Proceedings of the Fifth Canadian Workshop on Harmful Marine Algae

R. W. Penney (Ed.)

Department of Fisheries and Oceans
Science Branch
P. O. Box 5667
St. John's, Newfoundland
Canada, A1C 5X1

1996

Canadian Technical Report of Fisheries and Aquatic Sciences 2138

Fisheries and Oceans Pêches et Océans

Canada

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Editor's Address:

Department of Fisheries and Oceans
Science Branch
P. O. Box 5667
St. John's, Newfoundland
Canada, A1C 5X1

Tel: (709) 772-4704
Fax: (709) 772-3578
Internet: penneyr@athena.nwafc.nf.ca

Cat. No. Fs 97-6/2138E ISSN 0706-6457

Correct citation for this publication:

Penney, R. W. (Ed.). 1996. Proceedings of the Fifth Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci. 2138. xiii + 195p

DOMOIC ACID IN MEXICO

J. L. Ochoa, A. Sierra-Beltran, A. Cruz-Villacorta, and E. Nuñez

Center For Biological Research, Box 128, La Paz, BCS, 23000, Mexico

Abstract

For the last 20 years, several "red tides" have been observed in the Pacific coastline of Mexico (Cortes Altamirano and Nuñez-Pastén, 1992; Sierra-Beltrán *et al.*, 1996a; Ochoa *et al.*, 1996). Both, toxic and non-toxic events appear to be a regular phenomenon in the zone and yet the country lacks of an effective systematic monitoring program. The extension of the Mexican coastline, the difficulties to access it, and the lack of human and material resources, have made it impossible to implement control measurements that could have prevented the number of human casualties experienced throughout this period (533 poisonings, 16 deceased). In addition to its human impact, one cannot neglect the environmental effect that this kind of events have shown provoking the death of underestimated numbers of fish, marine animals and sea-birds. A recent example, constitutes the corroboration of the presence of Domoic Acid in the Peninsula of Baja California that killed over 150 pelicans, and which adds to the list of marine biotoxins (PSP, DSP, CTX, TTX) already detected in this region.

Introduction

Mexican aborigines appear to have been familiar with the incidence of "red tides" along the coastline of the country before the conquest. According to reports from Alvar Nuñez Cabeza de Vaca on a XVI century edition (Shipwrecks), the Indians avoided the eating of shellfish after a visible "red tide" event as a means to prevent gastrointestinal diseases. The report makes reference to the death of four Spaniard soldiers who disregarded the recommendations of the Indians. Furthermore, with few exceptions, Indians usually eat the callus and not the viscera of the mollusks, thus avoiding exposure to pathogens and toxins accumulated in such tissues and reducing the risk of a disease.

Only recently the microorganism, or fish, associated with "red tides", or poisoning events, in the Pacific coast of Mexico have been identified (Table I). Blasco (1977) and Turrubiates-Morales, (1992) describe *Gonyaulax polyhedra, Ceratium furca, Prorocentrum micans, Ceratium dens, Gonyaulax digitale* and *Gymnodinium Sanguineum*, as the main dinoflagellate components in "red tides" events observed in the West coast of the Baja California Peninsula. While *Prorocentrum sp.*, *Alexandrium sp.*, *Noctilluca scintillans* and *Phaeocystis sp.* are more typical in the Gulf of California (Sierra-Beltrán- et al, 1996a; PROFEPA, México, 1995). In addition, outbreaks of ciguatera and TTX poisoning have been registered in the Peninsula of Baja California in recent years in connection with consumption of contaminated fish

of *Serrinadae* and *Labridae* family (Lechuga-Deveze C., and Sierra-Beltran, A, 1995), as well as with puffer fish (*Sphaeroides annulatus, S. Lobatus, Arothron meleagris, Lagocephalus laevigatus* and *Canthigaster punctatissima*; unpublished). *Mesodinium rubrum*, *Protoperidinium sp.* which are non toxic species, and *Gymnodinium catenatum*, as well as *Gonyalaux monilata*, associated with PSP (Paralyzing Shellfish Poisoning), have been frequently linked to harmful algal blooms in Mazatlán Bay (Cortés-Altamirano and Nuñez-Pastén, 1992). and in the South of the Pacific, at the states of Oaxaca (Saldate-Castaileda *et al.*, 1991) and Guerrero (Cortes-Altamirano *et al*, 1993), *Gymnodinium catenatum* and *Pyrodinium bahamense* have been notoriously associated with the larger number of human casualties so far reported in connection with a "red tide" phenomenon in Mexico (Mee et al, 1986; Saldate-Catañeda et al, 1991; Colmenares and Barradas, 1996).

