

## Article

# Paralytic Shellfish Toxins of *Pyrodinium bahamense* (Dinophyceae) in the Southeastern Gulf of Mexico

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**Abstract:** In September and November 2016, eight marine sampling sites along the coast of the southeastern Gulf of Mexico were monitored for the presence of lipophilic and hydrophilic toxins. Water temperature, salinity, hydrogen potential, dissolved oxygen saturation, inorganic nutrients and phytoplankton abundance were also determined. Two samples filtered through glass fiber filters were used for the extraction and analysis of paralytic shellfish toxins (PSTs) by lateral flow immunochromatography (IFL), HPLC with post-column oxidation and fluorescent detection (FLD) and UHPLC coupled to tandem mass spectrometry (UHPLC-MS/MS). Elevated nutrient contents were associated with the sites of rainwater discharge or those near anthropogenic activities. A predominance of the dinoflagellate *Pyrodinium bahamense* was found with abundances of up to  $10^4$  cells  $L^{-1}$ . Identification of the dinoflagellate was corroborated by light and scanning electron microscopy. Samples for toxins were positive by IFL, and the analogs NeoSTX and STX were identified and quantified by HPLC-FLD and UHPLC-MS/MS, with a total PST concentration of  $6.5 \text{ pg cell}^{-1}$ . This study is the first report that confirms the presence of PSTs in *P. bahamense* in Mexican waters of the Gulf of Mexico.

**Keywords:** paralytic shellfish toxins; paralytic shellfish poisoning; saxitoxin; neosaxitoxin; *Pyrodinium bahamense*; Gulf of Mexico; HPLC-FLD; UHPLC-MS/MS; phytoplankton; dinoflagellates

**Key Contribution:** This study is the first report that confirms the presence of PSTs (STX and NeoSTX) in *Pyrodinium bahamense* in Mexican waters of the Gulf of Mexico.



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## 1. Introduction

The dinoflagellate *Pyrodinium bahamense* Plate 1906 (Dinoflagellata: Dinophyceae: Goniodomaceae) is a tropical–subtropical species that was originally described from the Atlantic Ocean at New Providence Island (Bahamas) [1]. The first record of paralytic shellfish poisoning (PSP) caused by *P. bahamense* was recorded in 1972 in Southeast Asia at Port Moresby, Papua New Guinea [2,3]. It is one of the main dinoflagellate species producing paralytic shellfish toxins (PSTs) that have caused PSP from the consumption of bivalves, planktivorous fish and gastropods in the world [4–8], mainly in tropical and subtropical areas [7,9–11]. Their proliferations have also been associated with epizootics

and massive mortalities in fish, sea turtles and other marine organisms [8,12–20]. Emslie et al. [21] suggested that mass mortality of the extinct cormorant *Phalacrocorax filyawii* Emslie, other seabird, fish and a seal species about 54 million years ago in coastal waters off Sarasota, Florida, United States, coincided with accumulation of *P. bahamense* cysts that have a stratigraphic range from the lower Eocene to the Holocene. The species was also described as a producer of the PSTs (saxitoxin and analogs) related to human intoxications due to consumption of puffer fish (Tetraodontidae) in Florida, in which, unexpectedly, tetrodotoxin and analogs were not detected as the cause of intoxication, thus establishing a new syndrome called saxitoxin puffer fish poisoning (SPFP) [22].

In the southeastern Mexican Pacific, *P. bahamense* has had the greatest impact on fisheries, tourism and coastal marine ecosystems due to the implementation of long sanitary bans, in which the extraction and commercialization of shellfish is prohibited [23]. Such incidents affect mainly bivalve harvesting with the purpose of protecting public health, because it has been the cause of 476 human poisonings and 18 deaths from PSP in the Mexican tropical Pacific [8,20,24–29]. In May 2022, a new case of PSP with 12 poisonings and two deaths was reported from the coastal waters of the state of Chiapas [30], and the health authorities of the Government of Chiapas issued a sanitary ban for the extraction and commercialization of bivalve mollusks [31].

Some authors distinguish two varieties or morphotypes in *P. bahamense* var. *compresum* and var. *bahamense*. Initially, var. *bahamense* was thought to be confined to the Atlantic Ocean and var. *compresum* to be limited to the Pacific Ocean [7,32]. However, differentiation between the two varieties based on morphological criteria is not irrefutable, as shown by a detailed morphological analysis of the populations from Papua, the Philippines, Jamaica and Puerto Rico [33]. In addition, the physiological criterion of toxin production versus no production is no longer applicable [11]. Therefore, segregated biogeography is no longer accepted because both varieties have been reported to coexist in several places, such as Costa Rica [34], the Pacific coast of Mexico [35] and along the eastern coast of Florida [22].

In the Gulf of Mexico and the Caribbean Sea, *P. bahamense* appears to have a continuous distribution [36–39], also being present in coastal lagoons [40,41], with a range of abundances of  $10^3$ – $10^5$  cells  $L^{-1}$ , generally in summer [38]. The species has been recorded along the central coast of Campeche (southwestern of Gulf de Mexico) with minimum abundances of  $2 \times 10^4$  cells  $L^{-1}$  recorded in October 2008 to a maximum of  $3.3 \times 10^5$  cells  $L^{-1}$  observed in August 2005 [41]. The first evidence of the presence of PSTs along the Campeche central coast with the profile of STX, NeoSTX, GTX2-3 and dcGTX2-3 was attributed to *P. bahamense*. This was inferred [42,43] indirectly from the examination of toxins in puffer fish *Spherooides* spp. and *Lagocephalus laevigatus* and thought to probably be transmitted via bivalves and other invertebrates that regularly constitute a part of the diet of these fish in Campeche [44].

A number of different methods have been employed globally for monitoring PSTs in bivalve mollusks. The traditional use of mouse bioassays (MBA) [45,46] has in recent years been replaced through regulations by instrumental chemical detection methods [47]. In North and South America, this is a post-column oxidation (PCOX) liquid chromatography with fluorescence detection (LC-FLD) method, whereas for European legislation, the formal reference method uses pre-column oxidation (Pre-COX) LC-FLD to quantify PSTs [48]. Both approaches have been subjected to validation, with advantages and disadvantages existing with each assay [49]. While other approaches based on receptor activity have also been validated and implemented into some monitoring programs [50], more recent developments have focused on the use of LC with tandem mass spectrometry (LC-MS/MS). One such method [51] utilizes a desalting clean-up step prior to acetonitrile dilution and analysis and has been subjected to both single-laboratory [52] and multi-laboratory validation [53].

In September and November 2016, eight marine sampling sites along the coast of the southeastern Gulf of Mexico were monitored for the presence of lipophilic and hydrophilic toxins [54,55]. Physicochemical parameters, inorganic nutrients and phytoplankton abundance were also determined. The aims of the study were to identify the toxin producer,

evaluate physicochemical factors that may be associated with its proliferation and confirm the presence of PSTs in this dinoflagellate in the southeastern Gulf of Mexico.

## 2. Results

### 2.1. Physicochemical Variables

The variation of the water temperature (Table 1) was low in both months; in November, at station 8, it reached a maximum of 38.63 °C. Salinity showed high variation both between stations and between months, with a minimum value of 17 at station 2 in November and a maximum value of 41.57 at station 4 in September. The pH showed a maximum value of 9.63 at station 7 in September and a minimum value of 6.5 at station 6.3 in November. Dissolved oxygen (DO) showed a minimum value of 4.1 mg L<sup>-1</sup> at station 7 in November.

**Table 1.** Variability of physicochemical characteristics from eight sampling sites along the central coast of Campeche, southeastern Gulf of Mexico. \* Water quality criteria for the protection of aquatic life [56].

