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Accumulation of diarrhetic shellfish poisoning toxins in the oyster *Crassostrea gigas* and the mussel *Choromytilus meridionalis* in the southern Benguela ecosystem

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Diarrhetic shellfish poisoning (DSP) poses a significant threat to the safe consumption of shellfish in the southern Benguela ecosystem. The accumulation of DSP toxins was investigated in two cultivated bivalve species, the Pacific oyster *Crassostrea gigas* and the mussel *Choromytilus meridionalis*, suspended from a mooring located off Lambert's Bay on the west coast of South Africa. The dinoflagellate *Dinophysis acuminata*, a known source of polyether toxins associated with DSP, was common through most of the study period. The toxin composition of the dinoflagellate was dominated by okadaic acid (OA) (91%), with lesser quantities of the dinophysistoxin DTX-1 (6.5%) and pectenotoxin PTX-2 (2.4%), and traces of PTX-2sa and PTX-11. The mean cell toxin quota of *D. acuminata* was 7.8 pg OA cell^{-1}. The toxin profile in shellfish was characterised by a notably higher relative content of DTX-1. The study showed the average concentration of DSP toxins in the mussels to exceed that in the oysters by approximately 20-fold. The results indicate a need to establish species-specific sampling frequencies in shellfish safety monitoring programmes.

Keywords: Benguela Current, *Dinophysis acuminata*, DSP toxins, LC-MS/MS, shellfish toxicity

Introduction

Dinoflagellate species of the genus *Dinophysis* Ehrenberg are the primary source of the lipophilic toxins responsible for diarrhetic shellfish poisoning (DSP), a severe gastrointestinal illness caused by the consumption of contaminated shellfish (Dominguez et al. 2010). DSP toxins in bivalve molluscs are subject to regulatory limits for human consumption in many countries around the world, and international trade in shellfish must also adhere to the accepted regulatory protocols regarding these toxins. The presence of DSP toxins presents a major problem to the shellfish industry as they are a common cause of closures to shellfish harvesting (Yasumoto 2000). Historically, three groups of lipophilic toxins have been associated with DSP: okadaic acid (OA) and dinophysistoxins (DTXs), pectenotoxins (PTXs) and yessotoxins (YTXs). Okadaic acid and its congeners DTX-1, DTX-2 and DTX-3 are acid polyethers and are considered primarily responsible for the DSP syndrome. The yessotoxins are disulphated polyethers, which are now known not to be diarrheagenic, and have therefore been removed from the DSP complex (Anon. 2004). Nevertheless, YTXs are separately regulated based on their high toxicity by intraperitoneal injection into mice. It is further recognised that YTXs are not produced by species of *Dinophysis* but rather by other dinoflagellates. The PTXs are polyether lactones produced by several species of *Dinophysis* and are included within the DSP complex. However, their classification as DSP toxins is also questioned as there is debate as to whether they cause diarrhea.

Although DSP is considered a worldwide problem impacting on seafood safety (Yasumoto 2000), the problems posed by DSP in the major eastern boundary upwelling systems vary appreciably (Trainer et al. 2010). Whereas DSP has not posed a significant problem in either the California Current or Humbolt Current systems, it has a considerable economic impact on shellfish harvesting in the Canary Current system where it has been the focus of extensive research. In Western Iberia, in particular, the species *Dinophysis acuminata* and *D. acuta* cause the most severe harmful algal bloom (HAB)-related impacts on shellfish harvests (Reguera et al. 1993). In the Benguela Current system, the first report of DSP was in 1991 (Pitcher et al. 1993) and, although the toxins associated with DSP pose a significant threat to shellfish harvesting (Pitcher and Calder 2000), they remain poorly investigated within this region.

Toxicity associated with DSP in the southern Benguela is usually attributable to either *D. acuminata* or *D. fortii*, which often form lesser components of blooms dominated by other dinoflagellates (Pitcher and Calder 2000). Plankton monitoring on both the West Coast (Elands Bay) and South-West Coast (Gordon's Bay, Figure 1) has revealed the intermittent presence of these species, as determined by the upwelling–downwelling cycle, particularly during the latter part of the upwelling season (Pitcher and Calder 2000). Presentation of the Gordon's Bay data, spanning 15 years (1992–2006), has demonstrated the marked interannual variability of *D. acuminata* abundance superimposed on the strong seasonal occurrence of this dinoflagellate, with elevated concentrations occurring from March through to May (Trainer et al. 2010). *Dinophysis acuminata* and *D. fortii* have also been shown to be widely distributed over the shelf, confined only by the 18 °C isotherm (Pitcher and Weeks 2006).

Direct measurements of toxins in *Dinophysis* species from the southern Benguela have been made only recently. Fawcett et al. (2007) found OA to be the dominant and only true DSP toxin in phytoplankton concentrates sampled from the West Coast and containing *D. acuminata* and *D. fortii*, whereas Hubbart (2010) also reported small amounts of DTX-1 in addition to OA in West Coast samples dominated by *D. acuminata*. Low amounts of PTX-2 were detected in both studies. Hubbart (2010) also reported on the composition of DSP toxins in shellfish collected from the West Coast, documenting increased levels of DTX-1 in relation to OA, compared to that measured in concentrates of phytoplankton, and the presence of trace amounts of PTX-2sa in addition to PTX-2.

