Phycotoxins might be involved in human diarrhoeal maladies in coastal areas of Nigeria

(A report to VLIZ for The Prize Dr Edouard Delcroix 2003)

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Abstract

The syndrome of phycotoxin-related diarrhoea is referred-to as diarrhetic shellfish poisoning (DSP). The epidemic of diarrhoeal symptoms is widespread in Nigeria's coastal area. Although microbial organisms are responsible for most diarrhoeal infections in Nigeria, phycotoxins-related diarrhoeal cases have been reported in many parts of the world. But the clinical symptoms of phycotoxin-related diarrhoea may, nevertheless, be mistaken for those of bacterial gastric infection. Known microalgae that produce DSP toxins belong to the dinoflagellate group and include *Prorocentrum* spp and *Dinophysis* spp. DSP is recognized as a threat to public health and commercial shellfish production in many countries, especially since more serious health risks might be associated with chronic exposure to DSP toxins. Public health problems associated with DSP-causing algal species make it very crucial for people and countries of the world to be aware of the syndrome, its symptoms and causative agents (i.e. microalgae and the biotoxins involved). DSP is common in nature with occurrence throughout the Pacific, Indian and Atlantic oceans. Thus, we suspected that some of the diarrhoeal cases in coastal areas of Nigeria are caused by toxic dinoflagellates. We also suspected that awareness about the toxic algal bloom phenomenon and its associated public health problems is lacking in Nigeria. Therefore, we studied the toxic algal bloom phenomenon in Nigeria with the aim of elucidating the level of awareness about the phenomenon in the country and to monitor her coastal waters for the presence of potential DSP-causing dinoflagellates. In order to accomplish our goal, we worked with questionnaires that were distributed to medical doctors, aquatic scientists and fishermen in Nigeria. We also analysed water samples from Nigeria's coastal waters for the presence of potential DSP-causing dinoflagellates. The results established the presence of potential DSP-causing dinoflagellates in Nigeria. They included five *Dinophysis* spp. and two *Prorocentrum* spp. Nearly all of them were first records for Nigeria. The results also suggested that phycotoxin-related diarrhoea might be occurring in Nigeria but, because awareness about the toxic algal bloom phenomenon is lacking in the country, the people there are not aware of it.

Introduction

The epidemic of diarrhoeal symptoms is widespread in Nigeria's coastal area. Although microbial organisms are responsible for most diarrhoeal infections in Nigeria, phycotoxins-related diarrhoeal cases have been reported in many parts of the world (e.g. Kat, 1985; Kumagai *et al.*, 1986; Quilliam *et al.*, 1993). In Nigeria, some diarrhoeal symptoms tend to be chronic and do not respond to treatments. This is because sufferers are treated for bacterial-related diarrhoea. Hallegraeff (1995) even observed that the clinical symptoms of phycotoxin-related diarrhoea may be mistaken for those of bacterial gastric infection. The syndrome of phycotoxin-related diarrhoea is referred-to as diarrhetic shellfish poisoning (DSP). It is acquired through the consumption of seafood (mostly shellfish) that had ingested toxic microalgae - microscopic aquatic plants that include phytoplankton, benthic algae, and epiphytic/periphytic algae.

To a large extent, microalgae form the basis of the aquatic food web and contribute immensely to the primary productivity of the world oceans. But not all of them are beneficial to the aquatic system and the organisms that live therein. Some of them form huge and extensive blooms that provoke aquatic biota kills *via* creation of anoxic conditions, reduction of the amount of sunlight penetrating into the water, and gill clogging. There are also highly toxic ones. The toxic species produce bioactive molecules (i.e. phycotoxins) that kill aquatic biota of commercial importance. Their toxins can also accumulate in the tissue of fish and shellfish and poison human consumers of seafood. Toxic microalgal species have been described among flagellates, diatoms and cyanobacteria. Already known microalgae that produce DSP toxins belong to the dinoflagellate group (Baden and Trainer, 1993; Steidinger, 1993). They include *Prorocentrum* spp (e.g. *P. concavuum, P. lima*, and *P. minimum*) and *Dinophysis* spp (e.g. *D. acuminata, D. acuta, D. caudate, D. fortii, D. norvegica, D. rotundata,* and *D. tripos*).

