Toxic and harmful marine phytoplankton and microalgae (HABs) in Mexican Coasts

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Harmful Algal Blooms (HABs) are becoming an increasing problem to human health and environment (including effects on natural and cultured resources, tourism and ecosystems) all over the world. In Mexico a number of human fatalities and important economic losses have occurred in the last 30 years because of these events. There are about 70 species of planktonic and non-planktonic microalgae considered harmful in Mexican coasts. The most important toxin-producing species are the dinoflagellates *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum*, in the Mexican Pacific, and *Karenia brevis* in the Gulf of Mexico, and consequently the poisonings documented in Mexico are Paralytic Shellfish Poisoning (PSP) and Neurotoxic Shellfish Poisoning (NSP). Although there is evidence that Amnesic Shellfish Poisoning (ASP), Diarrhetic Shellfish Poisoning (DSP) and Ciguatera Fish Poisoning (CFP) also occur in Mexico, these problems are reported less frequently. The type of phytoplankton and epiphytic microalgae, their toxins and harmful effects as well as current methodology used to study these phenomena are presented in this paper. As an experienced group of workers, we include descriptions of monitoring and mitigation programs, our proposals for collaborative projects and perspectives on future research.

Keywords: Biotoxins; harmful algal blooms; marine phytoplankton; Mexican coasts; microalgae.

Introduction

Mexico is a large country that has coasts in two Oceans: the Pacific and the Atlantic. Thus, there are many different environmental conditions (geological, climatologic and oceanographic ones) and diverse habitats and environs (oceanic zones, coastal areas with extensive or reduced shelves, coastal lagoons, deltas of various rivers, islands, coral reefs, mangroves) where diverse marine flora and fauna develop. These natural resources are very important, but have been subject of ongoing threat and deterioration. Recent increase of cases of toxic and harmful marine phytoplankton and microalgae is an issue that must be assessed to understand the consequent impact to human health, fisheries and tourism.

World research on toxic and harmful marine phytoplankton and microalgae has consequently augmented and various scientific organizations and international

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programs (e.g., Harmful Algal Bloom–HAB–program, from the International Oceanographic Commission–IOC–, of UNESCO, and subsequently ECOHAB, GEOHAB and EUROHAB) have been created to address correspondent investigations on the topic, trying to cover all possible disciplines related.^[1,2] During more than 30 years the world has observed species arising as toxic and harmful, causing disturbances and noxious effects on marine life and humans, new concepts and hypothesis proposed to explain the expansion and increase of HABs.^[1,3]

"Red tides" are regarded as bio-optical phenomena and "rely implicity on a threshold of plant biomass or light extinction," whereas blooms are rather ecophysiological phenomena "typically harmless" ("originally the regular spring growth in temperate waters")^[4] and where biomass and populations density are important issues.^[5] The term Harmful Algal Blooms (HAB) has become of common use among scientists and refers not only to phytoplankton, but also to non-planktonic algae.

The importance and diversity of the marine phytoplankton, microalgae that shift freely in the pelagic realm and which are mostly photosynthetic, has been already emphasized in Mexico,^[6,7] and other previous papers also dealt with the toxic and harmful marine phytoplankton, the phytoplankton that can produce toxins that affect marine life and also humans or can cause harmful cases due to high densities;^[8–13] other non-planktonic marine microalgae (e.g., tychoplanktonic, benthic, epiphytes) have been less treated and often are considered part of the phytoplankton.^[14]

Marine phytoplankton and epibenthic marine microalgae

Marine phytoplankton is an ambiguous term, which defines a community of microalgae, mostly photosynthetic, inhabiting the pelagic marine realm.^[7] The important ecological roles of the phytoplankton in the sea are as primary producers, linking inorganic sources of energy into upper trophic levels, and participating in fundamental cycles of some elements in nature.^[15,16]

The number of species within this community is nearly 5000 extant species:^[17] between 3444 and 4375 species,^[18] with 15 to 20 taxonomic classes (most classes of algae are represented in the plankton). In Mexico, an estimate of the number of marine phytoplankton taxa yielded approximately 1488, within 211 genera; this figure represents only the 33 to 42% of the total number proposed for the whole world.^[7] The most studied groups are diatoms (Bacillariophyceae) and thecate dinoflagellates (Dinophyceae), which largely contribute to the diversity and very often also to biomass in the marine phytoplankton. Two other groups less studied are silicoflagellates (Dictyochophyceae) and coccolithophorids (Haptophyceae); these 4 groups are "preservable" forms, which are usually collected and studied with no special proto-

col. The rest of the taxonomic groups are the so-called "phytoflagellates" (planktonic and photosynthetic flagellates) and cyanophytes (Cyanophyceae, Cyanobacteria), unique group of Procaryotes in the phytoplankton. From this large diversity, the species reported as forming "red tides" are 184–267 (about 300 species), whereas the species considered producing toxins are 60–78, from which the majority are dinoflagellates (73–75%).^[1,2,4,19]

Species of various groups produce toxins or are harmful to marine organisms and human health. Some species can be nuisance in some circumstances: dense populations may lead to high consumption of oxygen and liberation of toxic substances (e.g., hydrogen sulphide) that can kill fish, or interfere with the digestion of flagellates by shellfish.^[20] In Mexico, there are about 70 species of microalgae belonging to six taxonomic groups considered harmful in the littorals of the Atlantic and Pacific Ocean. Of them, the most diverse are the dinoflagellates: 47 taxa (46 species and one variety), diatoms (15 species), Raphidophytes (4–5 species), and 3 species of Cyanobacteria, 1 Haptophyte and 1 Dictyochophyceae (Table 1).

The dinoflagellates Gymnodinium catenatum and Pyrodinium bahamense var. compressum, in the Mexican Pacific, and *Karenia brevis* in the Gulf of Mexico (Figs. 1, 2), are known for their toxicity. Other dinoflagellates considered to be toxin producing (either to humans and marine fauna) and that have been found in Mexican waters, with preliminary or no documentation of having produced any toxic event in Mexico, are: Akashiwo sanguinea, Alexandrium acatenella, A. catenella, A. leei, A. minutum, A. monilatum, A. ostenfeldii, A. tamarense, A. tamiyavanichii, Cochlodinium polykrikoides, Dinophysis acuminata, D. caudata, D. fortii, D. mitra, D. rotundata, D. tripos, Gambierdiscus toxicus, Lingulodinium polyedra, Ostreopsis lenticularis, O.siamensis, Prorocentrum concavum, P. emarginatum, P. lima, P. mexicanum, P. minimum, P. rhathymum, Protoceratium reticulatum, Protoperidinium crassipes and Pyrodinium bahamense var. bahamense (Table 1, Figs. 1, 2). From this list, only Gambierdiscus toxicus, Ostreopsis species and most probably *Prorocentrum lima* are non-planktonic forms.^[21-23]

Gymnodinium catenatum is the only gymnodinoid dinoflagellate that produces saxitoxins and related toxins causing Paralytic Shellfish Poisoning (PSP). This is an athecate (naked), photosynthetic, chain- and cyst-forming dinoflagellate that has increased its global distribution all over the world (in temperate to subtropical areas). Originally described from the Gulf of California,^[24]G. catenatum is an important component of the phytoplankton along the Mexican Pacific coasts, from the Gulf of California^[25–27] (Figs. 1, 2).