In the present study, the accumulation of DSP toxins in the Pacific oyster *Crassostrea gigas* was compared with that in the mussel *Choromytilus meridionalis* at a site located off Lambert's Bay on the west coast of South Africa. *Crassostrea gigas* forms the basis of oyster cultivation in South Africa whereas *C. meridionalis* is one of two mussel species cultivated on the South African coast. The study also advances several aspects of our knowledge of the toxins and toxicity of *D. acuminata*, the dinoflagellate primarily responsible for DSP in the southern Benguela.

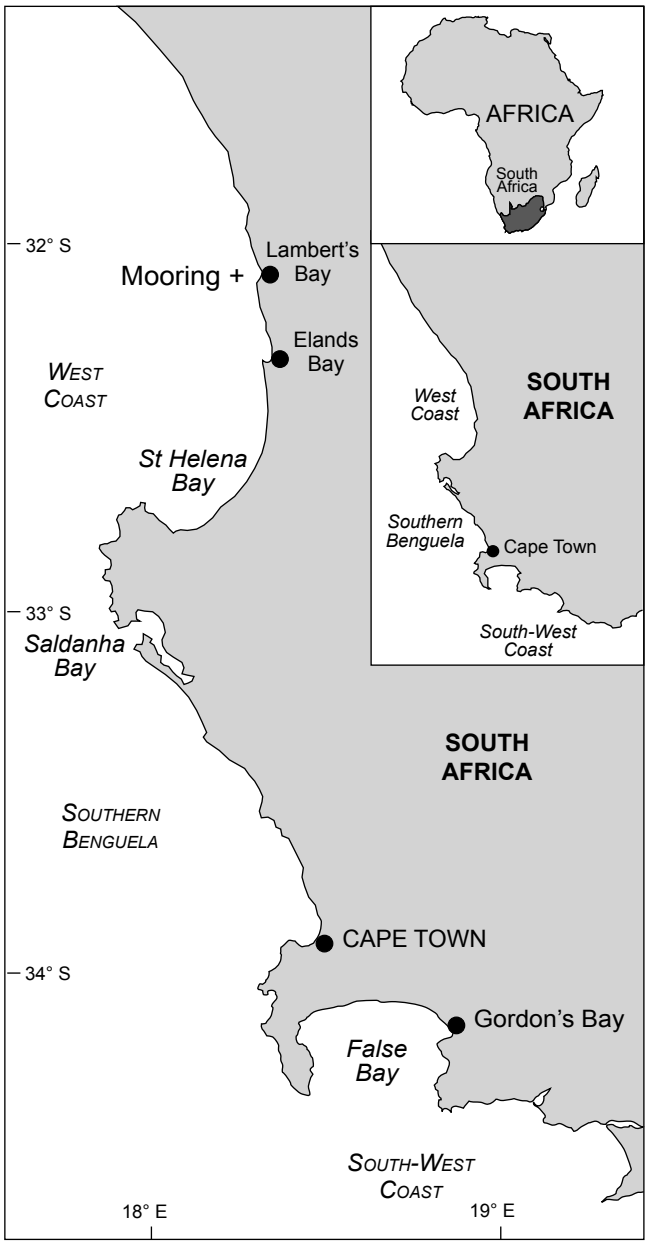

Figure 1: Map showing the mooring location off Lambert's Bay within the greater St Helena Bay region on the west coast of South Africa

Material and methods

Both oysters and mussels were collected on 21 February 2008 and suspended 1–3 m below the surface in meshed bags (15 individuals per bag) from a mooring located 3.5 km off Lambert's Bay at 50 m depth (Figure 1). The oysters were collected from an oyster farm in Saldanha Bay and the mussels were sourced from either Saldanha Bay or Lambert's Bay. One bag of oysters and one of mussels was intended for harvest each day for the period 4–25 March 2008. Owing to the loss of a number of bags, sampling of oysters was terminated on 21 March and mussels on 22 March 2008. Following harvest, the mussels and oysters were frozen and stored at −20 °C prior to analysis.

A Sea-Bird CTD with Wetstar fluorometer was used to profile the water column each day, providing a time-series of temperature and chlorophyll *a* for the duration of the experiment. Samples for the identification and enumeration of phytoplankton were taken daily at the mooring by means of NIO bottle samples at depths of 0, 5, 10, 15 and 20 m, fixed in buffered formalin at a final concentration of 0.5% and counted by the Utermöhl method (Hasle 1978). Samples of 10 ml were settled overnight and diagonal strips of the sedimentation chamber were counted. A count of >40 cells of species of interest was undertaken at a magnification of 160×.

Water samples were also collected for toxin analysis by filtration of 200 ml of seawater from the surface and from 5 m through Whatman GF/F filters, which were then frozen in liquid nitrogen prior to extraction. After analysis, cell toxin quotas were derived by dividing the measured toxin of the filtered phytoplankton concentrates by the corresponding concentration of cells putatively associated with the toxin.