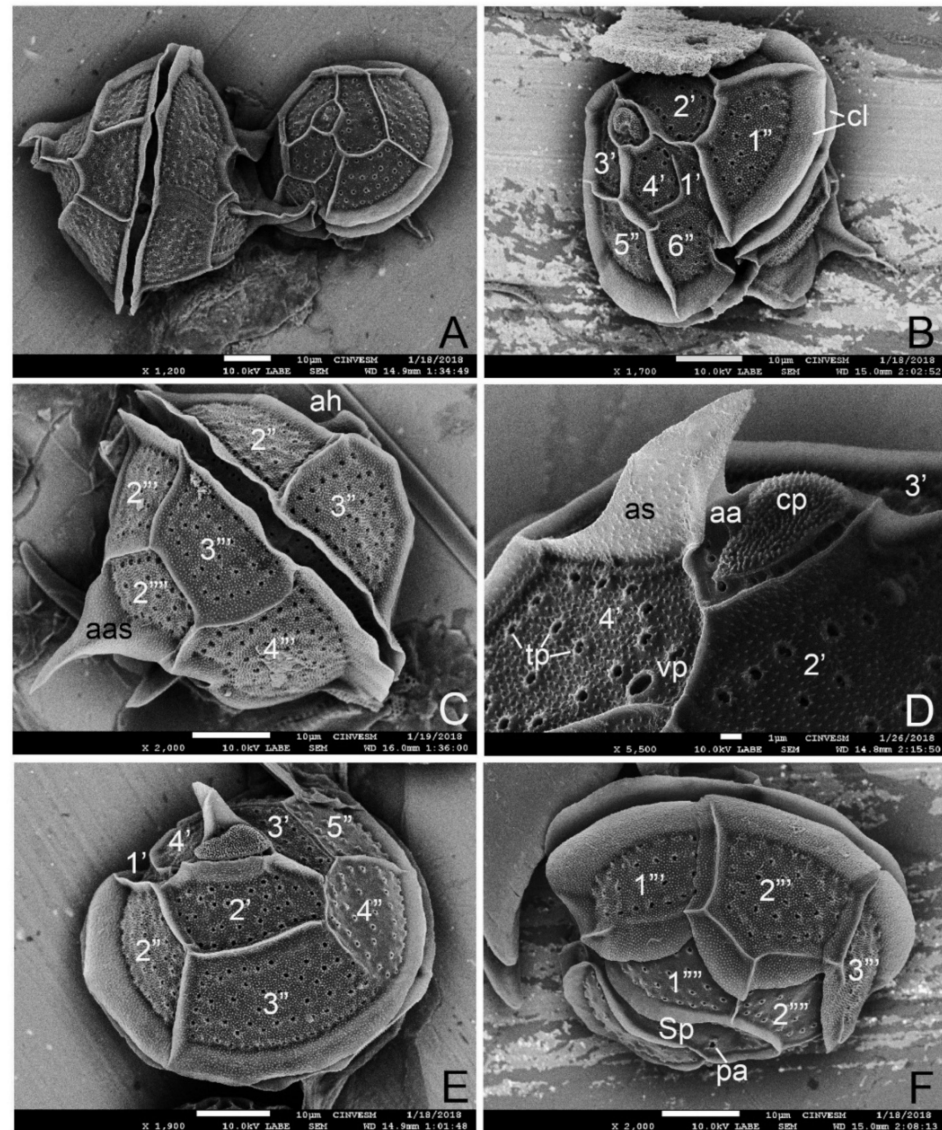
Physicochemical Variables									
Stations	T (°C)		Salinity		pH		DO (mg L <sup>-1</sup> )		
	September	November	September	November	September	November	September	November	
S1	31	27.5	37.8	24	9.29	7.06	6.2	6.9	
S2	31	28.6	39.95	17	9.26	6.9	6.7	4.12	
S3	31.37	28.5	40.54	21	9.05	6.5	7	5.7	
S4	31.31	28.8	41.57	39	9.37	7.7	7	7.45	
S5	31.79	29.4	40.01	30	9.29	6.6	7.3	7.53	
S6	32.06	29.4	28.24	18	9.14	7	7.5	6.53	
S7	32.8	30.1	38.42	24	9.63	9	7.9	4.1	
S8	32.31	38.63	39.47	35	9.29	7.3	7.7	6.79	
Mean	31.71	30.12	38.25	26	9.29	7.26	7.16	6.14	
SD	0.65	3.52	4.21	7.96	0.17	0.79	0.55	1.37	
Nutrients (µmol L <sup>-1</sup> )									
Stations	Nitrites		Nitrates		Ammonium		Orthophosphates		
	September	November	September	November	September	November	September	November	
S1	0.034	0.021	0.196	0.194	0.219	0.141	0.054	0.008	
S2	0.062	0.045	0.249	0.83	0.273	0.235	0.059	0.04	
S3	0.028	0.03	0.182	0.457	0.074	0.105	0.046	0.01	
S4	0.012	0.024	0.098	0.389	0.168	0.145	0.085	0.011	
S5	0.006	0.021	0.07	0.29	0.073	0.056	0.026	0.001	
S6	0.01	0.032	0.272	0.845	0.093	0.046	0.012	0.02	
S7	0.011	0.006	0.134	0.462	0.095	0.046	0.05	0.006	
S8	0.021	0.013	0.082	0.263	0.138	0.088	0.063	0.021	
Mean	0.023	0.024	0.160	0.466	0.142	0.108	0.049	0.015	
SD	0.018	0.012	0.076	0.247	0.073	0.065	0.022	0.012	
* Upper limits established for marine coastal waters	0.002		0.04		0.01		5		

In September, nitrites at all stations were above the maximum limit established for marine waters (MLEMW) [56]. As in November, a maximum value of 0.045 µmol L<sup>-1</sup> was recorded at station 2. In September and November, nitrates were above the MLEMW. At stations 1, 2, 4 and 8, the ammonium concentrations were above the MLEMW. At all stations, orthophosphates were well below the MLEMW (Table 1).

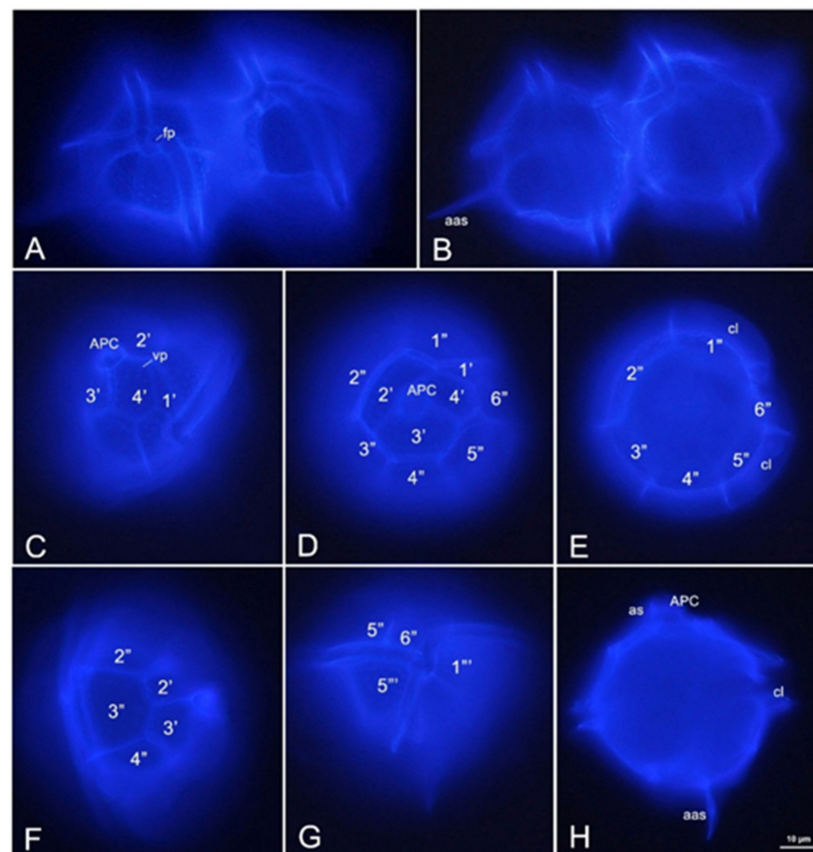
### 2.2. Identification and Abundance of *Pyrodinium bahamense*

The cell width was measured above or below the cingulum, not taking into account the cingular lists: length without spines, including the apical horn, 45–65 (56 ± 5.81) µm,

length with spines 52–95 ( $78 \pm 10.76$ )  $\mu\text{m}$ , width 44–53 ( $53 \pm 4.62$ )  $\mu\text{m}$ , length without spines/width ratio (L/W) 0.88–1.25 ( $1.06 \pm 0.06$ ). The apical horn was approximately 5  $\mu\text{m}$ . Cells were without spines, or with only one (apical or antapical) spine or with both. Almost all the cells were solitary; some two-celled chains (attached daughter cells) were observed. Only one cell with slightly different proportions was found (length without spines 54  $\mu\text{m}$ , length with spines 75  $\mu\text{m}$ , width 46  $\mu\text{m}$ , L/W 1.17) Figures 1 and 2, Table 2.



**Figure 1.** Thecal morphology of *Pyrodinium bahamense* (viewed with scanning electron microscopy). (A) two cells in dorsal (left) and apical-ventral left-side view; (B) cell in apical-ventral left-side view; (C) cell in dorsal left-side view; (D) a fragment of the epitheca with the apical pore complex and the apical spine; (E) cell in apical-dorsal left-side view; (F) cell in antapical left-side view. Plate labels: 1'–4', the apical plates; 1''–6'', the precingular plates; 1'''–5''', the postcingular plates; 1'''' and 2'''', the antapical plates; aa—the anterior attachment pore, aas—the antapical spine, ah—the apical horn, as—the apical spine, cl—the cingular lists, cp—the canopy (also known as the cover plate or the closing plate), pa—the posterior attachment pore, Sp—the posterior sulcal plate, tp—the trichocyst pores, vp—the ventral pore. The plates are named mainly according to Balech [33]. Scale bars: 10  $\mu\text{m}$  in (A–C,E,F), 1  $\mu\text{m}$  in (D).