The thecate dinoflagellate *Pyrodinium bahamense* var. *compressum* is considered a variety of the type species, *P. bahamense*, the latter shows no evidence of toxicity, until very recently.^[28] It has a number of tiny plates in the theca, cingulum and sulcus, and differs from the type variety for

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Table L.	HABS	species in	coasts (of Mexico

Causative species	Illness	Causative species	Illness
Dinoflagellates		Dinoflagellates	
Alexandrium acatenella (Whedon et Kofoid) Balech	PSP	Gambierdiscus toxicus Adachi et Fukuyo	CFP
Alexandrium catenella (Whedon et Kofoid) Balech	PSP	Ostreopsis lenticularis Fukuyo	CFP
Alexandrium leei Balech	PSP	Ostreopsis siamensis Schmidt	CFP
Alexandrium minutum Halim	PSP	Karenia brevis (Davis) Hansen et Moestrup	NSP
Alexandrium monilatum (Howell) Balech	PSP	Lingulodinium polyedra (Stein) Dodge	YTX
Alexandrium ostenfeldii (Paulsen) Balech et Tangen	PSP	Protoceratium reticulatum (Claparede et Lachmann) Bütschli	YTX
Alexandrium tamarense (Lebour) Balech	PSP	Protoperidinium crassipes (Kofoid) Balech	AZA
Alexandrium tamiyavanichii Balech	PSP		
Gymnodinium catenatum Graham	PSP	Diatoms	
Pyrodinium bahamense Plate var. Bahamense	PSP	Pseudo-nitzschia australis Frenguelli	ASP
<i>Pyrodinium bahamense</i> var. <i>compressum</i> (Böhm) Steidinger, Tester <i>et</i> Taylor	PSP	Pseudo-nitzschia delicatissima (Cleve) Heiden	ASP
Dinophysis acuminata Claparède et Lachmann	DSP	Pseudo-nitzschia fraudulenta (Cleve) Hasle	ASP
Dinophysis caudata Saville-Kent	DSP	Pseudo-nitzschia multiseries (Hasle) Hasle	ASP
Dinophysis fortii Pavillard	DSP	Pseudo-nitzschia pseudodelocatissima (Hasle) Hasle	ASP
Dinophysis mitra (Schutt) Abé	DSP	Pseudo-nitzschia pungens (Grunow ex Cleve) Hasle	ASP
Dinophysis rotundata Claparède et Lachmann	DSP	Pseudo-nitzschia subfraudulenta (Hasle) Hasle	ASP
Dinophysis tripos Gourret	DSP		
Prorocentrum concavum Fukuyo	DSP	Cyanobacteria	
Prorocentrum emerginatum Fukuyo	DSP	Anabaena spp.	CTP
Prorocentrum lima (Ehrenberg) Dodge	DSP	Microcystis aeruginosa Kütz	MC
Prorocentrum mexicanum Osorio-Tafall	DSP	Trichodesmium erythraeum (Ehrenberg) Gomont	MC
Prorocentrum rhathymum Loeblich, Sherley et Schmidt	DSP	Trichodesmium thiebautii Gomont ex Gomont	MC

*Illness or toxins: Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP), Ciguatera Fish Poisoning (CFP), Neurotoxic Shellfish Poisoning (NSP), Cyanobacterial Toxin Poisoning (CTP), Yessotoxin (YTX), Microcystin (MC), Azaspiracids (AZA).

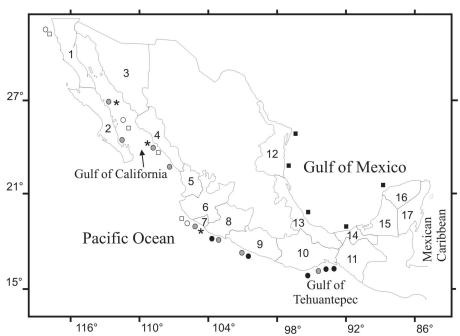


Fig. 1. Map showing Mexican States with littorals and general distribution of some harmful species. 1: Baja California, 2: Baja California Sur, 3: Sonora, 4: Sinaloa, 5: Nayarit, 6: Jalisco, 7: Colima, 8: Michoacán, 9: Guerrero, 10: Oaxaca, 11: Chiapas, 12: Tamaulipas, 13: Veracruz, 14: Tabasco, 15: Campeche, 16: Yucatán, 17: Quintana Roo. Gray circles: *Gymnodinium catenatum*, black circles: *Pyrodinium bahamense* var. *compressum*, white circles: *Lingulodinium polyedra*, white squares: *Alexandrium catenella* black squares: *Karenia brevis*, asterisks: *Cochlodinium polykrikoides*.

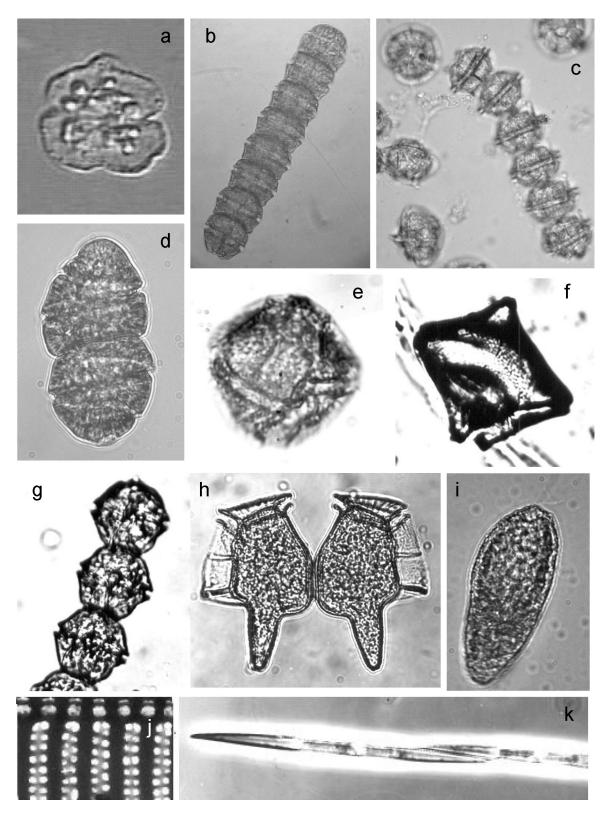


Fig. 2. Harmful species found in Mexican coasts (LM, except j. TEM). Dinoflagellates: a. *Karenia brevis*, b. *Gymnodinium catenatum*, c. *Pyrodinium bahamense* var. *compressum*, d. *Cochlodinium polykrikoides*, e. *Lingulodinium polyedra*, f. *Protoperidinium crassipes*, g. *Alexandrium catenella*, h. *Dinophysis caudata*, Raphidophyte: i. *Chatonella marina*, Diatom: j, k. *Pseudo-nitzschia pungens*, a valve in detail (TEM) and part of a chain.