Toxin analysis

Analytical reagents

Water was deionised and purified (Milli-Q, Millipore GmbH, Eschborn, Germany) to 18 MΩ cm^{-1} quality or better. Formic acid (90%, p.a.), acetic acid (p.a.) and ammonium formate (p.a.) were purchased from Merck (Darmstadt, Germany). Acetonitrile was high performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany), *n*-hexane, methanol, *n*-hexane and anhydrous sodium sulphate were p.a. quality (Merck, Darmstadt, Germany). A standard solution of lipophilic shellfish toxins (domoic acid, gymnodimine, 13-desmethyl spirolide C, okadaic acid, dinophysistoxin-1, pectenotoxin-2, pectenotoxin-2 seco acid, pectenotoxin-11, yessotoxin and azaspiracid-1) were kindly donated by Michael Quilliam of the Institute for Marine Biosciences, National Research Council, Halifax, Canada.

Sample preparation

Filters were cut and subsequently transferred into separate FastPrep tubes, each containing 0.9 g of lysing matrix D, followed by addition of 0.5 ml methanol. Samples were homogenised by reciprocal shaking at maximum speed (6.5 m s^{-1}) for 45 s in a Bio101 FastPrep instrument (Thermo Savant, Illkirch, France). After homogenisation, samples were centrifuged (Eppendorf 5415 R, Hamburg, Germany) at 16 100 × *g* at 4 °C for 15 min. The supernatant (400 µl) was transferred to a spin-filter (pore-size 0.45 µm, Millipore Ultrafree, Eschborn, Germany) and centrifuged for 30 s at 800 × *g*. The remaining pellet was extracted twice more with 0.5 ml methanol each. The combined filtrates were transferred into an autosampler vial and analysed by LC-MS/MS.

Two g of shellfish homogenate were weighed into a 50 ml centrifugation tube and homogenised for 2 min with 5 ml 80% aqueous methanol with an Ultraturrax tissue homogeniser (IKA, Hohenstauffen, Germany) and subsequently centrifuged (Eppendorf 5810, Hamburg, Germany) at 3 220 × *g* for 15 min. The supernatant was carefully transferred to a new 50 ml centrifugation tube and the remaining pellet was re-extracted with 5 ml 80% aqueous methanol. Both supernatants were combined and fat was removed by three subsequent extractions with 7 ml *n*-hexane each. The organic layers were discarded and 3.5 ml water was added to the aqueous phase. The sample was twice extracted with 7 ml chloroform and combined chloroform extracts were dried over anhydrous sodium sulphate overnight. Sodium sulphate was removed by filtration and filters were rinsed twice with 1 ml chloroform each. The sample was taken to dryness in a rotary evaporator (Büchi, Konstanz, Germany) and resuspended in 1 ml methanol. The methanolic extract was filtered through a spin-filter (pore-size 0.45 µm, Millipore Ultrafree, Eschborn, Germany) by centrifugation for 30 s at 800 × *g*. The filtrate was transferred into an autosampler vial and analysed by LC-MS/MS.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

LC-MC/MS analyses were conducted using the methodology in Krock et al. (2008) and are described here. Mass spectrometric experiments were performed on an ABI-SCIEX-4000 Q-Trap (Applied Biosystems, Darmstadt, Germany), triple quadrupole mass spectrometer equipped with a TurboSpray® interface coupled to an Agilent (Waldbronn, Germany) model 1100 LC. The LC equipment included a solvent reservoir, in-line degasser (G1379A), binary pump (G1311A), refrigerated autosampler (G1329A/G1330B), and temperature-controlled column oven (G1316A). After injection of 5 µl of sample, separation of lipophilic toxins was performed by reversed phase chromatography on a C8 phase. The analytical column (50 × 2 mm) was packed with 3 µm Hypersil BDS 120 Å (Phenomenex, Aschaffenburg, Germany) and maintained at 20 °C. The flow rate was 0.2 ml min^{-1} and gradient elution was performed with two eluents, where eluent A was water and eluent B was acetonitrile/water (95:5 v/v), both containing 2.0 mM ammonium formate and 50 mM formic acid. Initial conditions were 12 min column equilibration with 5% B, followed by a linear gradient to 100% B in 10 min and isocratic elution until 15 min with 100% B. The system was then returned to initial conditions until 18 min (total run time: 30 min).

The chromatographic run was divided into three periods: (1) 0–8.75 min for domoic acid (curtain gas: 20 psi, CAD: medium, ion spray voltage: 5 500 V, temperature: 275 °C, nebuliser gas: 50 psi, auxiliary gas: 50 psi, interface heater: on, declustering potential: 50 V, entrance potential: 10 V, exit potential: 15 V); (2) 8.75–11.20 min for gymnodimine and spirolides (curtain gas: 10 psi, CAD: medium, ion spray voltage: 5 500 V, temperature: ambient, nebuliser gas: 10 psi, auxiliary gas: off, interface heater: on, declustering potential: 50 V, entrance potential: 10 V, exit potential: 15 V); and (3) 11.20–18 min for okadaic acid, dinophysistoxins, pectenotoxins, yessotoxin and azaspiracid-1 (curtain gas: 10 psi, CAD: medium, ion spray voltage: 5 500 V, temperature: ambient, nebuliser gas: 10 psi, auxiliary gas: off, interface heater: on, declustering potential: 50 V, entrance potential: 10 V, exit potential:15 V).