Phycototoxins that cause DSP include the polyether compounds: okadaic acid (OA), dinophysistoxin-1 (DTX-1) (Murata *et al.*, 1982); DTX-2 (Hu *et al.*, 1992), and DTX-3 (Yasumoto *et al.*, 1985). The OA/DTX compounds are potent phosphatase inhibitors that probably cause diarrhoea by stimulating the phosphorylation of proteins that control sodium secretion by intestinal cells (Cohen *et al.*, 1990), or by enhancing phosphorylation of cytoskeletal or junctional elements which regulate permeability to solutes, thereby resulting in

passive loss of fluids (Dho *et al.*, 1990). The typical symptoms of DSP include acute diarrhoea, nausea, vomiting, and abdominal cramp. OA and the DTXs may, nevertheless, promote stomach tumours (Suganuma *et al.*, 1988) and thus produce chronic problems in shellfish consumers. DSP is, therefore, recognized as a threat to public health and commercial shellfish production in many countries (Carmody *et al.*, 1995), especially since more serious health risks might be associated with chronic exposure to DSP toxins (Aune and Yndestad, 1993).

Public health problems associated with DSP-causing algal species make it very crucial for people and countries of the world to be aware of the DSP syndrome, its symptoms and causative agents (i.e. microalgae and the biotoxins involved). This will help prevent DSP incidences by, for example, implementing regulatory measures like closure of shellfish fishery during toxic algal blooms. Nigeria is Africa's largest consumer of seafood (UNEP, 1994). Since it is reported that DSP is common in nature with occurrence throughout the Pacific, Indian and Atlantic oceans (Quilliam and Wright, 1995), we suspected that some of the diarrhoeal cases in coastal areas of Nigeria are caused by toxic dinoflagellates. We also suspected that awareness about the toxic algal bloom phenomenon and its associated public health problems is lacking in Nigeria and that this might result to misidentification of phycotoxin-related diarrhoea in the country. Therefore, we studied the toxic algal bloom phenomenon in Nigeria with the aim of elucidating the level of awareness about the phenomenon in the country and to monitor her coastal waters for the presence of potential DSP-causing dinoflagellates.

Materials and Methods

In order to accomplish our goal, we worked with questionnaires as well as analysed water samples from Nigeria's coastal waters. The questionnaire study was conducted in November/December 1999. The study area included Lagos, Enugu , Cross River, and Rivers, all in southern Nigeria (Fig. 1). Questionnaires were distributed to medical doctors in both private and government hospitals, aquatic scientists in universities and research institutes, and fishermen in three fishing communities (Fig. 2). In Enugu State, only medical doctors were interviewed. We did not work with any prepared list of physicians, scientists or fishermen. The interviews were based on spontaneous visits to hospitals, research institutes, universities

and the fishing communities. Therefore, all meetings with the contacted persons were highly based on chance and since they had no prior knowledge of the interview, we strongly belief in the responses they provided. C.C. Ajuzie personally handed the questionnaires to all respondents and even conducted oral interviews with most of them. The questionnaire for fishermen was translated into the local language of each community visited. The translations were recorded in audiocassettes and played to them in a small cassette player. In the questionnaire we gave a brief description of toxic algal blooms, the harm they cause to aquatic biota and to human consumers of seafood.

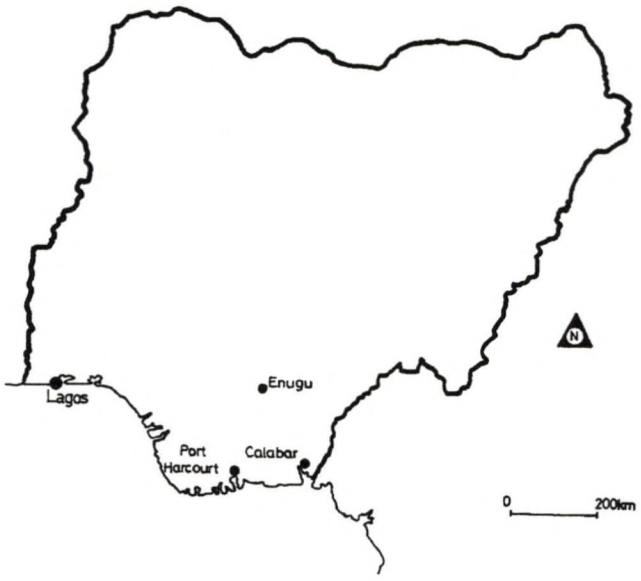


Fig. 1. Map of Nigeria showing the towns covered in the study

The questionnaire for the medical doctors read:

Name (optional):

Position (required):

Address of establishment (required):

a) Are you aware of the toxic algal bloom (TAB) phenomenon? Yes No

b) Are you aware of the human phycotoxin-poisoning syndromes? Yes No

c) Have you treated any case where you suspected phycotoxin poisoning? Yes No

d) Have you had patient(s) who took ill after consuming seafood? Yes No

e) If your answer to d is yes, what were the symptoms they exhibited, and what was the time lapse between the seafood consumption and the manifestation of the symptoms?

f) What type of seafood was consumed (fish, shellfish, and/or seaweed)?

g) Have you had patients that exhibited some combinations of the following symptoms: nausea, vomiting, diarrhoea, abdominal cramps, chills, headaches, muscle/joint pains, tingling/prickly sensation (or numbness around the lips, fingertips and toes), dizziness, confusion, hallucinations, short-term memory loss, seizures, difficulty in balance, digestive tract tumours, difficulty in breathing, double vision, difficulty in walking/swallowing (muscular paralysis)? Please, state the combinations, if yes is the answer.

The questionnaire for the aquatic scientists read:

Name (optional):

Position (required):

Address of establishment (required):

a) Are you aware of the toxic algal bloom (TAB) phenomenon? Yes No

b) Have TABs been recorded in Nigeria's coastal waters? Yes No

c) If your answer to b is Yes, in which year and area was the event recorded?

were there any aquatic biota kills associated with the event(s)?

were there any case of human seafood poisoning during the event?

d) Are you involved in TAB studies? Yes No

e) Are you aware of any TAB research laboratory in the country? Yes No

f) Has any TAB species been recorded in Nigeria's coastal waters? Yes No

g) If your answer to f is yes; do, please, name the species and state the places they were recorded

h) Do you know if anyone in the country had fallen sick after he or she consumed some kind of seafood? Yes No

i) If your answer to h is yes,

what kind of seafood (fish, shellfish, and/or seaweeds) was involved?

what symptoms did the affected person(s) exhibit?

The questionnaire for the fishermen read:

Name (optional):

Type of activity (required): Shellfish fishing, finfish fishing or both

Address (required):

a) Have you ever noticed a change in water colour in the sea around you? Yes No

b) If yes, what colour (red, brown, green) did you notice?

c) Was the change in water colour sudden (a few hours) or did it develop gradually (days)?

d) Did the colour change last longer than a few days?

e) How often does this change in water colour occur?

f) Is it seasonal (i.e. dry season vs. rainy season)?

g) Are there any local beliefs attached to it? Yes No

h) If yes, what are they?

i) Have you seen (or heard that) fish or shellfish died in huge numbers in your surrounding waters? Yes No

j) If yes, was such an incident caused by the use of illegal fishing means (the use dynamites or chemicals) or by an unknown means?

k) What is the frequency of occurrence of such kills?

l) Do such kills occur in coincidence with change in water colouration, or after heavy rains?

m) Have people in your community ever stopped some fisheries activity (e.g., the collection and consumption of shellfish)? Yes No

n) If yes, why? and does this occur regularly?

o) Do you know if anyone in your community took ill after he or she had consumed seafood? Yes No

p) If yes, what type of seafood was consumed (fish, shellfish, and/or seaweeds)?