being slightly depressed apical-antapicaly and usually forming short to long chains. *P. bahamense* var. *compressum* is now considered the most dangerous species in tropical regions because of its high toxicity and responsibility of 97% of human deaths by Paralytic Shellfish Poisoning (PSP). Hallegraeff and MacLean^[29] estimated that toxic *Pyrodinium* has been responsible for more than 1,000 human illnesses and 60 fatalities resulting from the consumption of contaminated shellfish as well as planktivorous fish such as sardines and anchovies; Rosales-Loessener^[30] pointed out that in coasts of Guatemala, 187 people were affected and 26 resulted in death in July, 1987, whereas in Costa Rica, 70 people were poisoned (December, 1999 to January, 2002).^[31]

The athecate dinoflagellate *Karenia brevis*, originally described as *Gymnodinium breve* Davis, later named *Ptychodiscus brevis* (Davis) Steidinger, and more recently proposed as a species of the genus *Karenia*,^[32] is a relatively small, ventral-dorsal flattened and photosynthetic form, with no known resting stages, which produces brevetoxins (or neurotoxins) associated to the Neurotoxic Shell-fish Poisoning (NSP). The species is currently considered an endemic microalga from the Gulf of Mexico: it is distributed from Texas, Alabama, Luisiana and Florida (USA) to the Peninsula of Yucatan (Mexico) (Figs. 1, 2). Effects on the marine fauna because of neurotoxins in New Zealand were reported, although the responsible species are different,^[3,33,34] and the brevetoxins varied in structure.^[35]

Among diatoms, in Mexico we can consider the following potentially toxic, planktonic forms: Pseudo-nitzschia australis, P. delicatissima, P. fraudulenta, P. multiseries, P. pseudodelicatissima, P. pungens and P. subfraudulenta^[36,37] (Table 1, Figs. 1, 2). Surveys on "phytoflagellates" and phytoplankton studies in Mexican coasts revealed the wide occurrence of the Raphidophytes: Chatonella marina, Ch. subsalsa, Fibrocapsa japonica and Heterosigma akashiwo,^[38] all of them considered to be toxic to fishes (Table 1, Figs. 1, 2), and with very recent reports of toxic events. Other "phytoflagellates" have been also detected in Mexican waters (Table 1), most of them innocuous, at the moment. Cyanobacteria potentially toxic that have been identified in Mexico are: Anabaena sp., Trichodesmium erythraeum and T. thiebautii^[39] (Table 1). Finally, it is important to include those considered non-toxic or innocuous "red tides"forming species that are common in Mexico, and often cause alarm. The main causing species are the ciliate Miryonecta rubra, some dinoflagellates of the genus Ceratium, Gonyaulax, Noctiluca scintillans, and various diatoms (Table 1).

Toxins

There are numerous toxins produced by planktonic and non-planktonic microalgae. They may be divided into four different categories, depending on their effects: (i) Human health, (ii) Natural and cultured marine resources, (iii) Tourism and recreational activities, and (iv) Marine ecosystem.^[2] The following is a classification based on chemical characters:

Lipophilic toxins

Azaspiracids (AZA). This is a new group of toxins which name derives with the ring Azaspir and the presence of a functional carboxyl. These are stable in solvents such as chloroform, under slightly alkaline conditions. They are produced by the thecate dinoflagellate *Protoperidinium crassipes*.

Brevetoxins (BTX). Brevetoxins are neurotoxic substances originally found in "red tides" and cultures of Karenia brevis. They are "ladder-shaped polycyclic ether compounds", with two types, A (defined as PbTx-1, -7, and -10) and B (defined as PbTx-2, PbTx-2 oxyded, -3, -5 -6, -8, and -9). Cells of K. brevis may break and release their toxins to the water, causing fish mortality in high concentrations, and aerosols are carried to the coasts producing human irritations. Additionally, Neurotoxic Shellfish Poisoning (NSP) may be produced when oysters or other marine products contaminated by brevetoxins are consumed by humans.^[40] In Mexico, the maximum permissible level of BTX is 20 MU 100 g⁻¹ of shellfish tissue.^[41]

Okadaic acid (OA). Okadaic acid and its analogues, Dinophysitoxins (DTX1 and DTX2), are the main toxins responsible for the Diarrhetic Shellfish Poisoning (DSP). These are produced by species of the dinoflagellates *Dinophysis* and *Prorocentrum.* Shellfish containing more than 2 μ g OA and/or 1.8 μ g DTX1 per gram of hepatopancreas are not considered fit for human consumption.^[3] No documented cases in humans are recorded in Mexico so far.

Pectenotoxins (PTX). The name of these toxins comes from the genus of Scalops (*Pectinopecten* and *Enssoensis*), from which they were first isolated, and are also polycyclic ether compounds. The toxins are atribuible to the thecate dinoflagellate *Protoceratium reticulatum*. The toxin homoyesotoxins (homo-YTX) is produced by another dinoflagellate, *Lingulodinium polyedra*. Stability of these toxins is not well known as yet.