Selected reaction monitoring (SRM) experiments were carried out in positive ion mode by selecting the following transitions (precursor ion > fragment ion), period 1: m/z 312>266 (collision energy [CE]: 20 V) and m/z 312>161 (CE: 30 V) for domoic acid; period 2: m/z 508>490 (CE: 40 V) for gymnodimine, m/z 692>164 (CE: 55 V) for 13-desmethyl spirolide C and spirolide G, m/z 692>150 (CE: 55 V) for spirolide A, m/z 694>164 (CE: 55 V) for 13-desmethyl spirolide D, m/z 694>150 (CE: 55 V) for spirolide B, m/z 706>164 (CE: 55 V) for spirolide C and 20-methyl spirolide G and m/z 708>164 (CE: 55 V) for spirolide D; and period 3: m/z 822>223 (CE: 55 V) for okadaic acid and DTX-2, m/z 836>237 (CE: 55 V) for DTX-1, m/z 946>223 (CE: 55 V) for OA diol ester, m/z 874>213 (CE: 55 V) for PTX-12 and PTX-14, m/z 876>213 (CE: 55 V) for PTX-2, m/z 892>213 (CE: 55 V) for PTX-1, PTX-4, PTX-8, PTX-11 and PTX-13, m/z 894>213 (CE: 55 V) for PTX-2 seco acid, m/z 842>824 (CE: 55 V) for AZA-1 and m/z 1160>965 (CE: 55 V) for YTX. Dwell times of 100–200 ms were used for each transition.

Results

The phytoplankton assemblage

The oceanographic conditions during the study period were characterised by two periods of stratification set apart by a brief but strong period of upwelling from 15 to 16 March 2008 at which time subthermocline water broke the surface

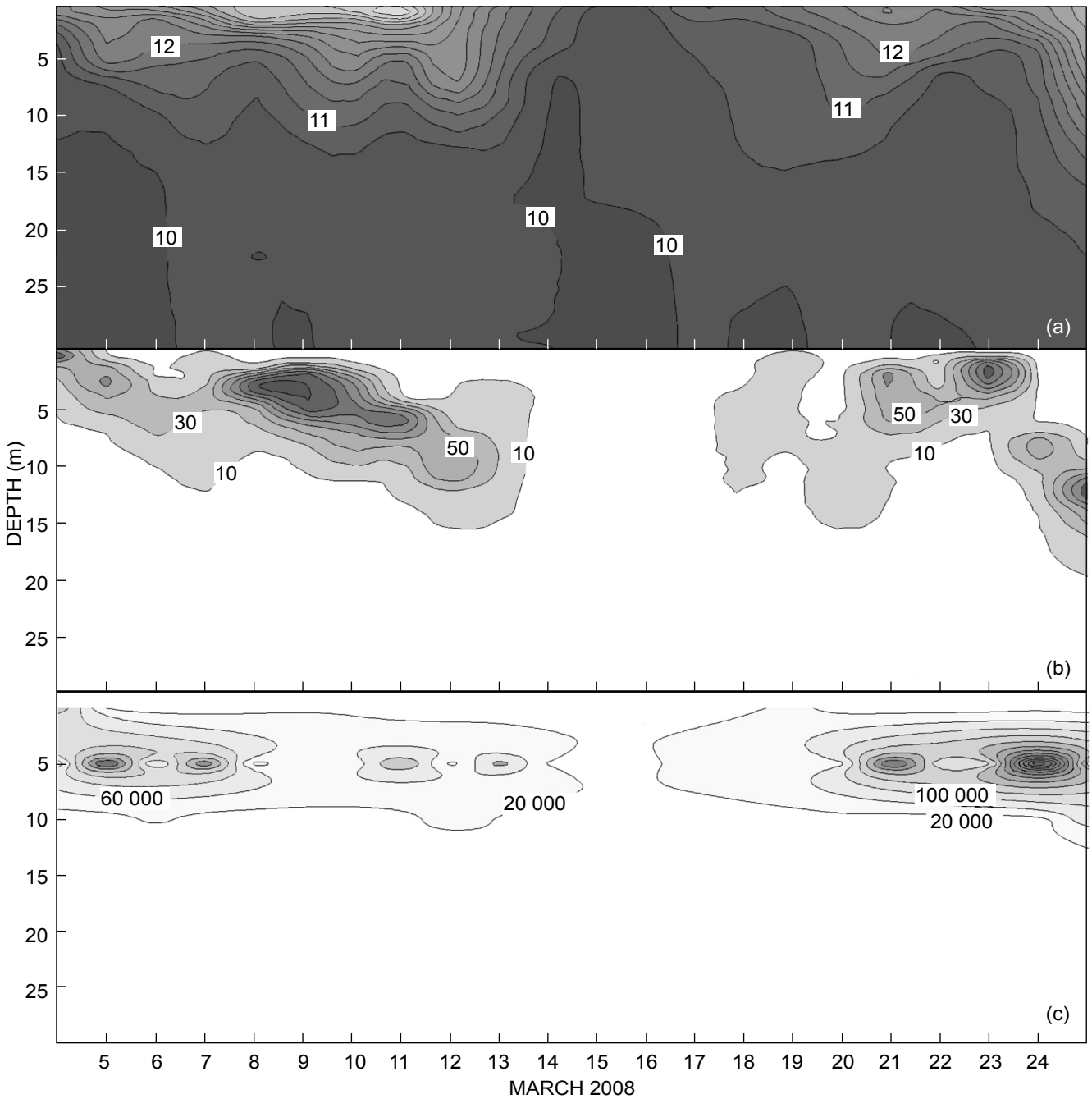

Figure 2: Time-series of (a) temperature (°C), (b) chlorophyll *a* (mg m^{-3}) and (c) *Dinophysis acuminata* (cells l^{-1}), 4–25 March 2008

(Figure 2a). The stratified periods were defined by a layer of warm water of approximately 10 m depth overlying a cold well-mixed bottom layer. Notable increases in phytoplankton biomass were associated with each period of stratification with chlorophyll *a* concentrations exceeding 100 mg m^{-3} (Figure 2b).