Fig. 2. One of the fishermen's communities visited

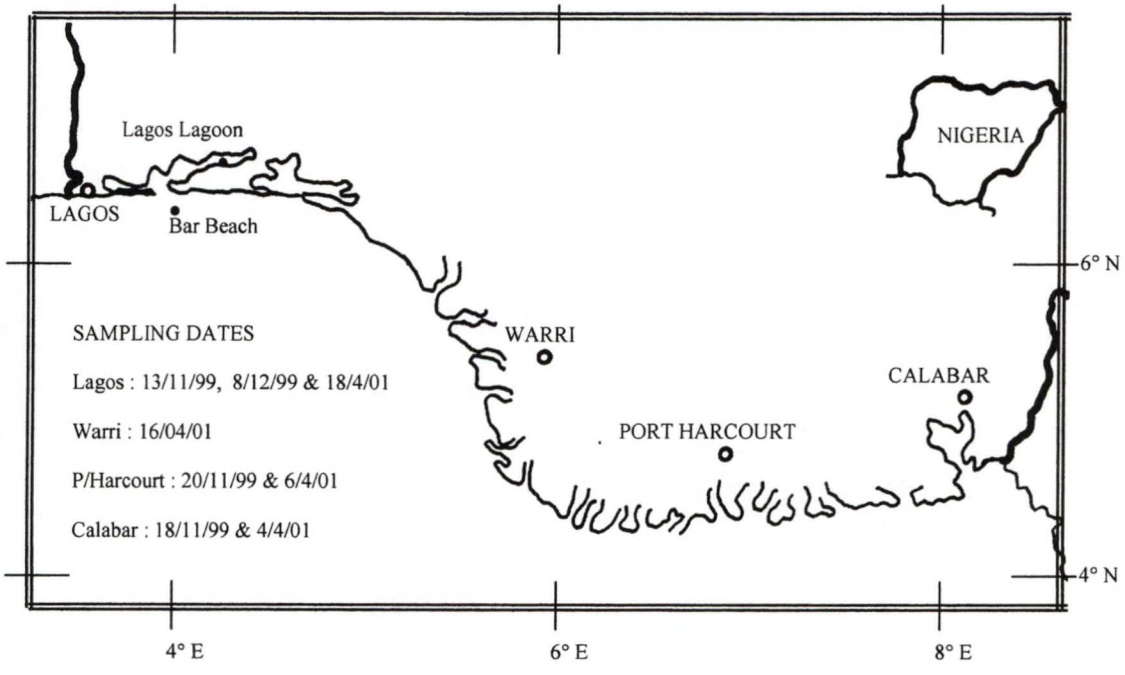


Fig. 3. Water Sampling locations

In order to qualitatively investigate the possible occurrence of potential DSP-causing microalgae in Nigeria's coastal waters, we collected water samples during the months of November/ December 1999 and April 2001. Sampling locations (Figure 3) were selected to assure a wider coverage of the Nigerian coast since this study, being the first of its kind in Nigeria's coastal waters, was of a reconnaissance type. The study area covered waters that are used for fishing, navigation, exploration of crude oil, etc. Near surface waters were collected at the sampling stations *via* plankton-net (20 µm-mesh) hauls. Benthic species were sampled with a sediment grab. Collected sediment samples were stored in a box until their arrival at our laboratory in Brussels. They were then transferred to a refrigerator until analysis. Before analysis, the sediment samples were suspended in water and washed through graded sifters, terminating with a 20 µm-mesh sieve. Identification of the potential DSP-causing dinoflagellates was performed on samples fixed in borax-buffered formaldehyde. All samples were examined under light microscopes (normal and inverted). Microphotographs were taken of all species of interest. Various reference materials, including Dodge (1982), Taylor, Hallegraeff *et al.* (1995), and Tomas (1997) were employed to identify the species of interest. During sampling, the physical (particularly temperature and salinity) and chemical (ammonium, nitrate, nitrite, and phosphate concentrations) characteristics of the waters were

determined on the spot, at each sampling station. The chemical parameters were measured using reagents (TESTSET™) for water quality assessment packaged by JBL®.

Results and Discussion

Two medical doctors refused to be interviewed. Most of the medical doctors that were interviewed (90 in all) knew nothing about the TAB phenomenon. Just 4.4 % of them were aware of the phenomenon. On the whole, 6.7 % of the doctors had received patients who took ill after consuming meals that contained a mixture of seafood (fish, shellfish and/or seaweed together). The symptoms they exhibited included diarrhoea, abdominal pains, weakness and raised body temperatures. The time lapse between their illness and the consumption of the seafood ranged from a few minutes to a few hours. However, only 2.2 % had suspected phycotoxin poisoning from among their patients.