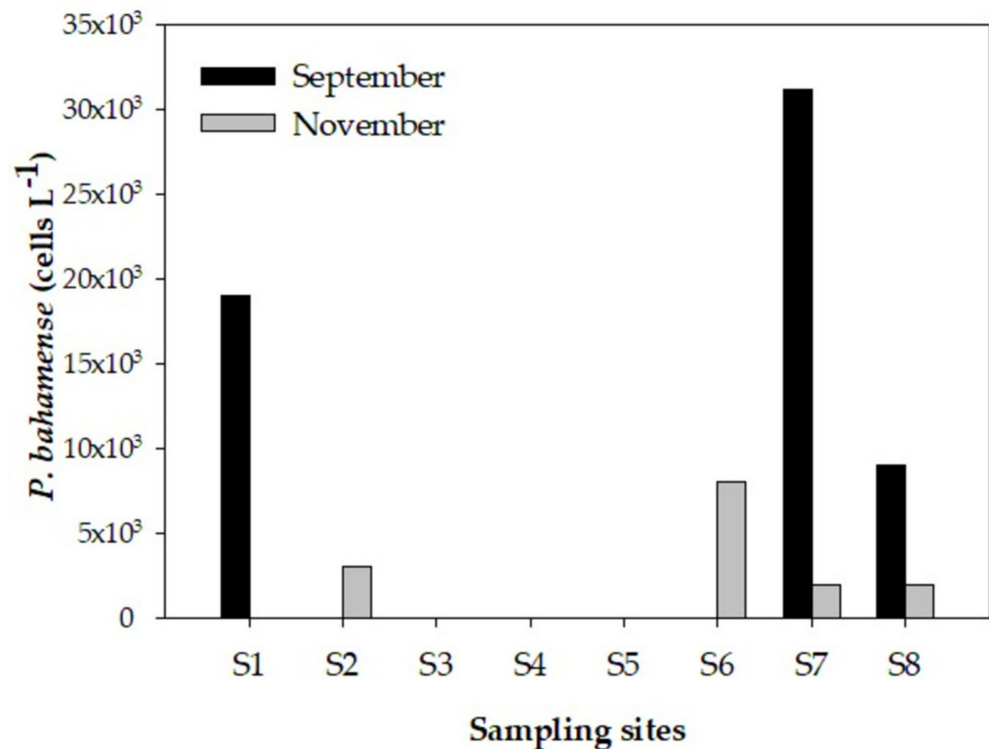


**Figure 2.** Thecal morphology of *Pyrodinium bahamense* (viewed with epifluorescence microscopy). (A,B) two cells in ventral view on different focal planes; (C) apical–ventral view; (D,E) apical view on different focal planes; (F) dorsal–apical view; (G,H) ventral view on different focal planes. Plate labels: 1'–4', the apical plates; 1''–6'', the precingular plates; 1'''–5''', the postcingular plates; Xaa—the anterior attachment pore, aas—the antapical spine, APC—the apical pore complex, as—the apical spine, cl—the cingular lists, vp—the ventral pore. Scale bar: 10  $\mu\text{m}$ .

**Table 2.** Vegetative cell measurements of *Pyrodinium bahamense* from the Mexican Pacific, the Greater Caribbean and Central America.

Length (Cell Body): Range or Average, $\mu\text{m}$	Width: Range or Average, $\mu\text{m}$	Locality	Reference
50	48	New Providence Island, the Bahamas	[1]
66 (86 with spines)	54	Gulf of Tehuantepec, east coast, and Pacific off El Salvador	[57]
33.8–77.1 (42.5 $\pm$ 10.8)	33.8–67.7 (39.9 $\pm$ 7.8)	Tampa Bay, West Florida	[37]
33–47	47–53	Gulf of Nicoya, Costa Rica	[34]
33–77	37–67	Douglas Cay and Twin Cay (coral-reef mangrove lagoons), Belize	[58]
41.9	43.8	Isla San José, Gulf of California	[59]
43 (without the apical horn)	41.5	Bahía de Campeche, Gulf of Mexico	[60]
33–47	37–52	Baja California Peninsula, southern coast	[35]
39–59 (48.6 $\pm$ 5.0); 48–90 (65.9 $\pm$ 11.1)	40–50 (45.3 $\pm$ 2.7)	Marinas along the northern Yucatan Peninsula, Gulf of Mexico	[61]
30–52	35–55	Mexican Pacific	[32]
27.30–81.88 (46.01 $\pm$ 9.35)	22.60–83.34 (48.11 $\pm$ 8.97)	Caribbean, Florida	[11]

In September, *P. bahamense* showed a maximum abundance of  $3.13 \times 10^4$  cells  $L^{-1}$  at station 7, followed by  $1.90 \times 10^4$  cells  $L^{-1}$  at station 2. At the other stations, the abundances ranged from undetected to  $10^3$  cells  $L^{-1}$  in November (Figure 3).



**Figure 3.** Temporal variation of *P. bahamense* at eight stations (S1–S8) along the Campeche central coast, southeastern Gulf of Mexico.

### 2.3. Toxicity

#### 2.3.1. Lateral Flow Immunochromatographic Test (Rapid PSP Test)

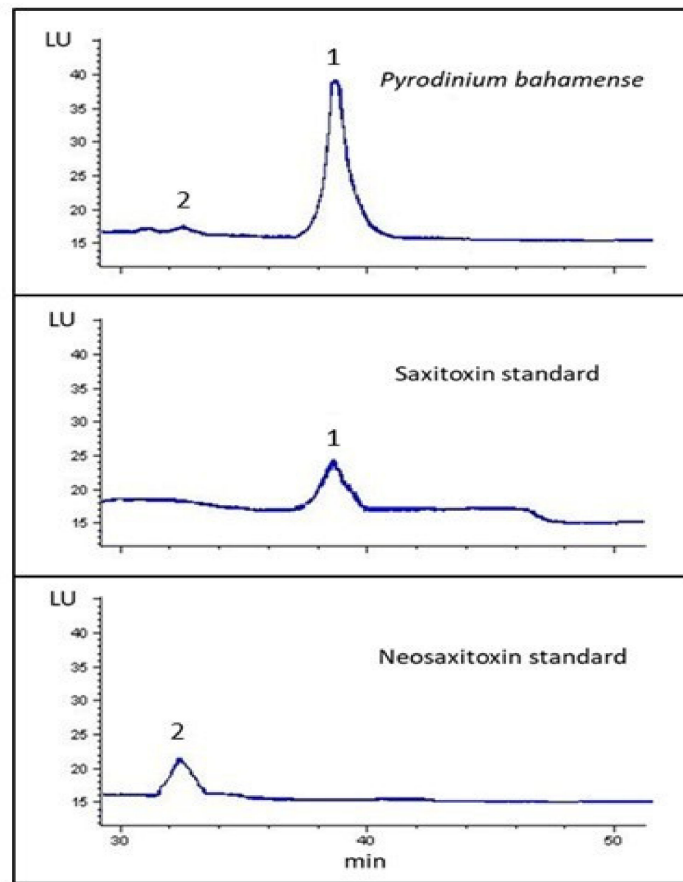
Positive identification of PSTs by qualitative analysis (saxitoxin and analogs) for lateral flow immunochromatography (LFIC) was found in phytoplankton extracts. The intensity of the marked line was similar to a positive control (STX, FDA Reference Standard STD, was obtained from the US National Institute of Standards and Technology (NIST, RM 8642).

#### 2.3.2. HPLC-FLD Analysis

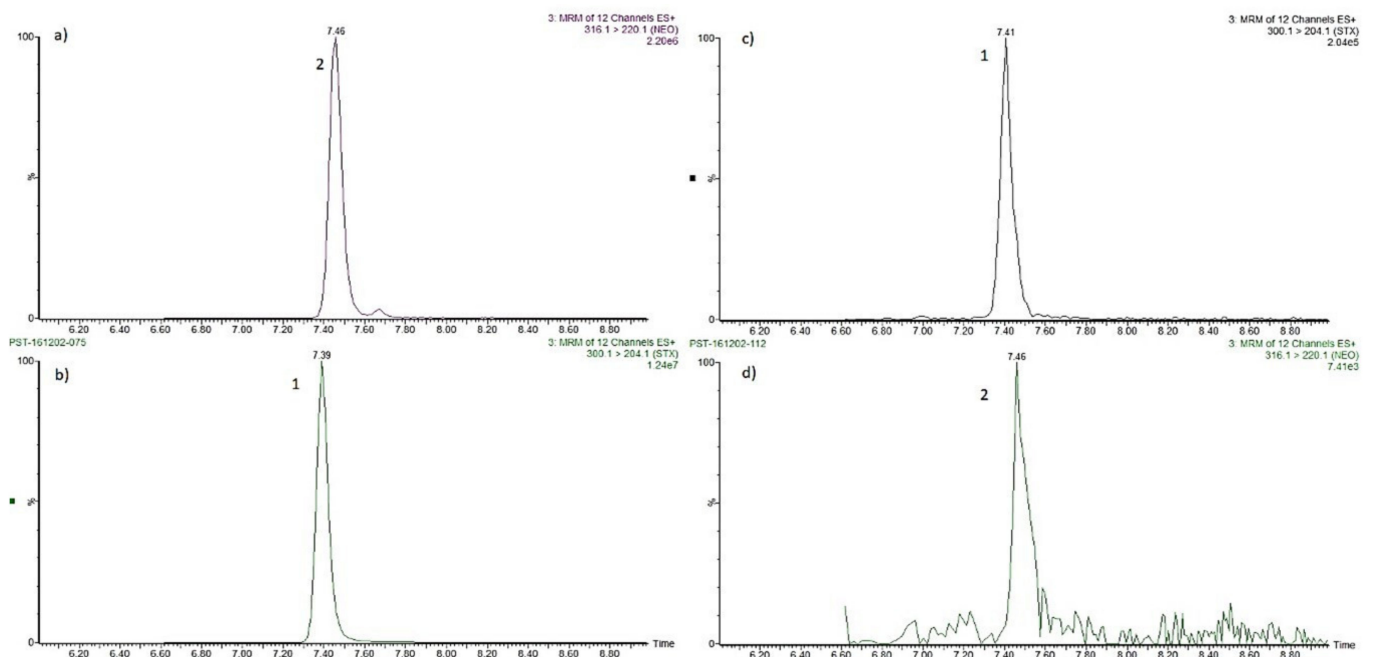
The toxin profile determined by post-column oxidation HPLC-FLD of *P. bahamense* in comparison to certified reference standards showed the presence of two analogs from the group of carbamoyl toxins (STX and NeoSTX; Figure 4). No sulfocarbamoyl and decarbamoyl toxins were detected in this study. The toxin content was quantified at  $6.5 \text{ pg cell}^{-1}$  (against external calibration standards. The molar percentages recorded for *P. bahamense* showed high molar percentages of carbamoyl toxins with 86.7% and 13.3% of STX and NeoSTX, respectively.