Hydrophilic toxins

Domoic acid (DA). Toxins causing ASP or Domoic Acid were originally isolated from the red macroalga *Chondria armata*, in Japan. After the incident in 1987 in Canada, when over 100 people were sickened and 3 died,^[42] this toxin was identified as the causative in poisonings for shell-fish consumption with very high levels. Shellfish containing more than 20 μ g DA per gram of shellfish meat are considered unfit for human consumption.^[3] At the moment, there

are 9 to 10 *Pseudo-nitzschia* species known to produce the toxin domoic acid.^[43]

Saxitoxins (STX). PSP is produced by a group of potent toxins termed saxitoxins, which include 2 dozens of different types. Toxins are produced by the dinoflagellates *Gymnodinium catenatum*, *Pyrodinium bahamense* var. *compressum* and species of the genus *Alexandrium*, although more recently they are also associated to the Cyanobaterium *Trichodesmium erythraeum*. This is one of the most common and dangerous poisoning around the world, that has caused numerous human fatalities, and some of the causative species have dispersed wider in the world (as *G. catenatum* and *P. bahamense* var. *compressum*).^[1,3]

Gárate-Lizárraga et al.^[44] provided PST's profiles on different strains of *Gymnodinium catenatum* from three bays in the Gulf of California. The toxin profile for the different strains of Mexico is: dcSTX, dgGTX2, GTX3, B1, B2 and C2 STX and neoSTX. Toxin content varied from 36.1 to 184 pg cell⁻¹; the strain from Mazatlan was the most toxic. The toxic composition appears not to be a conservative property in *G. catenatum*, however other studies have demonstrated a consistency in the toxin profile within population. Toxin composition may vary among microalgal species and strains with geographical locations, with environmental factors, and under different experimental conditions.^[45]

Other toxins

Ciguatera is a circum-tropical syndrome well-known in the Caribbean, French Polynesia and Australia, which is currently associated to the high populations of benthic dinoflagellates, such as *Gambierdiscus toxicus*, *Ostreopsis lenticularis*, *O. siamensis* and perhaps *Prorocentrum lima*. The toxins related to this poisoning include Ciguatoxins, Gambiertoxins and Maitotoxins, which accumulate through the food chain, from small fishes grazing on coral reefs or larger aquatic plants into muscle and organs of bigger fishes.

There are a number of other toxins which cause no effect on humans but in the marine fauna (mainly fish and shellfish), and eventually are nuisance to aquaculture, tourism and environment. Almost all marine Raphidophyte species are considered to be toxic to fish, by producing Reactive Oxygen Species (ROS) as superoxide anions, hydroxyl radicals, singlet oxygen, and hydrogen peroxide, which principally affect fish as other organisms,^[46] and other "phytoflagellates" produce other ichtyotoxins (Table 1). The symptoms of fish are not specific of raphidophyte bloom, both physical clogging of fish gills by mucus excretion as well as gills damage by hemolytic substances may be involved.^[47,48] Free fatty acid production, notably of eicosapentaenoic acid may also play a role in fish mortalities caused by these blooms.^[49]

Results and observations

Effects of HABs on human health

Typical poisonings in coasts of Mexico include NSP and PSP, but there are also some other poisonings that have been less documented and they are gaining importance: ASP, DSP and minusc Ciguatera. As these intoxications put at risk human health, popular concern grows, and thus these are the best documented poisoning.

Karenia brevis *and helvetic NSP*. In coasts from the Gulf of Mexico, mass fish mortalities have been reported since pre-hispanic times, and then frequently between 1797 and 1995.^[11] Many cases have been documented in Texas, Louisiana and Florida, USA since 1844.^[50] However, it was until 1947 when dinoflagellate *Karenia brevis* was identified as the species responsible for the production of neurotoxins (and NSP) and the causative of fish mortality.^[51]

In Mexican coastal waters there are only 2 official reports previous to 1995.^[8,52] However, during the period 1996– 2005 several blooms of *K. brevis* have occurred in Mexico, causing mass mortality of fish and other marine organisms and general respiratory effects in humans in Tamaulipas, Veracruz and Tabasco. The economic impact of these losses was not properly evaluated (e.g., fisheries, tourism, health), although banning of fishing oysters was determined.

Data recorded during 9 years indicate that October is the month with higher cases of "red tides" caused by *K. brevis* (Table 2) and that blooms of the species originally coming from Texas, USA, might be transported by coastal currents to Mexican coasts, affecting Tamaulipas and Veracruz.^[53] Different hydrographic and oceanographic processes do occur in the Gulf of Mexico, and a closely relation between physical processes and the blooms caused by *Karenia brevis* has been found.^[54]

Gymnodinium catenatum and PSP. Human fatalities in Mexico are associated, in most of the cases (if not all), to the paralytic toxins, in the Mexican Pacific. The 2 causative species are identified as Gymnodinium catenatum and Pyrodinium bahamense var. compressum, although their distribution apparently does not overlap: G. catenatum distributes from the upper Gulf of California to Acapulco, Guerrero, whereas P. bahamense var. compressum has a distribution from Costa Rica to Manzanillo, Colima, thus both species share a transition zone, but do not appear to occur at the same time.^[13] Alexandrium catenella is the causative dinoflagellate for PSP in western coast of Baja California; this species is a regular phytoplankton member, but rarely it is observed in high densities $(2.5 \times 10^4 \text{ cells L}^{-1} \text{ in sum-}$ mer), nevertheless, these lower cell concentrations are capable of generating very high level of PSP toxins in shellfish, although there is no documented evidence of human poisonings.

Date	Location (State)	Cell abundances $(cells L^{-1})$	Toxicity (MU 100 gr ⁻¹)	Remarks
1996 October	Tamaulipas	_	_	Losses of 4.5 Tons fish. Respiratory irritations.
1997 October	Tamaulipas	$13-279 \times 10^{3}$	10-89.6	Losses of 80 Tons fish. Respiratory irritations.
1999 October	Tamaulipas	$10-260 \times 10^{3}$	46	Short event
2000 October	Tamaulipas	5×10^{3}		Short event, fish mortality
2001 December	Veracruz	$3.5-739 \times 10^{3}$	10-207.2	Oyster beds closed from October, 2001 to February, 2002.
2005 May-July	Tabasco	$140-4143 \times 10^{3}$	10-306	Daily losses of 800 Kg fish. Respiratory irritations.