Phytoplankton assemblages during the study were therefore characterised by two 'blooms' separated by the short mid-March upwelling event. The first bloom was dominated by species of the centric diatom *Chaetoceros* and the small dinoflagellate *Gyrodinium zeta*, whereas the second bloom was dominated by the centric diatoms *Minidiscus trioculatus* and a *Coscinodiscus* sp. The toxigenic dinoflagellate *D. acuminata*, associated with DSP, was common through most of the study with the highest concentrations occurring subsurface at 5 m depth (Figure 2c). *Dinophysis acuminata* cell concentrations were lowest during the period of upwelling and highest during the subsequent period of stratification, reaching a maximum concentration of 5.7×10^5 cells l^{-1} on 24 March at 5 m.

Other species of *Dinophysis*, including *D. fortii*, *D. hastata* and *D. rotundata*, were also present but contributed only 0.3% to the *Dinophysis* population during the course of the study.

The toxin content of Dinophysis acuminata

Cell concentrations of *D. acuminata* and OA concentrations determined from the filtered concentrates of plankton corresponded closely at both the surface and at 5 m (Figure 3). Cell toxin quota of *D. acuminata*, derived by dividing the measured toxin (OA) of the filtered phytoplankton concentrates by the corresponding concentration of *D. acuminata* cells, is based on the assumption that this species represents the unique source of the toxin in the samples. The toxin content of *D. acuminata* thus determined ranged during the study from undetectable to 21.7 pg OA cell^{-1}, with a mean concentration of 7.8 pg OA cell^{-1} (Table 1). Although there was no apparent trend in the cell toxin quota over time, it was evident that cellular toxin content was significantly higher at the surface (10.7 pg OA cell^{-1}) than that determined from 5 m (5.4 pg OA cell^{-1}) depth (Table 1; $F = 4.11$, $p < 0.01$).

Toxin composition of plankton concentrates and shellfish

The toxin composition of *D. acuminata* as determined from filtered plankton concentrates was dominated by OA (91%), with lesser quantities of DTX-1 (6.5%) and PTX-2 (2.4%), and traces of PTX-2sa and PTX-11 (Figure 4). The toxins in shellfish were characterised by a notably higher content of DTX-1 (23.8% in *C. meridionalis*; 46.8% in *C. gigas*) and a corresponding lower relative content of OA (76.1% in *C. meridionalis*; 53.0% in *C. gigas*). Neither PTX-2 nor PTX-11 were detected in the shellfish but traces of PTX-2sa were evident.

Accumulation of DSP toxins in shellfish

A striking difference was observed in the accumulation of DSP toxins in *C. gigas* compared to that in *C. meridionalis* (Figure 5b); whereas toxin concentrations in *C. meridionalis* often exceeded the regulatory limit of 160 ng OA eq g^{-1} (mean 170 ng OA eq g^{-1}; range 92–267 ng OA eq g^{-1}; n = 19), toxin concentrations in *C. gigas* remained well below the regulatory limit (mean 8 ng OA eq g^{-1}; range 7–12 ng OA eq g^{-1}; n = 18).

The correspondence of cell concentrations of *D. acuminata* in the water column and toxins in the shellfish was poor (Figure 5). The mean cell concentrations (established from surface and 5 m samples) of *D. acuminata* varied substantially during the period of observation (mean 6.0×10^4; range 0–2.9×10^5 cells l^{-1}, n = 22). Shellfish were suspended at the mooring off Lambert's Bay 12 days prior to harvesting the first sample on 4 March 2008. At the time of first harvest, DSP toxins in mussels exceeded the regulatory limit, at which time the mean concentration of *D. acuminata* was 1.0×10^5 cells l^{-1}. Possible correspondence between cell concentrations and shellfish toxins was observed in that the lowest toxin concentrations in *C. meridionalis* were measured shortly after the upwelling event of 15–16 March and the subsequent marked decline in cell concentration of *D. acuminata.* No correspondence was observed between the concentrations of *D. acuminata* and the consistently low concentrations of DSP toxins in *C. gigas*.