Sixty one percent of the doctors hinted that they had received patients who exhibited two or more combinations of the following symptoms: nausea, vomiting, diarrhoea, abdominal cramps, chills, headaches, muscle/joint pains, tingling/prickly sensation or numbness (around the lips, fingertips and/or toes), dizziness, confusion, hallucinations, short-term memory loss, seizures, difficulty in balance, digestive tract tumours, difficulty in breathing, double vision, and difficulty in walking/swallowing (muscular paralysis). These are pooled symptoms of the human phycotoxin poisoning syndromes. They occur in different combinations according to the toxins involved (Hallegraeff, 1995). Nausea, vomiting, diarrhoea and abdominal cramp (which are symptoms of DSP) were the most registered group of symptoms (Fig. 4).

All the 20 Nigerian aquatic biologists contacted indicated that they were aware of the harmful algal bloom phenomenon, but none was aware of any HAB incidence or phycotoxin-poisoning case in Nigeria. None of them was aware of any in Nigeria. None of them was involved with HAB studies. They were not aware of any HAB researcher in the country. They were not aware of any described toxic algae in Nigeria's waters.

Thirty fishermen that fished for both fish and shellfish were interviewed in three different fishing communities, 10 from each community. The fishermen hinted that at certain times of the year they notice colour changes in their local waters: green waters that develop gradually

during the dry season and brown waters during the rainy season. The colour changes may reflect blooms of (or contain) potentially toxic algae. They also mentioned that there are neither taboos on the consumption of fish or shellfish species nor closure periods for shellfish collection in their waters. Climate is the only factor that determines what shellfish to harvest and when to harvest it. During the rainy season the water levels in the lagoons, creeks and estuaries are usually high, making shellfish harvesting difficult. The waters during this period are also turbulent and turbid in most areas. During the dry season shellfish collection is at its peak because the waters are calm, clear and shallower. Nevertheless, they disclosed that certain persons in their communities had fallen sick after consuming meals that included seafood.