We may include now to the above list, a diatom, *Pseudonitzschia* sp., and the fish *Scomber japonicum*, mackerel, as the vehicles for Domoic acid, the toxin responsible of the Amnesic Shellfish Poisoning syndrome that became famous after the incident in Prince Edwards Island, Canada, in 1987. However, and as occurred in Monterey and in Santa Cruz Bay, California, US, on 1991 and 1993, this time the local pelican (*Pelecanus occidentalis*) community at Cabo San Lucas, Mexico were the victims (Sierra-Beltrán *et al.*, 1996b).

Materials and Methods

Pelicans were collected from a site chosen apparently as cemetery by the sea-bird themselves since it is not the colony. nor the reproduction site, preferred by the pelicans. The mackerel fish came from catches carried out in the area were the pelicans were observed feeding. Both animals were dissected at the Marine Pathology Laboratory of this Center and extracts prepared from digestive tract and/or whole fish meal, as reported (Sierra-Beltrán *et al.*, 1996b). One dead pelican found in La Paz, Bay of normal causes, served as reference.

Mouse bioassay and HPLC analysis for Domoic acid was done as recommended by the IOC (International Oceanographic Commission, Training Course Report No. 29, Annex IV, pp. 5-6; and IN: Amnesic Shellfish Poisoning, ASP, HAB Publications Series, Vol. 1, Manual and Guides 31. UNESCO).

Results and Discussion

Following the events of human casualties in Eastern Canada on 1987 (Bird *et al*, 1988) attributed to the effect of a toxic aminoacid, namely Domoic acid, DA, and of the sea-bird mortality at Monterey Bay on 1991, (Work *et al.*, 1991, 1993), the most recent incident of DA outbreak causing a massive sea-bird mortality intoxication occurred off the Pacific coast of México, at Cabo San Lucas, in the tip of the Baja California Peninsula (Fig 1) on January 1996, as concluded from the results shown in Table 2 and Fig. 2. This is the first time that the sudden death

of pelicans in Mexico is explained since the phenomenon seems to have been observed before in the Peninsula of Baja California, although then the causes remained obscure (Morales-Chavez et al, 1994).

Domoic acid is a excitatory tricarboxilic aminoacid which has been known for about 40 years and to which some insecticide and antihelminthic activity has been ascribed. More recently, the syndrome expressed by persons intoxicated with DA, which includes some mutagenic actions as well as gastrotoxicity, has been designated as Amnesic Shellfish Poisoning, ASP, mainly due to its effect on the hyppocampus that causes memory loss. Domoic acid, was named after a Japanese seaweed, *Chondria armata domoi*, from which was first isolated; later, it has been also found in Chondria baileyana and in Alsidium *corallinum* (for review see: Todd, 1990). Very recently has been reported in diatom species previously considered harmless such as Pseudonitzschia *pungens f. multiseries*, P. *pseudodelicatissima*, P. *australis*, P. seriata, *Nitzschia actydrophila*, and *Amphora coffaeiformis* 1 (for review see: Wright and Quilliam, 1995 and ref. 7).

No obvious algal bloom in the event registered in Mexico early this year was observed. Yet, as in the cases of Monterey and Santa Cruz, CA, US, the pennate diatom Pseudonitzschia sp., is the suspected toxin source since it was found in the stomach content of contaminated mackerel fish, which were presumably eaten by the sea-birds affected. Also, the extract obtained fiom the pelican stomach showed a clear ASP syndrome in mice, with the characteristic scratching response.

At the time of the event, there were some local conditions that could have brought some upwelling into place, but this is only an appreciation resulting of climate charts and not fiom any direct observations or measurements. From such an incident we have learned that Mexico is not a DA-fiee country anymore; that monitoring of DA in the human food chain should comprise the mackerel fish; and, also, that local resources to carry out a sound monitor program are limited.

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Table I.- Harmfull events occurred in the Pacific Coast of Mexico during the last 20 years.