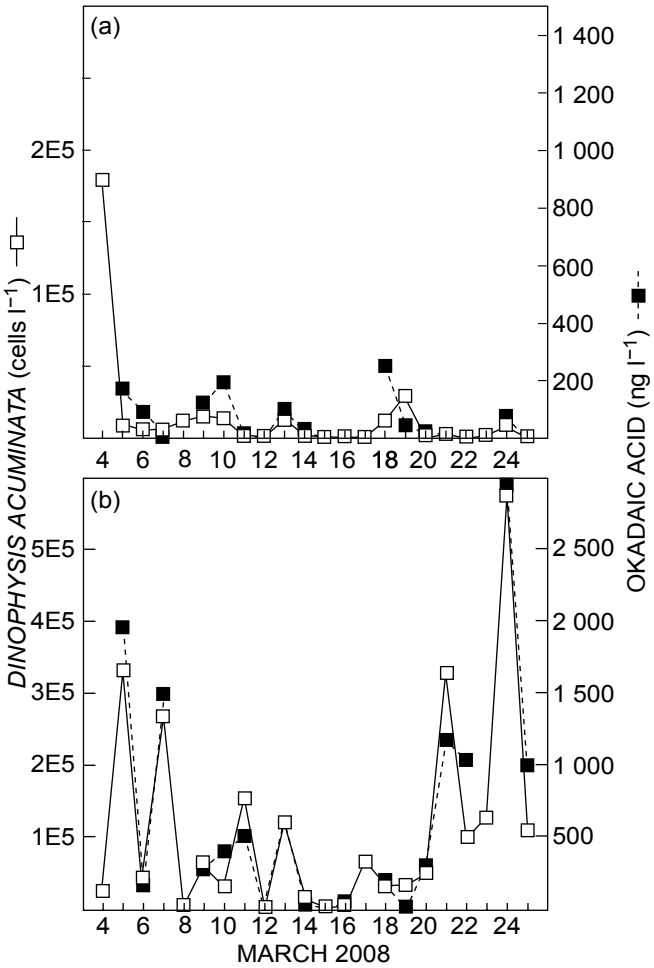

Figure 3: Daily time-series of *Dinophysis acuminata* cell concentrations and particulate concentrations of okadaic acid from (a) 0 m and (b) 5 m depth, 4–25 March 2008

Table 1: Estimated toxin cell quota of *Dinophysis acuminata* expressed as okadaic acid (OA) cell^{-1}, assuming that this species represents the sole source of the toxin

Sample depth	Mean (pg OA cell^{-1})	Range (pg OA cell^{-1})	*N*	SD
Surface and 5 m samples	7.8	0–21.7	31	5.8
Surface samples	10.7	0–21.7	14	6.9
5 m samples	5.4	0–13.5	17	3.4

Discussion

The association of species of *Dinophysis* with periods of relative warming and stratification in upwelling and other systems is well documented (e.g. Lassus et al. 1991, Delmas et al. 1992, Fawcett et al. 2007). This study serves to confirm the presence of *D. acuminata* as an important component of

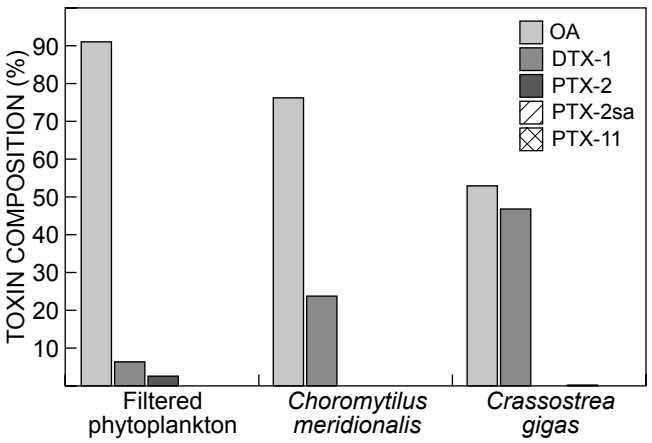

Figure 4: Toxin composition profile for *Dinophysis acuminata* (determined from filtered phytoplankton concentrates), the mussel *Choromytilus meridionalis* and the oyster *Crassostrea gigas*