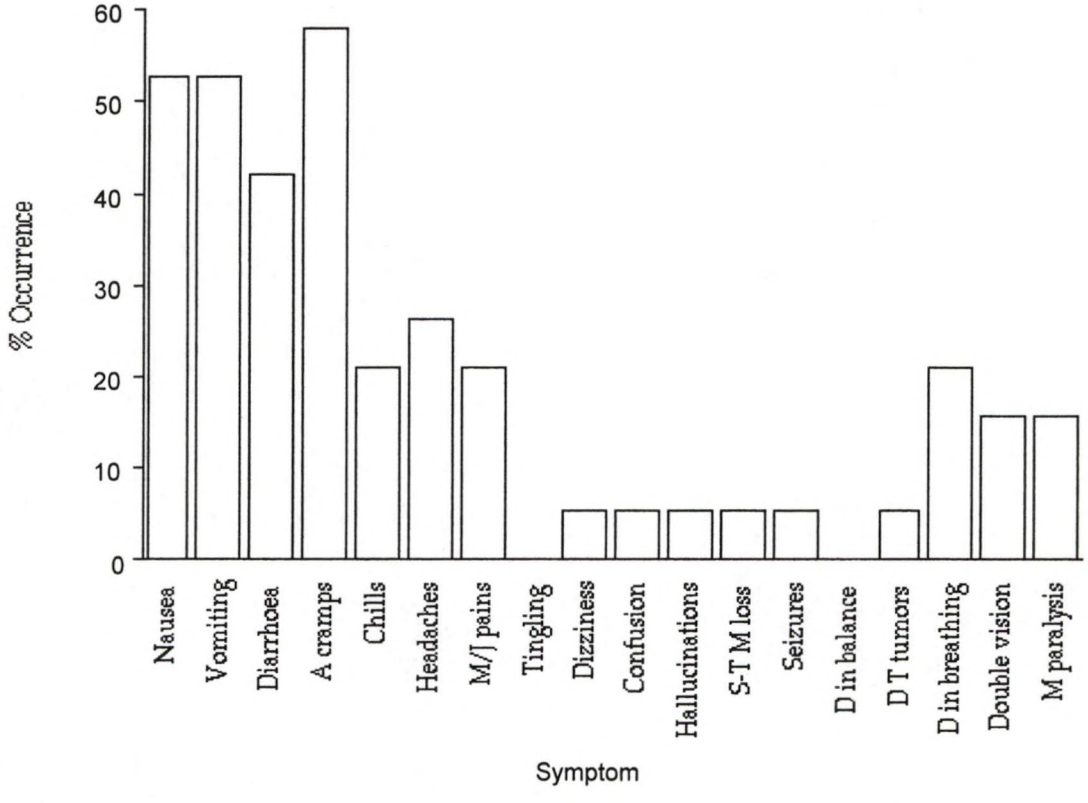


Figure 2.3. Percent occurrence of a HPPS symptom in combination with other HPPS symptoms

N/B: HPPS: human phycotoxin poisoning syndromes
A cramp: abdominal cramp
S-T M loss: short-term memory loss
D T tumors: digestive tract tumors
M paralysis: muscular paralysis
M/J pains: muscle/joints pains
D in balance: difficulty in balance
D in breathing: difficulty in breathing

Analyses of the water samples revealed the presence of potential DSP-causing microalgae in Nigeria's coastal waters. They included five species of *Dinophysis* (*D. acuta*, *D. caudata*, *D. rotundata*, *D. tripos* and a yet to be described *Dinophysis* sp; Figs. 5-9), and two species of *Prorocentrum* (*P. lima* and *P. minimum*; Figs. 10 & 11). All the *Dinophysis* spp were present in the Bar Beach. But *D. caudata* was also recorded in the Calabar area. *P. lima* was observed in sediment grabs from the Lagos Lagoon. *P. minimum* was present in the Lagos Lagoon and the Warri area. These algae tended to prefer waters where the N:P ratios were relatively high ($\geq$16). Thus, suggesting that N is very important for the blooming of these algae. The salinity of waters where the *Dinophysis* spp were present ranged from 13 to 34 ‰ for *D. caudata* and 26-34 ‰ for the others. Whereas *P. lima* was present in waters with salinities at 10 to 27 ‰, *P. minimum* was present in waters with salinity at 2 to 32 ‰. These salinity ranges match literature descriptions of their preferred habitats. All the species, with the exception of *Dinophysis caudata*, are first records for Nigeria. The recorded species strongly support our hypothesis that phycotoxins might be involved in some diarrhoeal illnesses in Nigeria. But further studies aimed at determining the toxigenicity of these algal species is strongly desired.

Dinophysis acuta Ehrenberg (Fig. 5)

This species is characterized by a rounded dorsal curvature and a posterior broad V-shaped lateral profile. Sulcal list extends to about two-thirds of the body length and ends around the deepest section of the cell below the midpoint. This species is mostly oceanic and neritic and has a worldwide distribution (Steidinger and Tangen, 1997). It has been implicated in DSP intoxications by many authors (Carmody *et al.*,1995; Pazos *et al.*, 1995).

Dinophysis caudata Saville-Kent (Fig. 6)

This species has a peculiar posterior projection or process. Cells are commonly seen in pairs, dorsally attached. Dorsal contour may be slightly or considerably curved, whereas the ventral margin might straight or undulated. Sulcal list extends the length of the main body. This is typically a neritic and estuarine species with worldwide distribution (Steidinger and Tangen, 1997). *D. caudata* produces DSP toxins and has caused DSP intoxications in several areas where shellfish consumption is cherished (e.g. Della Loggia *et al.*, 1993).

Dinophysis rotundata Claparede and Lachmann (Fig. 7)

Cells have a nearly rounded or ellipsoidal shape. The epitheca is low, fairly evenly rounded and convex. Sulcal list is narrow and extends to about half of the body. Food vacuole is

readily seen within the protoplasm. This species has a wide distribution (Taylor *et al.*, 1995) and produces DSP toxins (Lee *et al.*, 1989; Zhao *et al.*, 1993).