TIME	PLACE	ORGANISM	EFFECTS	TOXIN DETERMINED	REF.
1976 / Nov	Acapulco, Gro.	Unknown	7 humans / 2 deaths	Not done	SSA
1979 / Apr	Mazatlan, Sin.	*Gymnodinium catenatum*	19 humans / *3* deaths	Sax	9, SSA
1984 / Jun	La Paz, B.C.S.	*Lutjanus sp.* fish	200 humans	Ciguatoxins	12
1985 / Nov	Acapulco, Gro.	Unknown	5 humans / 2 deaths	51 µg Sax / g lioph. cells - 1720 µg Sax / 100 g shellfish	SSA
1989 / Dec	Salina Cruz and Huatulco, Oax.	*Pyrodinium bahamsnse var. compressurn* and *G. catenatum*	99 humans / 3 deaths, several turtles and fish	380-570 µg Sax / 100 g shellfish	14
1991 / Nov	Bahía Concepción, B.C.S.	?	several tons of shellfish lost	Not done	@
1992 / Jan & Apr	Ojo de Liebre, B.C.S.	?	several tons of shellfish lost	Not done	@
1992 / Jun	Ensenada, B.C.	Unknown	110 pelicans	Not done	
1992 / Oct	Bahía Magdalena, B.C.S.	Dinoflagellate	dolphins, sea lions, sea birds, fish & turtles	Not done	@
1992 / Nov	Pto. Madero, Chis.	*Pyrodinium bahamense var. compressum*	2 humans / 1 deaths	45 mg Sax / 100 g shellfish	6
1993 / Apr	La Paz, B.C.S.	*Oscillatoria sp.*	none	Hepatotoxins	@
1993 1 May	Bahia Concepción, B.C.S.	*Oscillatoria sp.*	none	No toxic	@
1993 / May	Alijos Rocks, B.C.S.	*Serranidae & Labridae* fish	7 humans	Ciguatoxins	8, @
1994 / Apr	Acapulco. Gro.	*Gymnodinium sp.* and *Gonyaulax sp.*	none	57-93 µg Sax 1 100 g shellfish	SSA
1994 / Apr & Jun	La Paz, B.C.S.	*M. rubrum*	none	No toxic	@
1994 / Jun	San Hipolito, B.C.S.	*Gymnodinium sanguineum*	fish & sea birds	No PSP	@
1995 / Jun	Vizczino, B.C.S.	*Sphaeroides sp.*	2 humans / 2 deaths	Not done	SSA
1995 / Oct	La Paz, B.C.S.	?	Fish (Ballistidae)	Highly potent liposoluble toxin	@
1995 / Jan	San Felipe, B.C,S,	*Phaeocystis sp.* and *Noctiluca scintillans*	birds and sea mammals: whales, dolphins,seals, etc. (more than 900)	Not done	13
1995 / Dec	Acapulco, Gro.	*Pyrodinium bahamense var. compressum,* and *Gonyaulax catenella.*	192 humans / 3 deaths	598-3091 µg / 100 g shellfish	4, SSA
1996 / Jan	Atil, Son.	*Microcystis*/LPPB	fish	Mucus & scum	@
1996 / Jan	Cabo San Lucas, B.C.S.	*Pseudonitzschia sp.*	brown pelicans	Domoic acid	11, 16, @
1996 / Feb	Loreto, B.C.S.	*Noctiluca scintillans & Pseudonitzschia sp.*	none	No toxic	@
1996 1 Feb	Sta. M. del Oro, Nay.	Cyanobacteria LPPB	fish	Oxygen depletion	@
1996 / Mar	Cabo San Lucas, B.C.S.	Cyanobacteria LPPB & *Chatonella sp.*?	bentonic fish	? Pmbably secondary infection.	@

SSA: Health Ministry @: Events assisted by CIBNOR

Table II. Results obtained with specimens collected at the onset of the event In Cabo San Lucas, B.C.S., Mexico. January, 1996.

Species/Locality	Organ/Tissue	Specimen Weight (g)	Mouw bloassay	HPLC Conc. (µg/g)
Pelecanus occidentalis/ CaboSanLucas	Liver	38	Scratching, salivation,tail wiggling, incoordination	Tracer,
Pelecanus occidentalis/ Cabo San Lucas	Digestive tract contents	5	Scratching, salivation,tail wiggling, Incoordination, diarrhoea, death.	37.17
Pelecanus occidentalis/ Cabo San Lucas	Liver	45	Scratching, incoordination	Traces
Pelecanus occidentalis/ C a b San Lucas	Digestive tract contents	II	Scratching, incoordintion	Traces
Pelecenus occidentalis/ La Paz (Negative Control)	Digestive tract contents	2	Negative	Negatlve
Scomber japonicum/ C a b San Lucas I	Digestive tract contents	3	Not *done*	142.85 @
Scomber japonicum/ C a b San Lucas II	Digestive tract contents	15	Not done	Negative

@ This figure represent 4.76 µg / fish on average.