Table 2. Information on HABs caused by Karenia brevis in coasts of Mexico from the Gulf of Mexico (1996–2005)

There have been 561 sickened and 38 dead people from 1970 to 2004, with a rate of mortality close to 6%. High concentrations of the species have been reported with few human fatalities, and also fish mortalities.^[25,55] The first documented case occurred in Mazatlan Bay in 1979, with several poisoned people^[55]; high densities of the species (up to 1.14×10^6 cells L⁻¹) and March and April as the species blooming period have been reported.^[56]

Pyrodinium bahamense *var.* compressum *and PSP.* In the southern Mexican Pacific, one of the first documented HAB produced by *Pyrodinium bahamense* var. *compressum* occurred in November, 1989, in the Gulf of Tehuantepec. It caused 99 poisoned people and 3 deceases by consumption of shellfish *Ostrea iridiscens* and *Chroromytilus palliopunctatus.*^[57]

More than 200 people have been affected and 6 have died for PSP due to saxitoxins produced by *P. bahamense* var. *compressum.* From November, 1995 to February, 1996, a bloom of the species reached coasts of Michoacán and Guerrero, causing 6 human fatalities and many more affected people by high toxicities were detected in *Ostrea iridiscens* (Table 3). From November, 2001 to August, 2002, HABs coming originally from Chiapas (patches of 1 357 km), reached coasts of Guerrero (Table 3). During 2001 HABs, in Puerto Madero, Chiapas, cysts of the taxon appeared in the water column 3 months before (January) than vegetative cells did (March). In May cysts disappeared from the water column and again were found in August with a maximum density (60,000 cysts L⁻¹), coinciding when the vegetative cells reached their maximum densities (180,000 cells L^{-1}). This was interpreted as a new generation of cysts.^[58]

In Mexico HABs by *P. bahamense* var. *compressum* have occurred with intervals of 3 to 5 years (winter 1989, November, 1992, November 1995 to February 1996, January 2001 to February 2002, and beginning of 2006) and always the first trace of the species started in coasts of Chiapas, in the Gulf of Tehuantepec, a region characterized by occurrence of winter upwellings. These HABs may be associated with those upwellings, but in some occasions this taxon is present in the water column by several months and the highest densities have been observed seven months after upwellings, during the rainy season (August).^[58]

Other poisonings

Ciguatera is a syndrome known mainly in the Mexican Caribbean, but there are few reports that it also occurs in the Mexican Pacific.^[59] Some of the most important causative species have been found in the Mexican Caribbean, such as the dinoflagellates *Gambierdiscus toxicus*, *G. belizeanus* and *G. yasumotoi*.^[22] The symptoms of this syndrome mask other illnesses and thus it is difficult to asses its real distribution and some other origins of toxins. In this area, it has been established that the most critical period for Ciguatera is July and August, which led to ban certain fisheries; the main spot where reports have been made is Isla Mujeres. In the last 5 years, 17 people have been reported to be poisoned, with no decision.

Table 3. Information on HABs caused by *Pyrodinium bahamense* var. compressum (with PSP affections) in the southern Mexican Pacific (1989–2006)

Date	Location (State)	Cell abundances $(cells L^{-1})$	Toxicity (µg SXTeq 100 gr ⁻¹)	Remarks
1989 November	Gulf of Tehuantepec	1.7×10^{6}	811	99 poisoned persons, 3 death
1995–1996 (Nov–Feb)	Michoacán and Guerrero	_	6 337	6 death persons
2001–2002 (Nov–Aug)	Chiapas to Guerrero	3.5×10^{6}	7 309	101 persons poisoned, 6 death. 48 Tons fish killed
2005–2006 (Dec–Mar)	Puerto Madero, Chiapas	1.8×10^{3}	200	_

Effects of HABs on the natural resources, environment and economy

No confident evaluation has been yet done on the economic loss due to HABs in Mexico, only in some cases this loss is calculated for the industry affected, therefore, there are not national statistics about losses caused by harmful algae events.

HABs in the Gulf of Mexico

Apart from the *Karenia brevis* reports in coasts of the Gulf of Mexico and Caribbean Sea, there is scarce documentation of HABs. A recent account on toxic and harmful dinoflagellates from the southern Gulf of Mexico has been given by Licea et al.,^[60] An innocuous "red tide" lasting 20 days appeared in the port of Veracruz, November, 2002, caused by the dinoflagellate *Peridinium quinquecorne*.^[61]

HABs in the Northern Mexican Pacific

In western coasts of Baja California, Lingulodinium polyedra is one of the dominant dinoflagellates, which has been historically documented from 1902 off La Jolla. California. USA.^[62] The conceptual model of L. polyedra was postulated by Orellana-Cepeda et al.,^[63] and the cysts were studied in Todos Santos Bay sediments by Peña-Manjarrez et al.,^[64] During blooms of L. polyedra, densities may be as high as 10^5 cells L^{-1} , and there is a dissolved oxygen deficit, causing the mortality of sensitive species on the bottom. When density reaches 10^6 cells L^{-1} , "marine snow" covers surface water and light cannot penetrate, causing ecologic disasters and fishes, crustacean, octopus and other benthic resources have died. By February, 1996, in Todos Santos Bay, Baja California, L. polvedra was the most abundant species $(7.93 \times 10^5 \text{ cells } \text{L}^{-1})$. The dinoflagellates *Proro*centrum micans and Akashiwo sanguinea are also regularly recorded, mainly during HABs and these species can also generate small quantities of hydrogen peroxide. Another dinoflagellate, Karenia mikimotoi, has a highest concentration in upwelling areas during the autumn. The raphidophytes Fribocapsa japonica, Chatonella antiqua, Ch. marina, Heterosigma akashiwo and the dinoflagellate Cochlodinium polvkrikoides are the most important potential ichtyotoxic species in the area, which have maximum densities on September.

The planktonic diatoms *Pseudonitzschia australis*, *P. multiseries*, *P. delicatissima*, *P. subfraudulenta* and *P. pungens* do occur in the area, with maxima values during spring and minima during the winter. *Pseudo-nitzschia* species have been associated with domoic acid production and poisonings of marine mammals. In 2002, 87 sea lions *Zalophus californianus* were found lying or dying on the beaches between the border with USA and Ensenada, Baja California, and they are assumed to be poisoned by domic acid. California anchovy is considered to be the vector of domoic acid to its predators as birds and marine mammals.

HABs in the Gulf of California

"Red Tides" are historical, frequent and periodical events in the Gulf of California, most of them are innocuous and produced by the ciliate *Myrionecta rubra* (widely distributed in the Mexican Pacific: from Punta San Hipólito -27° N, 114° W- to Oaxaca -15° 40'N, 96°30' W-), but it is perhaps in Mazatlan, where historical and intensive records and studies have been done in the Mexican Pacific.^[56,65] The highest number of blooms is recorded for Mazatlan and La Paz. Some patterns of blooms occurrence in Mazatlan shows *Gymnodinium catenatum* appearing by March– April, *Akashiwo sanguinea* by May-June, and *Cochlodinium polykrikoides* by September–October. Dinoflagellates may form considerable blooms with up to 20.45×10^6 cells L⁻¹.