#### 2.3.3. UPLC-MS/MS Analysis

Figure 5 illustrates the MRM chromatograms obtained following HILIC-UHPLC-MS/MS analysis of STX and NeoSTX standards and the sample of *P. bahamense*. Peaks were observed for both STX and NeoSTX in the algal sample, with retention times identical to those determined in the calibration standard. Ion ratios determined between standard and sample were found to be similar ( $\pm 10\%$  deviation).



**Figure 4.** Post-column oxidation HPLC-FLD chromatograms showing the presence of STX (1) and NeoSTX (2) in *P. bahamense* from the coastal waters of Campeche.



**Figure 5.** Confirmation of PSTs in *P. bahamense* from the coastal waters of Campeche for UPLC-MS/MS. (a) NeoSTX in calibrant, (b) STX in calibrant, (c) STX in sample, (d) NeoSTX in sample.

### 3. Discussion

The physicochemical variables (temperature, salinity, pH and DO) measured at the end of the rainy season and at the beginning of the windy season coincided with those reported by Poot-Delgado et al. [62], who concluded that their variation was influenced by the shallow depth in the study area (~1 m), recording higher values compared to the area more than 2.5 km offshore [63–66]. Similarly, the registered values of nitrogenous compounds are above the MLEMW, which could be influenced by the proximity of the study area to point sources of wastewater discharges resulting from various anthropogenic activities [67,68].

Phlips et al. [69] described, as physiological limits for *P. bahamense* in Florida, a temperature of 20 °C as the lower temperature limit and a tolerance to salinity between 10 and 45. The bloom potential of this dinoflagellate was most closely associated with shallow ecosystems with long water residence times, and peak biomass levels were correlated to nutrient concentrations in regions of high abundance.

In Mexico, *P. bahamense* has been reported from various states from the Mexican Pacific (Baja California, Baja California Sur, Guerrero, Oaxaca, Chiapas; see [32,35,59,70–73]) and in the Gulf of Mexico from the states of Campeche and Yucatan [60,61]. Although several publications have been documented with light micrographs [32,35,59,70–74], SEM micrographs are still rare: [59,74]—vegetative cells, [72]—cysts.

In the present study, we followed [33], who found no significant morphological differences between the two varieties, var. *bahamense* and var. *compressum* [37]. Mertens [11] suggested that instead, they are probably different life stages and that *P. bahamense* is a species complex represented by two clearly separated ribotypes, Indo-Pacific and Atlantic–Caribbean ones.

Cusick and Duran [75] reported the existence of genotypes in natural *P. bahamense* subpopulations in Florida and Puerto Rico, with relevant insights on underlying genetic factors influencing the potential for toxin variability among natural subpopulations of HAB species.

The toxicity profile of *P. bahamense* is relatively simple, with most isolates from Indo-Pacific producing only dc-STX, STX, NeoSTX, B1 and B2 toxins [7]. This toxin profile is typical of both natural environmental samples and cultures [76–79] (Table 3).

Nevertheless, the isolates from Guatemala contained STX, neoSTX, B1, GTX2, GTX3 and GTX4 [81] (Table 3); in Florida, *P. bahamense* most likely produced STX, dc-STX and B1 as detected in puffer fish and HAB of *P. bahamense* [22] (Table 3), and one strain from Isla San José in the Gulf of California produced only STX [72] (Table 3).

Studies on bivalves contaminated by *P. bahamense* in Malaysia and the Philippines showed that there were few differences in toxin profiles of dinoflagellate and shellfish [7,82,83].

Chromatographic profiles have been determined by HPLC-FLD and UHPLC-MS/MS from shellfish sampled during the main bloom of *P. bahamense*, which had the greatest impact on public health in Mexico [14,55]. A concentration of 7396 µg eq STX kg<sup>-1</sup> was determined in the rock oyster *Crassostrea iridescens* (Hanley) from the Bay of Acapulco, Guerrero, during the 1995 HAB event; between 8889.3 and 32,272.5 µg eq STX kg<sup>-1</sup> occurred in the clam *Donax gracilis* Hanley from Zapotal and 14,445 µg eq STX kg<sup>-1</sup> in the mangrove mussels *Mytella strigata* (Hanley) from Puerto Madero, both in Chiapas, during the HAB of 2001 (regulatory limit for PSTs in flesh is 800 µg saxitoxin equivalents (STX eq.) per kg of shellfish flesh). The profiles by HPLC-FLD were STX, GTX2, dcSTX, dcGTX2, dcGTX3 and B1 in *C. iridescens*, STX, NeoSTX, GTX2, GTX3 and B1 in *D. gracilis* and STX, GTX2, GTX3 and B1 in *M. strigata*. In Salina Cruz, Oaxaca, in 2002, 306.3 µg eq STX kg<sup>-1</sup> was still detected in the spiny oyster *Spondylus calcifer* Carpenter, with only STX present. Additionally, UHPLC-MS/MS analysis was used to detect GTX1, GTX5, GTX6, doSTX, dcNEO and C1 [14,55]. The two PST detection methods in this study are both thoroughly validated and have been found to generally compare well when applied to shellfish samples. A recent study assessing the use of six detection methods for analysis of shellfish from Latin America, including Mexico, showed good correlations between all instrumental methods applied.



The HPLC-FLD method has been previously validated for a wide range of PST analogs, although the LC-MS/MS has the further advantage of incorporating a larger number overall. Both methods are, however, well suited to the accurate quantitation of individual PST analogs and the summation of PSTs to estimate total toxin levels.

**Table 3.** Toxin content and PST profile of natural samples and cultures of *Pyrodinium bahamense*.

Locality, Year	Profile	Toxin Content (pg STX per Cell)	Reference
Palau, 1980	Natural: STX, NeoSTX, GTX5	$1.5\text{--}1.4 \times 10^{-4}$ MU/cell *	[80]
Guatemala, 1987	Natural: STX, NeoSTX, B1, GTX2, GTX3 and GTX4	—	[79,81]
Sabah, Malaysia, 1991	Culture: STX, NeoSTX, GTX5, GTX6 and dcSTX	0.66–3.98	[82]
Masinloc Bay, Philippines, 2000 and 2002	Natural: STX, NeoSTX, B1	0.66–5.35	[83]
	Culture: STX, NeoSTX, dcSTX, B1 and B2	0.54–1.33	
Indian River Lagoon, Florida, U.S., 2005	Natural: STX, B1 and dcSTX	3.28	[22]
	Culture: STX and B1	2.02–12.74	
Isla San José, Gulf of California, 2008	Culture: STX	0.31	[72]
Lagoon, Red Sea, 2013–2014	Culture: STX	2	[84]
Campeche, southeastern Gulf of Mexico, 2016	Natural: STX and NeoSTX	6.5	This study

\* 1 MU (mouse unit) = 0.16 µg STX eq (equivalent).

Puffer fish (Tetraodontidae) from southeastern Mexico can also be vectors of PSTs associated with HABs of *P. bahamense* and could be the cause of SPFP in human cases [44,55] reported in the Yucatan Peninsula [85]. The profiles of sodium channel blocker neurotoxins (TTX and analogs + STX and analogs) in five species of puffer fish of the genus *Sphoeroides* and *Lagocephalus* were recently evaluated along the central coast of Campeche, are attributed to *P. bahamense*, and were composed of PSTs (STX, NeoSTX, GTX2, GTX3, dcGTX2 and dcGTX3 [42] and TTX analogs. The presence of TTX and STX analogs was also confirmed by UHPLC-MS/MS, detecting TTX, 4-epiTTX, MonodeoxyTTX, 4,9-anhydroTTX and 11-norTTX-6(R)-ol as well as STX and dcSTX [54,55].

This was observed by Núñez-Vázquez et al. [42,54] indirectly in puffer fish, probably due to the toxins transmitted via bivalves and other invertebrates that regularly make up part of the diet of these fish in Campeche coastal waters [44]. Previous studies by [22,86] demonstrated the presence of high concentrations of PSTs in puffer fish from Florida, resulting from the *P. bahamense* HAB event.