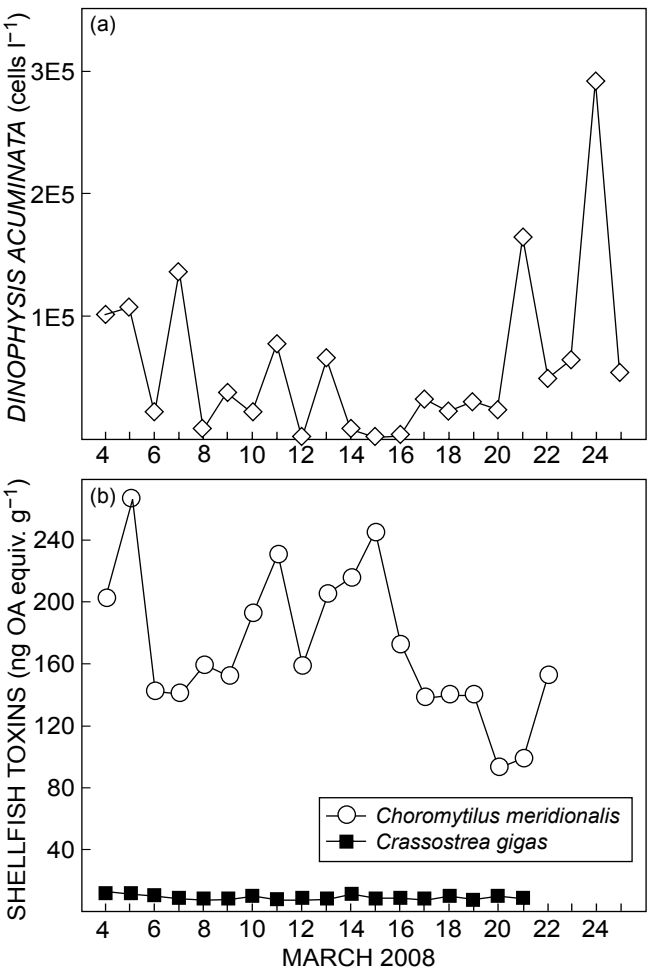

Figure 5: Time-series of (a) the mean cell concentration (surface and 5 m) of *Dinophysis acuminata*, and (b) DSP toxins in the mussel *Choromytilus meridionalis* and the oyster *Crassostrea gigas*, 4–25 March 2008

the phytoplankton of the southern Benguela under conditions of intensified stratification, particularly toward the end of summer and during early autumn. This investigation further corroborates past studies that have implicated *D. acuminata* as the most common cause of DSP in this region.

Toxins and toxicity of Dinophysis acuminata

The toxin composition of *D. acuminata* as determined during this study is similar to that reported by Hubbart (2010), for samples collected from the same locality in 2007, in that OA dominated the toxin profile (>90%) with smaller quantities of DTX-1 and PTX-2. The only other account of the toxin profile of *D. acuminata* from the Benguela is that of Fawcett et al. (2007) that similarly reported the dominance of OA, but did not indicate the presence of DTXs. Whereas OA is often reported as the dominant toxin produced by *D. acuminata*, only some of these reports specify the presence of DTXs (e.g. Lee et al. 1989, Masselin et al. 1992, Blanco et al. 1995, Dahl et al. 1995, Sato et al. 1996, Fernández et al. 1998), whereas others report their general absence (e.g. Fernández et al. 2001, Vale and Sampayo 2000, 2002, Moroño et al. 2003). The presence of PTXs was originally reported in association with *D. fortii* (Lee et al. 1989, Draisci et al. 1996), but more recently these toxins have been found to be prominent in several other species of *Dinophysis*, including *D. acuminata* (e.g. MacKenzie et al. 2005). Consistent with the findings of this study, PTX-2 is typically found to be the most common PTX in phytoplankton. The dominance of PTX-2 in *D. acuminata* from New Zealand, as reported by MacKenzie et al. (2005), provides evidence of the considerable global variation in the toxin profile of *D. acuminata*.

The control of cell toxin quota in *Dinophysis* and other toxic dinoflagellates is poorly understood, although it is recognised that the balance or imbalance between toxin production and cell division is important. For *D. acuminata*, reported toxin content varies considerably, from non-detectable (e.g. Lee et al. 1989, Hoshiai et al. 1997) to >50 pg OA cell^{-1} (e.g. Andersen et al. 1996). The toxicity of *D. acuminata* measured during this study is similar to that reported in several other studies (e.g. Lee et al. 1989, Blanco et al. 1995, Maneiro et al. 2000, Fernández et al. 2001, Klöpper et al. 2003, Moroño et al. 2003, Lindahl et al. 2007, Reizopoulou et al. 2008), but is notably higher than that previously measured in the southern Benguela (Hubbart 2010).

Of particular interest during this study was the higher cellular toxicity of *D. acuminata* at the surface compared to cells at 5 m depth. Lindahl et al. (2007) similarly demonstrated higher cellular toxin content in *D. acuminata* in the surface waters of Koljö Fjord (Sweden) compared to that of cells in the pycnocline. Lindahl et al. (2007) demonstrated a negative correlation between the population cell concentration and the cell toxin quota of *D. acuminata*, i.e. the higher the cell concentration the lower the toxin content per cell. Off Lambert's Bay, the typically higher concentration of *D. acuminata* at 5 m and the lower toxin content of cells at this depth suggest a similar relationship. It is possible that the differences between cellular toxicity at the surface and at 5 m are a function of growth conditions and nutrition. For example, Johansson et al. (1996) have shown higher toxin production by *D. acuminata*

when growth is limited by nitrogen. The expected presence of lower nitrogen concentrations in the surface waters off Lambert's Bay therefore supports the finding of more toxic cells at the surface. Lindahl et al. (2007) suggested that the lower density surface populations may be autotrophic, slow-growing and toxin-producing, whereas the higher density deeper populations were either mixotrophic or heterotrophic, fast-growing and produce less toxins.

The processes responsible for the reported variability in cellular toxicity need to be further addressed. It is plausible that much of this variability is a function of the methods of determination. For example Jørgensen and Andersen (2007) have ascribed the variability in cellular toxin content, determined from concentrates of phytoplankton, to the loss of toxins during filtration due to cell damage. Therefore, the greater the volume filtered the more toxin lost, resulting in an underestimate of cell toxin quota. Conversely, Pizarro et al. (2009) have suggested that estimates of cellular toxins from concentrates of phytoplankton may lead to overestimates as a result of the inclusion of extracellular toxins during the filtration process. Overestimate of cellular toxins may also result from the inclusion of other particulates, such as faecal pellets or zooplankton, in the concentrates of phytoplankton.