Almost all main types of marine biotoxins produced by HABs in the world also occur in the Gulf of California: PSP, DSP, ASP, CFP, and other toxins as tetrodotoxin, conotoxin and freshwater toxins as the microcystins and others organisms types.^[44] Fauna mortality in the Gulf of California includes whales, seals, dolphins, pelicans, turtles and fishes among others. One important event was the mortality of sea lions, dolphins and turtles close to Mazatlan Bay in 2003, during red tides of *Gymnodinium catenatum*, *Gymnodinium instriatum* and *Pseudo-nitzschia* spp. Events of mass mortality of fishes happened in several localities in Sinaloa and Sonora during *Chattonella* spp. blooms.^[66] Fish mortality is common on *Cochlodinium* spp. blooms in coasts of Sinaloa.

During the last 10 years, the mariculture in Mexico has faced some problems due to HABs. Mariculture basically consists in shrimp production and an incipient fish culture activity in the Gulf of California. Numerous cases of diseases, shrimp mortality and other problems associated to toxic and harmful phytoplankton have happened in the East of Gulf of California.^[67] Shrimp mortality associated to phytoplankton blooms in hatcheries and ponds has been reported since 1997,^[25] and in subsequent cases, Gymnodinium catenatum blooms were occurring in the water supply during these mortality events.^[68] Other common cause of mortality of shrimp in culture is the anoxia caused by high densities of phytoplankton in the ponds. Previous conditions for forming blooms are often nutrients in excess, changes in salinity, daylight time, wind or the income of sewage waters waters. Fish culture has already experienced the effects of HABs. Since 2002, the red tides formed by Cochlodinium polykrikoides in the coast, in natural and culture cause the c.a. 30% of fish mortality.^[69]

HABs in the Central Mexican Pacific

Since 1986, systematic observations on HABs have been made in Manzanillo Bays, Colima. From 1999 there has

been a marked increase of these HABs both in distribution and duration.^[70] In this area, 10 dinoflagellates dominate "red tides," but also two potentially harmful dinoflagellates, two diatoms, one silicoflagellate and one ciliate have been recorded. Higher densities of phytoplankton reached to more than 2×10^6 per liter, however, no records of poisonings or toxin were made. From the dominant species recorded in Manzanillo Bays, *Cochlodinium polykrikoides* has been the most recurrent and abundant, with its blooms occurring during March and April. Maximum bloom intensity in terms of time and extensions coincided with water temperatures between 21°C and 23°C and salinity ranging from 34.56 to 34.68. The location of HABs reported for Bahia Banderas and Mazatlan Bays agreed well.^[71]

The composition of potentially harmful dinoflagellates has been recently studied in coasts of Michoacán.^[27] This study includes the species: *Akashiwo sanguinea*, *Alexandrium catenella*, *Amylax triacantha*, *Ceratium furca*, *C. divaricatum var. balechii*, *Dinophysis caudata*, *D. fortii*, *D. mitra*, *Gambierdiscus toxicus*, *Gonyaulax polygramma*, *G. spinifera*, *Gymnodinium instriatum*, *Lingulodinium polyedra*, *Noctiluca scintillans*, *Prorocentrum micans*, *P. triestinum*, *Protoperidnium crassipes*, *Scrippsiella trochoidea*.

HABs in Southern Mexican Pacific

Apart from the records of HABs caused by *P. bahamense* var. *compressum*, there are few studies on other potentially harmful planktonic and epibenthic microalgae in the Gulf of Tehuantepec. Phytoplankton species composition has been studied in that area,^[72] and more recent reports indicate the presence of 24 "red tides"-forming species, from which four taxa are important, the diatoms *Skeletonema costatum* complex and *Pseudonitzschia delicatissima* complex, and the potentially toxic dinoflagellates *Alexandrium acatenella* and *A. tamarense*, with their maxima densities reaching to 1.1×10^5 and 2.2×10^5 cells L⁻¹, in May, 2004 and January, 2005, for the diatoms, and 5.55 and 6.14×10^3 cells L⁻¹ by summer, 2006 for the dinoflagellates (Barón-Campis, comm. pers.).

Conclusions

Perspectives and future studies

Technical innovations to improve our understanding of HABs are needed, including integrated techniques to envisage physical, chemical and biological variability. In Mexico, we would need long term monitoring programsthat can indicate certain tendencies of HABs and how they associate with human activity. Opportune detection would also help to protect areas dedicated to aquaculture and may serve as a signal to justify other investigations aimed at characterizing together the distribution and physiological stage of the phytoplankton in an oceanographic context.^[73]

Technical and methodological advances

Positive identification and counting of phytoplankton cells are a fundamental issue in studying HABs, for toxic and harmful events are specific-species (e.g., species produce specific toxins or may not be toxic at all, even if they are responsible for "red tides"). Traditional and classic methods include the analysis of water samples by microscopy (either conventional or inverted microscopes). However, well-trained personnel and time for confident analysis are badly needed, because often quick results are necessary to take important decisions (e.g., determining whether or not the bloom may be toxic, mitigation plan). Technical advances have contributed to less time-consuming analysis, as for example the incorporation of the Flow Citometry to biological oceanography.^[74] Study of resting stages (basically cysts produced by many dinoflagellates) is an approach to investigate possible distribution and blooming of certain species. Only recently, this study has started in Mexico.^[26,64]

Pigment signatures are closely related to phytoplankton species composition, and specific pigments (also often termed "fingerprints") might be useful in HABs monitoring programs.^[75,76] This approach is possible with new developments in High Performance Liquid Chromatography (HPLC) techniques.^[75,77] Most species forming blooms either toxic or not in Mexican waters (especially in the Mexican Pacific) have pigment signatures.^[76] Vertical and temporal distributions can now be determined following this method.

For toxin analysis, there are numerous methods using Thin Layer Chromatography, HPLC, capillary electrophoresis, mass spectrometry, fluorimetry, or molecular tools,^[40,78,79] although the mouse bioassay is an established method, which is the "official" method for some authorities and countries, and continues to be used in many laboratories.

Molecular tools have become very important to study HABs. Molecular probes to potentially toxic species, which are very often difficult to identify by conventional microscopical methods, are now widely used worldwide.^[80–82] Many species of the genera *Pseudo-nitzschia*, *Alexandrium* and *Chattonella*, show morphological characters only observed in detail by electron microscopy in many cases, and are now routine monitored using this technique. Other detection and counting methods include use of lectins and antibodies.^[81]

Remote sensing instruments utilize electromagnetic radiation to study surface processes on earth, and various advantages of this method are accessing difficult locations, rapidly mapping and a panoramic view.^[83,84] The ocean color imagery commonly used for studying HABs is produced from SeaWiFS estimates of surface Chl *a*concentrations. Using a sophisticated radiometer of a very high resolution and satellite equipment, Aguirre-Gómez et al.^[73] recorded successfully the evolution of a HAB episode in the Mazatlan Bay.