PSP has been the main public health problem due to bivalves in Mexico [14,28,55]. The HABs of *P. bahamense* have caused 92.78% of PSP cases, with 476 human intoxications, all occurring along the southeastern Mexican Pacific coast [55].

Studies that confirm the presence of PSTs in natural samples are scarce, and few reports are available that have confirmed toxin-producing microalgae, either from environmental samples or laboratory cultures (Table 3) worldwide with this species. This study, in addition to generating data on the microalgal toxin profile, has allowed for comparison with those toxin profiles from other regions of the world and from other species of dinoflagellates (e.g., *Alexandrium* spp. and *Gymnodinium catenatum*) that produce PSTs. *Pyrodinium bahamense* is one of the harmful microalga that has caused the highest number of human

poisonings and deaths from PSP at global and regional levels (8). To the best of our knowledge, this is only the second time that the production of PSTs by *P. bahamense* has been confirmed in the Atlantic of the American continent, and worldwide, it is one of the few cases of toxins confirmation in this species. As such, this study contributes to the evaluation of possible risks to public and animal health in the area, as well as socioeconomic impacts related to fisheries and aquaculture industries. Additionally, the knowledge of the toxin profile of *P. bahamense* has allowed for confirmation of the possible etiology of these toxins that can be transferred to other trophic levels, including for example, puffer fish that are consumed and have caused human poisonings in this region.

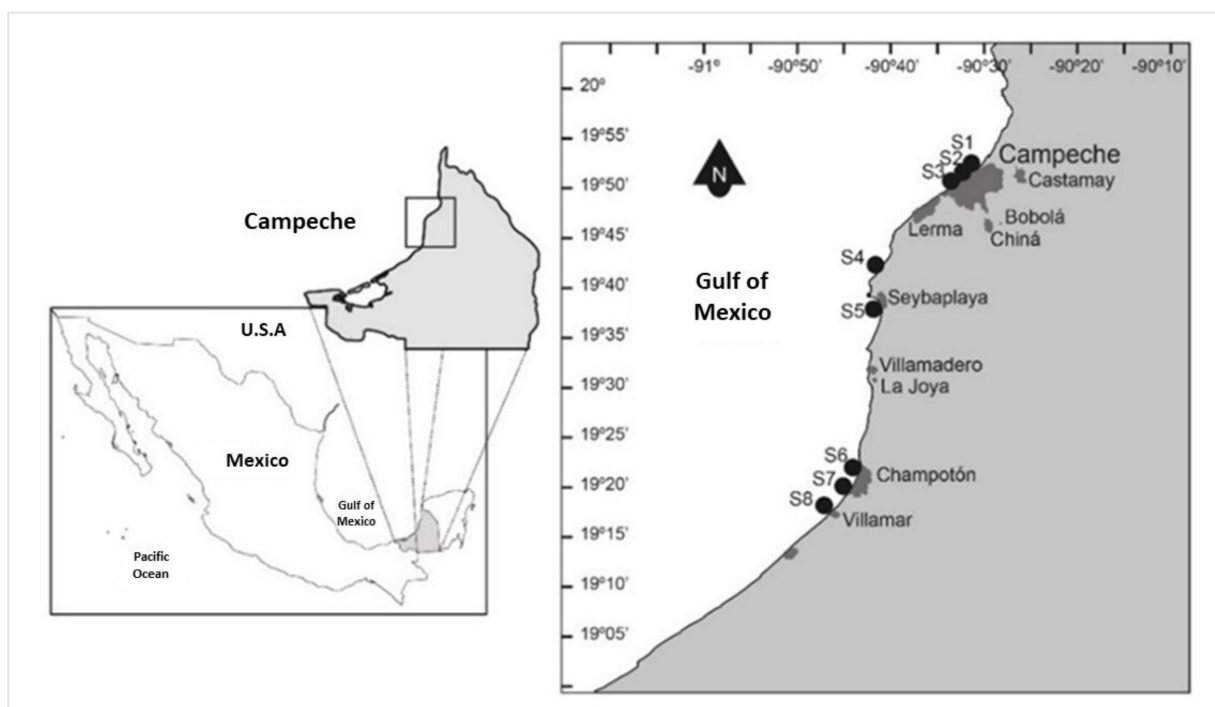
#### 4. Conclusions

The occurrence of the gonyaulacoid dinoflagellate *P. bahamense* in the southeastern Gulf of Mexico using light microscopy and SEM was related to elevated nutrient content at the sites of rainwater discharge or those near anthropogenic activities. STX and NeoSTX were detected by chromatographic techniques (IFL, HPLC-FLD and UHPLC-MS/MS). This study is the first report that confirms the presence of PST in *P. bahamense* in Mexican waters of the Gulf of Mexico.

#### 5. Materials and Methods

##### 5.1. Seawater Samples

In September and November 2016, seawater samples were taken at eight stations (site depth ca. 1 m) located along the central coast of the state of Campeche. All sampling sites were influenced by rainwater discharge or were near the sites with anthropogenic activities (Figure 6).



**Figure 6.** Location of study area and sampling sites (S1–S8) in the coastal waters of the state of Campeche.

##### 5.2. Phytoplankton Sampling and Nutrient Determination

At each site, surface seawater samples were collected with a 1 L plastic bottle; a 100 mL aliquot was used to determine cell abundances of phytoplankton taxa. Immediately after collection, samples were fixed with a neutral Lugol solution and subsequently preserved by

adding 37% neutralized formalin to a final concentration of 4% [87]. Cells were counted after sedimentation in a 10 mL cylinder following Reguera et al. [88]. Additionally, horizontal tows were taken with a 20 µm mesh conical hand net. The material collected for the *P. bahamense* analysis was placed in glass vials and fixed using the same procedure as for the cell counting. Net samples were also examined under a light microscope for accurate identification of *P. bahamense*. In situ water temperature (°C), salinity, pH and DO were measured using a Hanna multiparameter probe, model HI9828, equipped with a sensor, model HI769828, and a Hach multiparameter probe, model HQ40d (Hanna Instruments Inc., Woonsocket, RI, USA). Orthophosphate, ammonium, nitrite, nitrate and silicate analyses were performed in the laboratory following [89].

*Pyrodinium bahamense* cells were quantified according to the Utermöhl technique [90] using an inverted Zeigen microscope (Xiamen, China) equipped with phase-contrast objectives 10×/0.25 Ph1 ADL and LD 25×/0.30 Ph1. The cell abundance was expressed in cells L<sup>-1</sup>.

To make the thecal plate arrangement of *P. bahamense* visible, selected cells were stained with Calcofluor White M2R [91] by adding a drop of a 0.2% stain in aqueous solution to a water mount. Cells were observed under epifluorescence microscopy (Axio Scope.A1, Carl Zeiss, Oberkochen, Germany) with filter set 18 shift free EX BP 390–420 (excitation), BS FT 425 (optical divider) and EM LP 450 (emission), using the Plan-Neofluar 40×/0.75 and 63×/0.95 Korr objectives (total magnification 400× and 630×, respectively). Photomicrographs were taken with a Carl Zeiss AxioCam 506 color camera (6 MP) using the ZEN 2012 SP2 program (Carl Zeiss Microscopy GmbH, Göttingen, Germany).

### 5.3. Identification of *Pyrodinium bahamense*

Cell measurements ( $n = 50$ ) were made using the 40×/0.65 objective and an Olympus BX51 compound microscope. Four samples were examined in a JEOL JSM-7600F scanning electron microscope (SEM) at a working distance of 15 to 21 mm and a voltage of 1.2 to 5.0 kV after a preliminary wash in distilled water, followed by dehydration in a series of ethanol solutions of increasing concentration (30, 50, 70, 90 and 100%), air drying on 0.5'' aluminum mounts and sputter coating with gold–palladium using a Polaron SC7640 High Resolution Sputter Coater (Quorum Technologies, Newhaven, UK).

### 5.4. Toxicity

#### 5.4.1. Lateral Flow Immunochromatography Test (Rapid PSP Test)

Analysis for paralytic shellfish toxins (saxitoxin and analogs) were conducted by Lateral flow immunochromatography (LFIC) using rapid test kits provided by Scotia Rapid Testing (Scotia Rapid Testing Ltd., Nova Scotia, Canada), a qualitative lateral flow test for detection of PST in shellfish and phytoplankton for PSP (SRT PSP). The development and validation of the SRT PSP kit using the standard protocol is described by Scotia test [92].