Species-specific differences in toxin composition and toxicity in shellfish

The higher proportion of DTX-1 in the shellfish harvested off Lambert's Bay, compared to that measured in the phytoplankton concentrates, is consistent with the composition of DSP toxins reported by Hubbart (2010) in *C. meridionalis* sampled from the same locality in 2007. Reports of DTXs forming a significant portion of the toxin content in bivalves are common (e.g. Carmody et al. 1995), an observation further influenced by the bivalve species. For example, the higher proportion of DTXs in oysters compared to those in mussels, as noted in this study, has been previously reported (Dominguez et al. 2010). Whether toxin compositional differences among shellfish species versus ambient plankton composition containing *Dinophysis* spp. in the southern Benguela ecosystem reflect biotransformation processes or selective retention/release of particular DTX toxin analogues remains unclear.

DSP toxins are regulated in the European Union by Regulation No. 853/2004/EC of the European Commission, which establishes the maximum permitted level of 160 µg OA equivalents kg^{-1} of shellfish for OA, DTXs and PTXs in combination. This regulatory level is also applied by the South African Molluscan Shellfish Monitoring and Control Programme. Consequently, the DSP toxins as measured during this study in *C. meridionalis* more often than not exceeded the permitted regulatory limit, whereas these toxins measured in *C. gigas* were well below this level. The study therefore clearly demonstrated the greater susceptibility of the mussel *C. meridionalis* compared to the oyster *C. gigas* to the accumulation of DSP toxins, with the average concentration in the mussel exceeding that in the oyster by approximately 20-fold. Although comparisons of this nature are not common, similar studies consistently show higher DSP toxin concentrations in mussels compared to those in oysters and several other filter-feeders (e.g. Poletti et al. 1998, Svensson et al. 2000, Vale and Sampayo 2002, Reizopoulou et al. 2008). In a study very similar to that reported here, Lindegarth et al. (2009) also showed DSP toxins to be 10–50 times lower in the European flat oyster *Ostrea edulis* compared with the blue mussel *Mytilus edulis*.

Marked interspecific variation in the accumulation, biotransformation and depuration of toxins by shellfish is well established, although the reasons for these differences remain speculative. Differences in feeding rates, selective feeding, and different rates of ingestion and assimilation, are all likely to influence shellfish toxicity (e.g. Shumway and Cucci 1987). Sidari et al. (1998) have, for example, shown that mussels may select for dinoflagellates rather than diatoms, and further describe a selection preference by the mussel *Mylilus galloprovincialis* for the genus *Dinophysis*. Species-specific differences in metabolism of toxins, in selective retention or elimination of specific toxins, and in transformation of toxins, all further contribute to species-specific differences in shellfish toxicity. In general, mussels tend to more rapidly accumulate and release phycotoxins (reviewed by Bricelj and Shumway 1998 for paralytic shellfish toxins), and perform comparatively less biotransformation of toxins than other bivalve molluscs when exposed to natural toxic blooms or when fed in controlled laboratory simulations.

Although certain studies have shown a defined relationship between the cell concentrations of *Dinophysis* and shellfish toxicity (e.g. Sidari et al. 1998, Séchet et al. 1990, Dahl et al. 1995, Godhe et al. 2002, Klöpper et al. 2003, Jorgensen and Andersen 2007), others have found this relationship to be inconsistent (e.g. Lassus et al. 1991, Jackson and Silke 1995, Dahl and Johannessen 2001, Marcaillou et al. 2001, 2005). The difficulty of relating toxic cell count with shellfish toxicity is often considered to be a function of the variability in cell toxin content (Marcaillou et al. 2005). Consequently, when attempting to relate toxic cell concentration and shellfish toxicity, care should be taken to estimate intrapopulation specific toxicity (Marcaillou et al. 2005). The apparent discrepancy of toxic cell concentration and shellfish toxicity may also be a mere function of comparison of an integrated signal, as provided by assessment of toxins in shellfish, with that obtained from samples collected from discrete depths at selected time intervals, i.e. a sampling strategy that does not accurately reflect the food environment and continuous feeding regime of the shellfish.

Conclusion

The results presented here represent the first attempt to quantify the differences in the accumulation of DSP toxins in two of the most commonly cultivated bivalve mollusc species in South Africa. As in the Iberian upwelling system, DSP poses a major threat to shellfish cultivation on the South African coast. Despite the moderate toxin content of *D. acuminata* in the southern Benguela, this dinoflagellate is the most common cause of DSP within the region, frequently rendering shellfish unsafe for consumption because of its presence at relatively high cell concentrations. The threat posed by DSP is, however, very dependent on the particular shellfish being cultivated. The results of this study show the mussel *C. meridionalis* to be a high-risk

species for DSP contamination in that the regulatory limit for toxins was often exceeded, whereas the oyster *C. gigas* was a low-risk species, for which the regulatory limit was never exceeded. The almost 3-fold variation in the toxicity of *C. meridionalis* over the course of sampling, without any obvious trend or correspondence with the cell concentrations of *D. acuminata*, further demonstrate the difficulties in obtaining representative samples and in determining appropriate sampling frequencies. The results do nevertheless indicate that regulatory authorities should consider establishing species-specific sampling frequencies in the design of monitoring programmes for the safety assurance of shellfish for human consumption.

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