Monitoring and management programs: toxins and phytoplankton

Health Ministry (Secretaría de Salud) through the "Comisión Federal para Riesgos Sanitarios" (COFEPRIS -Federal Commission for Sanitary Risks-) and State sanitary jurisdictions in Mexico, with the support of other institutions of the Federal government, launched since 1984 the "Programa Mexicano de Sanidad de Moluscos Bivalvos" (PMSMB-Mexican Program of Bivalve Moluscs Sanity), with the purpose of monitoring toxin levels in "red tide" or blooms events, mainly using the mouse bioassay method and taking adequate procedures to avoid human poisonings. Phytoplankton and toxins sampling by the INP has been done since 1996, however these activities were suspended from 2003. Since 1996 COFEPRIS continues monitoring "red tides" and their impacts by K. brevis in Tamaulipas, Veracruz and Tabasco. Historical records are found in the web site: (http://www.cofepris.gob.mx/marea Roja).

In Yucatan, the "Programa de Monitoreo de Florecimientos Algales Nocivos" (Monitoring Program of HAB) was launched since 2000, with the participation of the Oceanographic Research Station of Progreso (Secretaría de Marina) and CINVESTAV-IPN, Unidad Mérida. More recently, remote sensing imagery is used as an additional tool for this purpose, with the "in real-time" added component.

Other program established nation-wide is the Red Tide Surveillance Program, through the web of Centros Tecnológicos de Mar (CETMAR) with 36 small centers, depending of the Dirección General de Estudios en Ciencia y Tecnología del Mar de la Secretaria de Educación Publica (DGECyTM, SEP), which started in 1998, to investigate and inform on the events in Mexican coasts. There is a web site that gives general information and recent records: (http://fans.cicese.mx).

In the southern Mexican Pacific, since 1998 there is a Surveillance and Monitoring Program of HAB's in CETMAR No. 24 (Center of Marine Technologic Studies), at Puerto Madero, Chiapas, in a fixed station, three km off the coast (14°42′43″N and 92°25′08″W). Mouse bioassays are also regularly made. This established monitoring allowed to follow presence and abundance of cysts suspended in the water column, preceding the vegetative cells of the blooms by *Pyrodinium bahamense* var. *compressum*, in 2001–2002 and 2006, and it has greatly aided evaluation of saxitoxins and consequent fisheries banning.^[85]

Monitoring in the central Mexican Pacific considered phytoplankton and chlorophyll *a* collected form surface water and CTD casts performed at the same stations. HABs spreading have been detected by satellite data to prepare maps of SSC distribution between $21.4^{\circ}-17^{\circ}$ N and $101^{\circ}-109^{\circ}$ W. These maps were used to detect possible bloom areas and their extension. The resulting SSC maps were projected with a resolution of 1 km, and a scale ranging form 0 to 20 μ m L⁻¹.

Collaborative approaches

A formal evaluation of HABs impact in Mexico is not available, although an increase in number, periodicity, and impact of HABs is widely recognized. National newspapers have reported locations such as La Paz, Mazatlán, Manzanillo, Acapulco and Huatulco frequently stricken by HABs, but they do not mention the socioeconomical cost involved. Nevertheless, because of the lack of an appropriate and systematic monitoring system, scientific records about HABs in Mexico are scarce.^[86] Only 2 certified laboratories authorized by the Health Ministry are able to detect toxins in mollusk samples. With a coastline of about 11,600 Km, this capacity is obviously insufficient to provide an opportune warning and assistance for HABs off coast of Mexico. The official records of the last 22 years indicate at least 500 cases of hospitalization and 20 casualties, which may be just the tip of a formidable iceberg of HABs health impact in Mexico.^[30, 55]

Aquaculture, fisheries and even tourism are strongly affected by HABs. The noticeable synchrony in the occurrence of different HABs of *Pyrodinium* in the Central American Pacific, from Costa Rica to Mexico, suggested that they represent a regional event.^[58] A formal regional project has started involving phytoplankton researchers and personnel of Health department in Central American countries, including Costa Rica, El Salvador, Guatemala and Mexico (States of Chiapas, Oaxaca and Guerrero), with the purpose of analyzing oceanographic and environmental variables' features related to the occurrences of blooms and their toxicity.

Only multinstitutional and multidisciplinary approaches to study HABs may guarantee a better understanding of the mechanisms and processes that lead to developing and keeping HABs, the possible causes and factors that enhance these phenomena, the role of human activities in the coastal zone, the responses of species involved, possible trends and seasonality, biology and ecophysiology (including life cycles and life histories), and the possibility of developing accurate methods to forecast their occurrences.

Local and global factors

Increase of cases of HABs in the world and Mexico are currently related to various factors, most of then of anthropologic origin: rapid increase of human population, increased utilization of coastal waters for aquaculture, cultural eutrophication and pollution, transport of resting stages (cysts, resting spores) in ships' ballast waters;^[1,3,20,87,88] different hydrographic conditions, global climatic change and unusual climatologic conditions (leading to a wide range of global, regional and local effects: oceanographic patterns, rain regimes, El Niño Southern Oscillation –ENSO– events) are also involved in "stimulation" of algal blooms at local and global scale.^[1–3,20] An important amount of information proceeding from monitoring, scientific research and collaboration is still required to propose mitigation policies conducing to reduce risks in health and economic losses.

Anthropologic influence

Coastal eutrophication in the north of Yucatan represents a big environmental problem.^[89,90] The increase of ammonia and urea due to waste water may promote blooms of toxic and harmful species, replacing other phytoplankton species that regularly occur there. Evidence of blooms caused by innocuous species, such as *Ditylum brightwelli* and species of *Bacteriastrum, Guinardia, Leptocylindrus, Ornithocercus, Pleurosigma, Proboscia, Protoperidinium, Rhizosolenia,* that precede HABs, may be used as an indicator of their imminent appearance and also to establish their temporal occurrence in Yucatan. This fact, together with oceano-graphic processes such as upwellings, seems to contribute to the developing of HABs.

Sufficient evidence exists that eutrophication has accelerated in Mexican coastal waters, and we still need to know how this can aid HABs developments. For instance, we speculate that the increase of HABs in Manzanillo Bays, Mexican Pacific, is associated to the increased activities of the port. On the other hand, in the area of Mazatlan bay, also in the Pacific, the waters are mostly eutrophic, with presence of red tides close to the submarine waste water distributor.^[91]

Introduction of potentially harmful species is a mechanism that has been proposed for the recent finding of the naked dinoflagellate *Cochlodinium polykrikoides*,^[71] of wide distribution in the Mexican Pacific, which was only observed from 1999 in Manzanillo, Colima,^[70] and no previous record in Mexican waters.^[10] The hypothesis includes ships' ballast waters.