Dinophysis tripos Gourret (Fig. 8)

Cell is characterized by two posterior V-shaped processes, one being longer than the other. The terminal end of both processes often bears minute protuberances. Sulcal list is broader posteriorly. It is a neritic, estuarine and oceanic species, and particularly present in warm temperate to tropical waters (Steidinger and Tangen, 1997). It produces the DSP toxin known as dinophysistoxin-1 (Lee *et al.*, 1989).

Prorocentrum lima (Ehrenberg) Dodge (Fig. 10)

This is an egg-shaped, smooth surface dinoflagellate that is usually broadest postmedian. Anterior ends of the cell are indented. This benthic/epiphytic species colonizes neritic and estuarine waters and has a worldwide distribution (Faust, 1993a,b; Steidinger and Tangen, 1997). This species has been implicated in several DSP episodes by many authors (e.g. Jackson *et al.*, 1993; Lawrence *et al.*, 1998).

Prorocentrum minimum (Pavillard) Schiller (Fig. 11)

This is relatively small *Prorocentrum* species. It is typically heart-shaped, but some may appear triangular or oval in shape. It is mostly estuarine and neritic, and has a worldwide distribution (Steidinger and Tangen, 1997). It has caused shellfish poisoning and fish kills in some localities. Baden and Trainer (1993) suggested that *P. minimum* produces DSP toxins.

Conclusion and recommendation

The level of awareness about the toxic algal bloom phenomenon is very low in Nigeria. Majority of the medical doctors interviewed knew nothing about the phenomenon. They had no idea of the symptoms and syndromes associated with human phycotoxin poisoning. This finding corroborates the observation of Nwankwo (1997) who was of the opinion that information on dinoflagellate-induced harmful algal blooms is not available in Nigeria. He attributed this to limited awareness of the danger they pose, on their occurrence, distribution and taxonomy. The needed awareness about the toxic algal bloom phenomenon must be brought to Nigeria for the protection of her citizens against the dangers inherent in the

consumption of seafood that is laden with phycotoxins. Awareness on this phenomenon is also needed for the prevention of harvesting and exporting of seafood that may be infested with phycotoxin(s). This is a very important point, especially when one considers the regulations bordering exportation of goods meant for human consumption. For example, any country that exports seafood must certify that the product(s) being exported contain(s) no phycotoxin. How can a country with limited awareness about toxic algal blooms fulfil this requirement. Nigeria is at the moment exporting pink shrimps to Europe and the United States.

Symptoms of DSP syndrome were recorded most, suggesting phycotoxin-related diarrhoea may be common among the coastal dwellers of Nigeria. But the potential fear is that medical doctors in Nigeria are mistaking phycotoxin-related diarrhoea for microbial-related cases. It should be borne in mind that the fishermen hinted that certain persons in their communities had fallen sick after the consumption meals that included seafood. We should also remember that while 6.7 % of the medical doctors contacted admitted to have had patients that took ill after they had consumed meals containing seafood, only 2.2 % of all the doctors considered here hinted that they had suspected phycotoxin poisoning among their patients. World Bank (1996) reported that diarrhoeal deaths from water-related contamination in Africa are the highest in the world. Nigeria, it should be noted, has the highest population in Africa, and majority of her people, which are found in the coastal zone, rely heavily on seafood as a chief source of protein.

This study offered us the opportunity to know the locations along the Nigerian coast where mollusc fisheries are highest. Such places included Ibese in Lagos State where oysters are fished at sandy bottoms of open waters, Tombia in Port Harcourt where oysters are fished on propping mangrove roots, and Itu in the Calabar area where surf clams are fished in huge quantities. It is now possible to map fixed stations for routine monitoring of potentially toxic microalgal species and biotoxins in seafood. These inshore waters were sampled because it is the occurrence of toxic blooms along coastlines that can lead to widespread ecological devastation, economic hardship and human health risks (Franks, 1995).