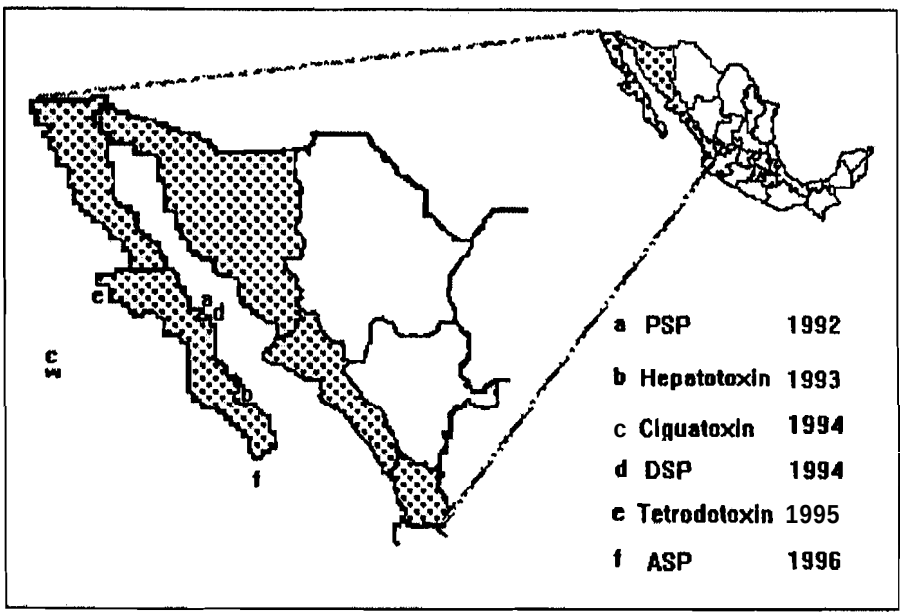

FIG.1.- Marine Biotoxins already detected at the Northwest Pacific Coast of Mexico depicting the place and year of confirmation. Note the presence of DA at the tip of the Peninsula, a naturally recurring upwelling area.

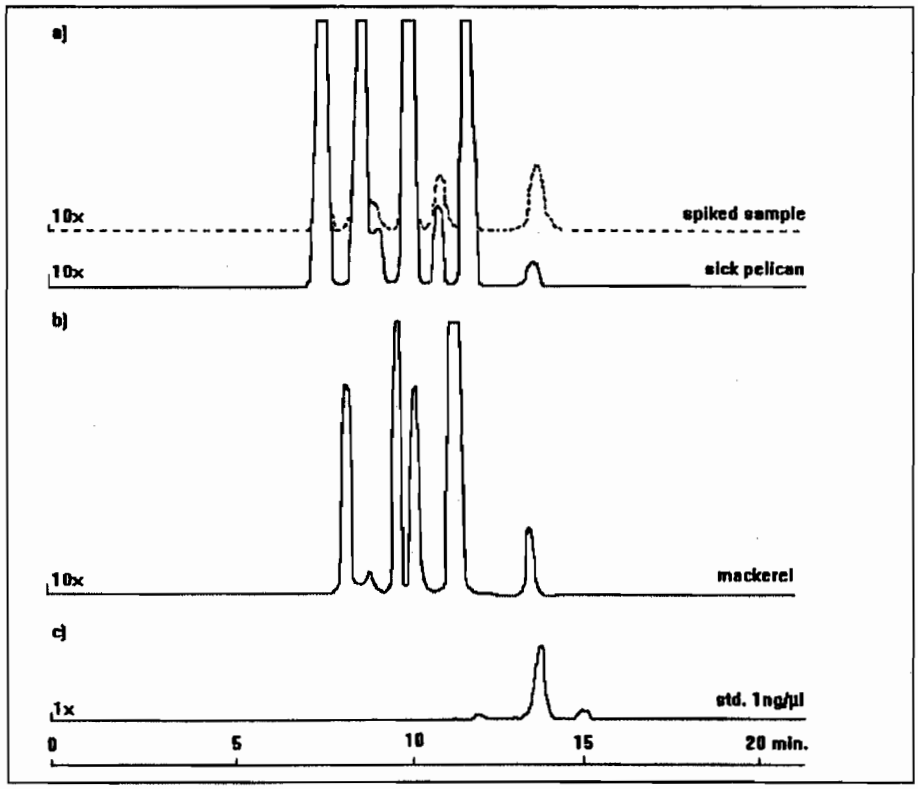

Fig. 2.- HPLC analysis was performed according to Quilliam's method (Ref. 7). Pure domoic acid was added to the sick pelican extract to confirm the authenticity of the peak obtained before spiking the sample. Negative control pelican as well as uncontaminated fish (sardine) did not show any peak corresponding to DA mobility (not shown). Gain: 1x and 10x absorbance at 242 nm. Retention time in minutes.