We have a general understanding of the movement of biotoxins through the food chain. Some blooms initiate offshore in more oceanic zones: microalgae increase in number as the bloom is moved toward shore, driven by wind and currents. The bloom is initiated and sustained by upwelling in the continental shelf, which brings up cooler water rich in nutrients required by the cell for reproduction. Small pelagic (herbivorous fish), such as anchovies and sardines, consume phytoplankton, accumulate toxin if it is present, and they in return pass it onto predator species such as marine mammals or humans. On the other hand, benthic filter feeders (bivalve mollusks: mussels, clams and oysters) also accumulate toxins. It is necessary to quantify the toxin pathway through food net to obtain better models.

Oceanographic and climatic processes

Upwelling events are usually short-time events. Some local and regional phenomena regarded HABs are undoubtly related to their patterns in some areas (e.g., western coasts of Baja California, the Gulf of California and the Gulf of Tehuantepec). We have to gain knowledge on the mechanisms that regulate blooms in these areas and also the role of post-upwelling periods and mixing of waters in adjacent. In coasts of Yucatan, HABs seem to be associated to upwellings from the Mexican Caribbean, which come to the Gulf of Mexico, bordering the northern coast in the State. The different types of water combined may favour microalgal blooms.

In Manzanillo Bays, an inverse correlation was found between surface chlorophyll *a* and temperature. There is a remarkable spatial distribution in red tides: one bay showed red tide caused by one species, whereas the other was caused by another species or showed no event. The observed increase of HABs between 1999 and 2000 in the Pacific Mexican coastal zones coincided with the lowest temperatures recorded in the area and was probably related to the negative anomalies of temperature encountered in the North Pacific Ocean during the period of La Niña 1999–2000.

El Niño (ENSO) plays a complex role in plankton biomass variation along the Peninsula of Baja California, the Gulf of California, [55,92] and the Gulf of Mexico and the Mexican Caribbean. El Niño seems to cause a decline in upwelling-based primary productivity along the western coast of Baja California. In contrast, in the mouth of the Gulf of California, temperatures above the normal (29– 30°C) attributed to the presence of El Niño, favour the replacement of nutrient-rich waters by oligotrophic waters causing a very low productivity. The strong tidal mixing and upwelling events that tend to mask the effect of such a climatic phenomenon should be considered to draw any conclusion about the El Niño influence in primary productivity in the Gulf of California. González-López^[92] associated^[14] El Niño events in the Gulf of California, identifying^[18] phytoplankton species to find "markers."

El Niño and HABs incidence in coasts of Mexico is, however, not yet clear. Cortés-Altamirano^[39] reported that El Niño favours the development of some blooms in Mazatlan Bay, but not in the Gulf of California.^[93] Also Manrique & Molina^[94] have found an inverse relationship between El Niño and HABs occurrences in the Gulf of California. The analysis of 43 cases of HABs during the last 25 years by these authors point out to Noctiluca scintillans, Gymnodinium catenatum, Lingulodinium polyedra and Miryonecta *rubra* as dominant species, with no evidence of toxicity during blooms occurring in November, December, and January each year. The diatom Pseudonistzchia australis and the toxin domoic acid were detected in bodies of stranded animals in Sinaloa beaches derived from a mass mortality of sea-birds, fish, and sea mammals, during a strong El Niño event. As it appears, El Niño, with the exceptions of 1976, 1984 and 1997 events, generally attenuates the blooms in such an area.^[94]

The occurrence of the El Niño events seem to be more frequent and intense: the 1997–1998 EL Niño and 1998– 2000 La Niña were the most intense on record, showing evidence of climatic change and its impacts. The exceptional HABs recorded in coastal line and offshore in Mexican Central Pacific Waters during the 1999–2000 La Niña event, might give an orientation towards what could be with the recurrent presence of this events in the area.

Approaching new concepts and paradigms

The recent established concepts about the term "species" should be considered from now on in studying HAB. Concepts like "species complex," "cryptic (and semicryptic) species" and morphological and genetic variation of species require actualization, discussion and new research proposals. Athecate dinoflagellates (*Karenia, Karlodinium, Takayama*), and species of many thecate genera (with a potential thread to the environment in terms of invasion, toxicity and harm) such as *Alexandrium, Gambierdiscus, Heterocapsa, Scrippsiella* are poorly known in Mexico, and recent proposals of "cryptic species" of the diatoms genera *Pseudonitzschia* and *Skeletonema*^[95,96] call for further studies on morphology and taxonomy of these groups.

A particular issue to be investigated is the identity of *Pyrodinium bahamense* var. *bahamense* and its relationship to the var. *compressum* in the Mexican Pacific. The former has been detected since 1942^[97] and more recently.^[98] Vargas & Freer^[31] found both taxa in an extensive bloom in 2001, in Costa Rica: they believed both taxa correspond to stages in the life cycle of a given taxon.

Mixed species and the synergic effect^[46] have been much less studied in Mexican waters: the only available example is the finding of certain species associations, such as *Ceratium furca-Prorocentrum micans*, *Alexandrium catenella*, *Dinophysis acuminata*.^[99]

An important issue that has not been dealt with is the knowledge of the biology of the species involved in HABs. In Mexico we know few details of the physiology and ecology, life cycles, life histories, cyst or resting stages production, range of environmental conditions of causative species of HABs. More detailed studies should be made on the proposed evolutive strategies that seem to give competitive advantages to many of the HABs species. Some of them were summarized by Smayda:^[19] (1) nutrient retrieval migrations, (2) mixotrophic tendencies, (3) allelopchemical competition, and (4) allelopathic, antipredation defence mechanisms. These studies imply ecophysiological research both in the field and experimental, controlled conditions.

The low toxicity of regional strains needs to be studied by molecular techniques. We do know the relationship between algal growth and toxin production depending on nutrients (silicates, different nitrogen sources, phosphates, perhaps iron), organic matter (e.g., vitamins) and temperature. The precise values for predictive models, has yet to be developed. In related fields, histopathologic studies of the acute and chronic exposition to PSP and NSP toxins are currently being developed, based on the model of mouse, and toxin accumulation and depuration in shrimp. Toxicity and biological activity of *Amphidinium carterae*, *Cochlodinium polikrikoides*, *Gymnodinium catenatum*, *Karenia bre*- vis, Prorocentrum lima, P. minimum, Prorocentrum sp., Pyrodinium bahamense var. compressum are current subjects for investigation.^[69] These investigations may eventually contribute to redefine the Norma Oficial Mexicana (Official Mexican Norm) with regards to toxicity levels in marine products to human consumption.

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