7	2,56	99,42	560,3417
Taurospongin A	741,5078	78719	0,5	2,64	99,63	741,4846
Tedanolide	628,3686	15008	0,9	4,97	98,98	610,3348
Theopederin A	526,3010	22820	0,17	4,93	99,97	543,3043
Thorectandramine	438,2052	85140	-0,28	2,67	99,92	438,1819
Tumonoic acid A; Me ester	336,2531	53235	0,54	4,37	99,77	353,2564

199
200

First detection of OA, PTX2 and possible presence of DTX1 in African coastal marine environments.

Presence of *Dinophysis caudata* and *Prorocentrum spp* in seawater consistent with passive sampler findings.

HRMS analysis used for chemical profiling of marine environments using passive sampler.

Untargeted analysis pointed towards presence of a cyanobacteria community.

Ethical statement for

Algal toxin profiles in Nigerian coastal waters (Gulf of Guinea) using passive sampling and liquid chromatography coupled to mass spectrometry

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This paper has not been published in or submitted to any other journal. No animals have been used in any of our experimentations.

On behalf of the authors,

Zita ZENDONG