#### 5.4.2. HPLC-FLD Analysis

Phytoplankton samples from station 7 were harvested by filtration through glass fibers (GF/F Whatman) and immediately frozen at −20 °C. These filters were used for toxin analysis. PSTs were analyzed by high-performance liquid chromatography and fluorescence detection (HPLC-FLD) [77] modified by [93]. PSTs were extracted by adding 2 mL of acetic acid (0.03 N) to each *P. bahamense* sample containing the filter and sonicated three times for 5 min each. The extract was centrifuged for 15 min at 14,000 rpm (HERMLE Z 216 microcentrifuge, Labortechnik GmbH, Wasserburg, Germany), and the supernatant was filtered through 0.45 µm filters using syringe filters of glass fiber (PVDF Millex membrane, 25 mm diameter). For the derivatization, 150 µL of each extract was mixed with 37 µL of hydrochloric acid 1 M and heated for 15 min at 90 °C. Cool to room temperature, after adding 75 µL of 1 N sodium acetate. The extracts were injected in the liquid chromatograph in separate runs for the identification and quantification of the PSTs (standards of saxitoxin

(STX), neosaxitoxin (NeoSTX), goniautoxin-1,4 (GTX 1,4), decarbamoylsaxitoxin (dcSTX), decarbamoylgoniautoxin-2,3 (dc GTX 2,3), and N-sulfocarbamoyl-11-hydrosulfate (C1,2).

The chromatography system used was an HP 1100 (Agilent Technologies, Santa Clara, CA, USA) consisting of an autosampler, degasser, quaternary pump, two binary pumps used for post-column reactions, a fluorescence detector, a C-18 column, and a post-column reactor. The PSTs were detected using an excitation wavelength of 333 nm and an emission wavelength of 390 nm. Two criteria were used for the identification of the PSTs: the retention times and co-elution with commercial standards of (STX), (NeoSTX) (National Research Council Canada, Institute for Marine Biosciences, Certified Reference Material Program, Halifax, NS, Canada).

#### 5.4.3. UPLC-MS/MS

A Waters (Manchester, UK) Acquity UPLC I-Class coupled to a Waters Xevo TQ-S tandem quadrupole mass spectrometer (MS/MS) was used for UHPLC-MS/MS analysis. A Waters 1.7  $\mu\text{m}$ ,  $2.1 \times 150$  mm Waters Acquity BEH Amide UPLC column held at  $+60$  °C was used for chromatographic separation of toxin analogs in conjunction with a Waters VanGuard BEH Amide guard cartridge. Cleaned-up sample extracts were injected onto the UHPLC with an injection volume of 2  $\mu\text{L}$ . Mobile phases and UHPLC gradient conditions were exactly as described by [52]. The MS/MS tune parameters were as follows: 150 °C source temperature, 600 °C desolvation temperature, 1000 L h<sup>-1</sup> desolvation gas flow, 7.0 Bar nebulizer gas flow, 150 L h<sup>-1</sup> cone gas flow and 0.15 mL min<sup>-1</sup> collision gas flow. Capillary voltage was held at 0.5 and 2.5 kV for positive and negative ionization modes, respectively. Multiple Reaction Monitoring (MRM) transitions were exactly those described by [52,94]. Quantitation of toxins was performed against toxin standards available as certified reference standards. Five additional analogs (C3, C4, dcGTX1, dcGTX4 and GTX6) were incorporated into the method, with quantitation performed using the calibrations generated from their nearest structural analog, using experimentally determined relative response factors [51]). Toxicity equivalence factors (TEFs) were taken from EFSA recommendations where feasible [94], with others used as described by [94]. The method has been validated in shellfish internationally through collaborative study with well defined method performance characteristics [53]. Assessment of the method when applied to water or algae samples has previously demonstrated the absence of any significant matrix effects.

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## References

1. Plate, L. *Pyrodinium bahamense* n. g., n. sp. die Leucht-Peridninee des “Feusersee” von Nassau, Bahamas. *Arch. Protistenk.* **1906**, *7*, 411–429.
2. Maclean, J.L. Red tide and paralytic shellfish poisoning in Papua New Guinea. *Papua New Guinea Agric. J.* **1973**, *24*, 131–138.
3. Worth, G.K.; Maclean, J.L.; Price, M.J. Paralytic shellfish poisoning in Papua New Guinea, 1972. *Pac. Sci.* **1975**, *29*, 1–5.
4. Hallegraeff, G.; Maclean, J.L. (Eds.) Biology, epidemiology and management of *Pyrodinium* red tides. In Proceedings of the Management and Training Workshop, ICLARM Conference Proceedings 21, Bandar Seri Begawan, Brunei, 23–30 May 1989; p. 286.
5. FAO. Marine Biotoxins. In *FAO Food and Nutrition Paper*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2004; p. 80.
6. Deeds, J.R.; Landsberg, J.H.; Etheridge, S.M.; Pitcher, G.C.; Longan, S.W. Non-traditional vectors for paralytic shellfish poisoning. *Mar. Drugs* **2008**, *6*, 308–348. [[CrossRef](#)]
7. Usup, G.A.; Ahmad, A.A.; Matsuoka, B.K.; Lim, P.T.; Leaw, C.P. Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*. *Harmful Algae* **2012**, *14*, 301–312. [[CrossRef](#)]
8. Band-Schmidt, C.J.; Durán-Riveroll, L.M.; Bustillos-Guzmán, J.J.; Leyva-Valencia, I.; López-Cortés, D.J.; Núñez-Vázquez, E.J.; Hernández-Sandoval, F.E.; Ramírez-Rodríguez, D.V. Paralytic toxin producing dinoflagellates in Latin America: Ecology and physiology. *Front. Mar. Sci.* **2019**, *6*, 42. [[CrossRef](#)]
9. Azanza, R.V.; Taylor, F.J.R. Are *Pyrodinium* blooms in the Southeast Asian region recurring and spreading? A view at the end of the millennium. *AMBIO* **2001**, *30*, 356–364. [[CrossRef](#)]
10. Llewellyn, L.; Negri, A.; Robertson, A. Paralytic shellfish toxins in tropical oceans. *Toxin Rev.* **2006**, *25*, 159–196. [[CrossRef](#)]
11. Mertens, K.N.; Wolny, J.; Carbonell-Moore, C.; Bogus, K.; Ellegaard, M.; Limoges, A.; De Vernal, A.; Gurdebeke, P.; Omura, T.; Al-Muftah, A.; et al. Taxonomic re-examination of the toxic armored dinoflagellate *Pyrodinium bahamense* Plate 1906: Can morphology or LSU sequencing separate *P. bahamense* var. *compressum* from var. *bahamense*? *Harmful Algae* **2015**, *41*, 1–24. [[CrossRef](#)]
12. Orellana-Cepeda, E.; Martínez-Romero, E.; Muñoz-Cabrera, L.; López-Ramírez, P.; Cabrera-Mancilla, E.; Ramírez-Camarena, C. Toxicity associated with blooms of *Pyrodinium bahamense* var. *compressum* in Southwestern Mexico. In *Harmful Algae, Proceedings of the 8th International Conference on Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo, Spain, 25–29 June 1997*; Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T., Eds.; Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO: Vigo, Spain, 1998; p. 60.
13. Ramírez-Camarena, C.; Rojas-Crisóstomo, R.; Muñoz-Cabrera, L.; Sarniento-Nafate, S.; Juárez-Ruiz, N.O. Mortandad de peces e intoxicaciones humanas en la costa de Chiapas en el 2001. In *IX Congreso Nacional de Ciencia y Tecnología del Mar*; Nuevo Vallarta: Nayarit, México, 2002; pp. 1–2.
14. Núñez-Vázquez, E.J.; Bustillos-Guzmán, J.J.; Ramírez-Camarena, C.; Hernández-Sandoval, F. Perfiles cromatográficos de toxinas paralizantes en moluscos bivalvos asociados a *Pyrodinium bahamense* var. *compressum* en el Pacífico Sur Mexicano. In Proceedings of the II Taller sobre Florecimientos Algales Nocivos (CICESE-CETMAR), Ensenada, Mexico, 21–23 November 2007; p. 18.
15. Licea, S.; Navarrete, A.; Rodríguez, R.; Bustillos-Guzmán, J.; Martínez, B.; Ramírez, C. Monitoring a bloom of *Pyrodinium bahamense* var. *compressum* in El Salvador and the southern coast of Mexico (November 2005–March 2006). In Proceedings of the 12th International Conference on Harmful Algae, Copenhagen, Denmark, 4–8 September 2006; pp. 219–220.
16. Licea, S.; Navarrete, A.; Castañeda, V.; Bustillos-Guzmán, J.J. Monitoring program for harmful algal blooms in Salvadoran waters: Report of *Pyrodinium bahamense* from November 2009 to June 2010. In *Proceedings of the 14th International Conference on Harmful Algae, International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO 2013*; Pagou, K.A., Hallegraeff, G.M., Eds.; IOC-UNESCO: Hersonissos, Greece, 2012.
17. Amaya, O.; Ruiz, G.; Espinoza, J.; Rivera, W. Saxitoxin analyses with a receptor binding assay (RBA) Suggest PSP Intoxication of sea turtles in El Salvador. *Harmful Algal News* **2014**, *48*, 6–7.
18. Amaya, O.; Dechraoui-Bottein, M.Y.; Leighfield, T.; Ruiz, G. Five years of application of the Receptor Binding Assay (RBA) on seafood products and threatened turtles during outbreaks HABs in El Salvador. In *Marine and Fresh-Water Harmful Algae, Proceedings of the 17th International Conference on Harmful Algae, Florianópolis, Brazil, 9–14 October 2016*; Proença, L.A.O., Hallegraeff, G.M., Eds.; ISSHA, IOC-UNESCO: Florianópolis, Brasil, 2017; pp. 30–132.
19. Amaya, O.; Quintanilla, R.; Stacy, B.A.; Dechraoui-Bottein, M.Y.; Flewelling, L.; Hardy, R.; Dueñas, C.; Ruiz, G. Large-scale sea turtle mortality events in El Salvador attributed to paralytic shellfish toxin-producing algae blooms. *Front. Mar. Sci.* **2018**, *5*, 411. [[CrossRef](#)]