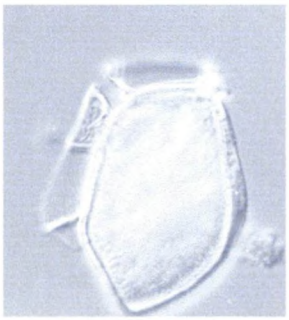


Fig.5. *Dinophysis acuta*

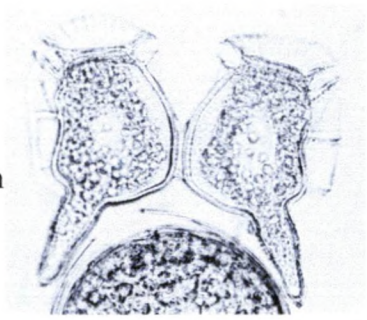


Fig. 6. *Dinophysis caudata*

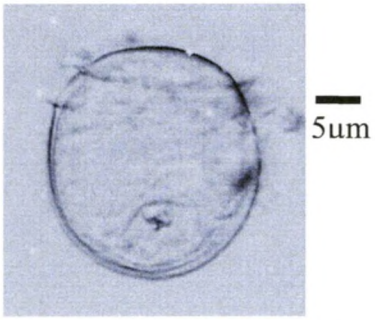


Fig. 7. *Dinophysis rotundata*

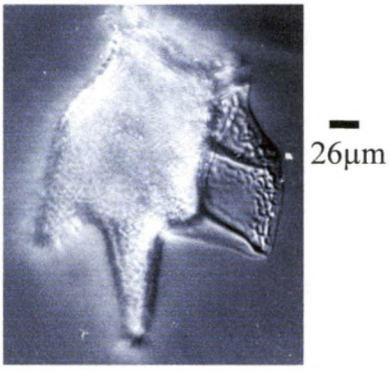


Fig. 8: *Dinophysis tripos*

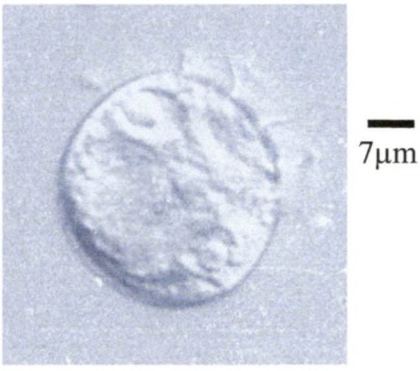


Fig. 9: *Dinophysis* sp

Fig. 10. *Prorocentrum lima*

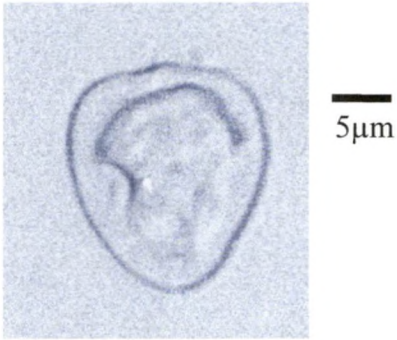
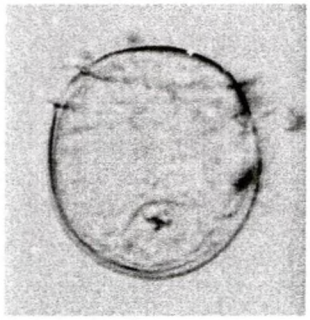


Fig. 11. *Prorocentrum minimum*

26μm

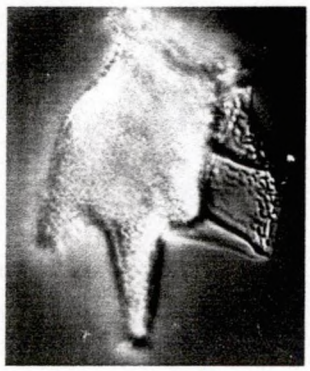
Fig.5. *Dinophysis acuta*

Fig. 6. *Dinophysis caudata*



5um

Fig. 7. *Dinophysis rotundata*



26μm

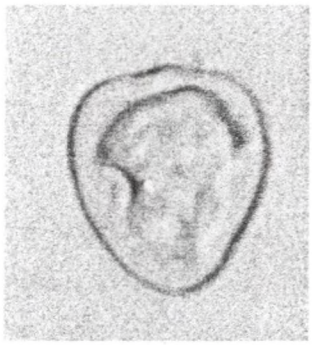
Fig. 8: *Dinophysis tripos*

7μm

Fig. 9: *Dinophysis* sp

10μm

Fig. 10. *Prorocentrum lima*



5μm

Fig. 11. *Prorocentrum minimum*

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