20. Band-Schmidt, C.J.; Bustillos-Guzmán, J.J.; López-Cortés, D.J.; Núñez-Vázquez, E.J.; Hernández-Sandoval, F.E. El estado actual del estudio de florecimientos algales nocivos en México. *Hidrobiológica* **2011**, *21*, 381–412.
21. Emslie, S.D.; Allmon, W.D.; Rich, F.J.; Wrenn, J.H.; Defrance, S.D. Integrated taphonomy of an avian death assemblage in marine sediments from the late Pliocene of Florida. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **1996**, *124*, 107–136. [[CrossRef](#)]
22. Landsberg, J.H.; Hall, S.; Jahannessen, J.N.; White, K.D.; Conrad, S.M.; Abbott, J.P.; Flewellyn, L.J.; Richardson, R.W.; Dickey, R.W.; Jester, E.L.E.; et al. Saxitoxin puffer fish poisoning in the United States, with the first report of *Pyrodinium bahamense* as the putative toxin source. *Environ. Health Perspect.* **2006**, *114*, 1502–1507. [[CrossRef](#)]
23. García-Mendoza, E.; Quijano-Scheggia, S.I.; Olivos-Ortiz, A.; Núñez-Vázquez, E.J.; Pérez-Morales, A. Introducción general. In *Florecimientos Algales Nocivos en México*; García-Mendoza, E., Quijano-Scheggia, S.I., Olivos-Ortiz, A., Núñez-Vázquez, E.J., Eds.; CICESE: Ensenada, México, 2016; pp. 10–20.
24. Ochoa, J.L.; Sierra-Beltrán, A.; Alonso-Colmenares, G.; Barrada-Sánchez, H.; Cruz-Villacorta, A.; Núñez-Vázquez, E.J. “Biotoxins in the Pacific Coast of Mexico” in *Mycotoxins and Phycotoxins Developments in Chemistry, Toxicology and Food Safety, International Union Purity Analytical Chemistry (IUPAC)*. In *IX IUPAC International Symposium on Mycotoxins and Phycotoxins*; Miraglia, M., Van Egmond, H., Brera, C., Gilbert, J., Eds.; Alaken, Inc.: Fort Collins, CO, USA, 1998; pp. 441–448.
25. Sierra-Beltrán, A.; Cruz, Á.; Núñez-Vázquez, E.; Del Villar, L.M.; Cerecero, J.; Ochoa, J.L. An overview of the marine food poisoning in Mexico. *Toxicon* **1998**, *36*, 1493–1502. [[CrossRef](#)]
26. Núñez-Vázquez, E.J.; Cordero-Tapia, A.; Arnaud, G. Origen e impacto de las Biotoxinas Marinas en la Salud de Tortugas Marinas Salud Pública. In *Proceedings of the 1er Encuentro Internacional de Medicina de la Conservación*, Vitoria, Spain, 3–7 August 2007.
27. Núñez-Vázquez, E.J.; Almazán-Becerril, A.; López-Cortés, D.J.; Heredia-Tapia, A.; Hernández-Sandoval, F.E.; Band-Schmidt, C.J.; Bustillos-Guzmán, J.J.; Gárate-Lizárraga, I.; García-Mendoza, E.; Salinas-Zavala, C.A.; et al. Ciguatera in Mexico (1984–2013). *Mar. Drugs* **2019**, *17*, 13. [[CrossRef](#)]
28. Morquecho, L. *Pyrodinium bahamense* One the Most Significant Harmful Dinoflagellate in Mexico. *Front. Mar. Sci.* **2019**, *6*, 1. [[CrossRef](#)]
29. Alonso-Rodríguez, R.; Mendoza-Amézquita, E.; Velásquez-López, S.A.; Seim, J.A.; Martínez-Rodríguez, V.M. Florecimientos algales nocivos producidos por *Pyrodinium bahamense* en Oaxaca, México (2009–2010). *Salud Pública México* **2015**, *57*, 343–351. [[CrossRef](#)]
30. Mueren dos Menores Tras Consumir Moluscos Contaminados en Chiapas. Available online: <https://www.youtube.com/watch?v=F359KQrArSs> (accessed on 29 October 2022).
31. Salud Establece Veda Sanitaria por Presencia de Marea Roja en Puerto Madero: Dr. Pepe Cruz. Available online: <https://saludchiapas.gob.mx/noticias/post/salud-establece-veda-sanitaria-por-presencia-de-marea-roja-en-puerto-madero-dr-pepe-cruz> (accessed on 29 October 2022).
32. Gárate-Lizárraga, I.; Pérez-Cruz, B.; Díaz-Ortíz, J.A.; López-Silva, S.; González-Armas, R. Distribución del dinoflagelado *Pyrodinium bahamense* en la costa pacífica de México. *Rev. Latinoam. Ambiente Las Cienc.* **2015**, *6*, 2666–2669.
33. Balech, E. A revisión de *Pyrodinium bahamense* Plate (Dinoflagellata). *Rev. Palaeobot. Palynol.* **1985**, *45*, 17–34. [[CrossRef](#)]
34. Vargas-Montero, M.; Freer, E. Co-occurrence of different morphotypes of *Pyrodinium bahamense* during an extensive bloom in Gulf of Nicoya, Costa Rica. In *Molluscan Shellfish Safety*; Villalba, A., Reguera, B., Romalde, J.L., Beiras, R., Eds.; Xunta de Galicia and IOC-UNESCO: Vigo, Spain, 2003; pp. 211–217.
35. Gárate-Lizárraga, I.; González-Armas, R. Occurrence of *Pyrodinium bahamense* var. *compressum* along the southern coast of the Baja California Peninsula. *Mar. Pollut. Bull.* **2011**, *62*, 626–630. [[CrossRef](#)] [[PubMed](#)]
36. Del Merino-Virgilio, F.C.; Okolodkov, Y.B.; Aguilar-Trujillo, A.C.; Osorio-Moreno, I.; Herrera-Silveira, J.A. Florecimientos algales nocivos en las aguas costeras del norte de Yucatán (2001–2013). In *Golfo de México. Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*; Botello, A.V., Rendón von Osten, J., Benítez, J.A., Gold-Bouchot, G., Eds.; UAC, UNAM-ICMyL, CINVESTAV-Unidad Mérida: Mérida, Mexico, 2014; pp. 161–180.
37. Steidinger, K.A.; Tester, L.S.; Taylor, F.J.R. A redescription of *Pyrodinium bahamense* var. *compressa* (Bohm) stat. nov. from Pacific red tides. *Phycologia* **1980**, *19*, 329–337. [[CrossRef](#)]
38. Gómez-Aguirre, S. First record of *Pyrodinium bahamense* (Dinoflagellata) in brackish waters of the Mexican Caribbean Coast. *Annal. Inst. Biol. UNAM Ser. Zool.* **1998**, *69*, 121–123.
39. Gómez-Aguirre, S.; Licea, S. Blooms of *Pyrodinium bahamense* (Dinophyceae) in coastal lagoons of the southern Gulf of Mexico and Mexican Caribbean. In *Harmfull Algae*; Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T., Eds.; Xunta Galicia and IOC-UNESCO: Vigo, Spain, 1998; pp. 61–62.
40. Poot-Delgado, C.A.; Okolodkov, Y.B.; Aké-Castillo, J.A.; Rendón-von Osten, J. Annual cycle of phytoplankton with emphasis on potentially harmful species in oyster beds of Terminos Lagoon, southeastern Gulf of Mexico. *Rev. Biol. Mar. Oceanogr.* **2015**, *50*, 465–477. [[CrossRef](#)]
41. Poot-Delgado, C.A. Florecimientos algales nocivos en la costa de Campeche, Golfo de México. *Investig. Cienc. Univ. Autónoma Aguascalientes* **2016**, *68*, 91–96. [[CrossRef](#)]
42. Núñez-Vázquez, E.J.; Poot-Delgado, C.A.; Dominguez-Solis, G.; Hernández-Sandoval, F.E.; Bustillos-Guzmán, J.J. Toxicidad de los botetes silvestres *Sphoeroides* spp. y *Lagocephalus* spp. de las costas de Campeche, México. In *Proceedings of the Memorias del XX Congreso Nacional de Ciencia y Tecnología del Mar*, Cabo San Lucas, Mexico, 1–4 October 2013; pp. 1–5.

43. Núñez-Vázquez, E.J.; Ramírez-Camarena, C.; Poot-Delgado, C.A.; Almazán-Becerril, A.; Aké-Castillo, J.A.; Pérez-Morales, A.; Hernández-Sandoval, F.E.; Fernández-Herrera, L.J.; Heredia-Tapia, A.; Band-Schmidt, C.J.; et al. Toxinas marinas en el Golfo de México: Orígenes e impactos. In *Florecimientos Algales Nocivos en México*; García Mendoza, E., Quijano Scheggia, S.I., Olivos-Ortiz, A., Núñez-Vázquez, E.J., Eds.; CICESE: Ensenada, Mexico, 2016; pp. 308–321.
44. Colmenero, L.M.; Yáñez-Arancibia, A.; Amezcua-Linares, F. Taxonomía, biología y ecología de los tetraodontidos de la Laguna de Términos, Sur del Golfo de México (Pisces: Tetraodontidae). *Ann. Inst. Cien. Mar Limnol.* **1982**, *9*, 161–212.
45. Anon. Official method 959.08. Paralytic shellfish poison. Biological method. Final action. In *AOAC Official Methods for Analysis*, 18th ed.; Natural Toxins; Truckses, M.W., Ed.; AOAC International: Gaithersburg, MD, USA, 2005; Chapter 49; pp. 79–80.
46. Anon. *Official Method 2005.06 Quantitative Determination of Paralytic Shellfish Poisoning Toxins in Shellfish Using Pre-Chromatographic Oxidation and Liquid Chromatography with Fluorescence Detection*; AOAC International: Gaithersburg, MD, USA, 2005.
47. Anon. Official method 2011.02 determination of paralytic shellfish poisoning toxins in mussels, clams, oysters and scallops. In *Post-Column Oxidation Method (PCOX). First Action 2011*; AOAC International: Gaithersburg, MD, USA, 2011.
48. Anon. *Official Method 2005.06. Report on the Study on the Determination of PSP Toxins in Shellfish Including GTX6 After Hydrolysis Report from the Community Reference Laboratory for Marine Biotoxins*; Ministerio de Sanidad y Consumo: Vigo, Spain, 2007.
49. Turner, A.D.; Hatfield, R.G.; Maskrey, B.H.; Algoet, M.; Lawrence, J.F. Evaluation of the new European Union reference method for paralytic shellfish toxins in shellfish: A review of twelve years regulatory monitoring using pre-column oxidation LC-FLD. *Trends Anal. Chem.* **2019**, *113*, 124–139. [[CrossRef](#)]
50. Anon. *Official Method 2011.27. Paralytic Shellfish Toxins (PSTs) in Shellfish, Receptor Binding Assay*; AOAC International: Gaithersburg, MD, USA, 2011.
51. Boundy, M.J.; Selwood, A.I.; Harwood, D.T.; McNabb, P.S.; Turner, A.D. Development of a sensitive and selective liquid chromatography–mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction. *J. Chromatogr. A* **2015**, *1387*, 1–12. [[CrossRef](#)]
52. Turner, A.D.; McNabb, P.S.; Harwood, D.T.; Selwood, A.I.; Boundy, M.J. Single laboratory validation of a multitoxin LC-hydrophilic interaction LC-MS/MS method for quantitation of Paralytic Shellfish Toxins in bivalve shellfish. *J. AOAC Int.* **2015**, *98*, 609–621. [[CrossRef](#)]
53. Turner, A.D.; Tarnovius, S.; Hatfield, R.G.; Teixeira Alves, M.; Broadwater, M.; Van Dolah, F.; Garcia-Mendoza, E.; Medina, D.; Salhi, M.; Goya, A.B.; et al. Application of Six Detection Methods for Analysis of Paralytic Shellfish Toxins in Shellfish from Four Regions within Latin America. *Mar. Drugs* **2020**, *18*, 616. [[CrossRef](#)]
54. Núñez-Vázquez, E.J.; Poot-Delgado, C.A.; Turner, A.; Ley-Martínez, T.; Domínguez-Solís, G.; Cahuich-Sánchez, Y.; Hernández-Sandoval, F.E.; Bustillos-Guzmán, J.J.; Balart, F.E. Presencia de toxinas marinas en peces comerciales de las costas de Campeche. In *Memorias IX Foro Científico de Pesca Ribereña*; Espino-Barr, E., Gaspar-Dillanes, T., López-González, L., Zúñiga-Flores, M.S., Martínez-Becerril, V.H., Eds.; Instituto Nacional de Pesca y Acuicultura Núm. IX.: Mexico City, Mexico, 2018; pp. 27–28.
55. Núñez-Vázquez, E.J.; Turner, A.D.; Ramírez-Camarena, C.; Hernández-Sandoval, F.E.; Bustillos-Guzmán, J.J.; Poot-Delgado, C.A. Profile of paralytic shellfish toxins of *Pyrodinium bahamense* and first detection of tetrodotoxin in Mexican bivalve mollusks. In *Proceedings of the 12th International Conference on Molluscan Shellfish Safety (ICMSS)*, Ensenada, BC, Mexico, 9–13 September 2019; p. 49.
56. CNA Ley Federal de Derechos. *Disposiciones Aplicables en Materia de Aguas Nacionales 2016*; Secretaría de Medio Ambiente y Recursos Naturales: Mexico City, Mexico, 2016; p. 173.
57. Osorio-Tafall, B.F. Notas sobre algunos dinoflagelados planctónicos marinos de México con descripción de nuevas especies. *Ann. Esc. Nac. Cienc. Biol.* **1942**, *2*, 435–450.
58. Faust, M.A.; Litaker, W.; Vandersea, M.W.; Kibler, S.R.; Tester, P.A. Dinoflagellate diversity and abundance in two Belizean coral-reef mangrove lagoons: A test of Margalef’s mandala. *Atoll Res. Bull.* **2005**, *534*, 104–131. [[CrossRef](#)]
59. Morquecho-Escamilla, L. Morphology of *Pyrodinium bahamense* Plate (Dinoflagellata) near Isla San José, Gulf of California, Mexico. *Harmful Algae* **2008**, *7*, 664–670. [[CrossRef](#)]
60. Poot-Delgado, C.A.; Novelo-Salazar, R.A.; Pérez-Cruz, B. Primer reporte de *Pyrodinium bahamense* var. *bahamense* (Gonyaulacales: GoniDOMATAceae), dinoflagelado tóxico, en la Bahía de Campeche, México. In *Proceedings of the Resúmenes del XVI Congreso Nacional de Ciencia y Tecnología del Mar*, Colima, Mexico, 5–7 October 2009; p. 2.
61. Merino-Virgilio, F.D.C.; Aguilar-Trujillo, A.C.; Osorio-Moreno, I.; Okolodkov, Y.B.; Herrera-Silveira, J.A.; Espinosa-Matías, S. *Pyrodinium bahamense* var. *bahamense* en el norte de la península de Yucatán. In *Proceedings of the Resúmenes del 1er Congreso Nacional de la Sociedad Mexicana para el Estudio de Florecimientos Algales Nocivos*, Mazatlán, Sinaloa, 16–18 November 2011; p. 50.
62. Poot-Delgado, C.A.; Okolodkov, Y.B.; Rendón-von Osten, J. Spatio-temporal variation of harmful planktonic microalgae and cyanobacteria along the central coast of Campeche, southeastern Gulf of Mexico. *Bull. Environ. Contam. Toxicol.* **2021**, *108*, 15–23. [[CrossRef](#)]
63. Licea-Duran, S.; Luna-Soria, R. Spatio-temporal variation of phytoplankton on the continental margin in the SW Gulf of Mexico. *Rev. Soc. Mex. Hist. Nat.* **1999**, *49*, 83–99.
64. Gío-Argaez, F.; Machain-Castillo, M.L.; Gaytan-Caballero, A. Los ostrácodos de la zona económica exclusiva de México. Parte I. La bahía de Campeche. *Jaina* **2002**, *13*, 1–11.

65. Poot-Delgado, C.A.; Rosado-García, P.I.; Guzmán-Noz, Y.A. Fitoplancton marino potencialmente nocivo en las aguas costeras de Campeche. In *Golfo de México. Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*; Botello, A.V., Rendón vonOsten, J., Benítez, J.A., Gold-Bouchot, V., Eds.; UAC, UNAM-ICMyL, CINVESTAV-Unidad Mérida: Mérida, Mexico, 2014; pp. 117–132.
66. Gracia, A.; Vázquez, G.F.; Enciso-Sánchez, G.; Alexander-Valdés, H.M. Composición y volumen de contaminantes de las descargas costeras al Golfo de México. In *Golfo de México. Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*; Botello, A.V., Rendón vonOsten, J., Benítez, J.A., Gold-Bouchot, V., Eds.; UAC, UNAM-ICMyL, CINVESTAV-Unidad Mérida: Mérida, Mexico, 2014; pp. 787–816.
67. Gómez-Figueroa, J.A. Fitoplancton como Bioindicador de la Calidad de Agua: Evaluación de Ecosistemas Acuáticos del Estado de Campeche. Master's Thesis, Universidad Autónoma de Campeche, Campeche, México, 2020; p. 115. (In Spanish).
68. Poot-Delgado, C.A.; Okolodkov, Y. Bloom of *Cylindrotheca closterium* originating from shrimp farming discharges in the SE Gulf of Mexico. *Harmful Algae News* **2020**, *66*, 10.
69. Philips, E.J.; Badylak, S.; Bledsoe, E.; Cichra, M. Factors affecting the distribution of *Pyrodinium bahamense* var. *bahamense* in coastal waters of Florida. *Mar. Ecol. Prog. Ser.* **2006**, *322*, 99–115. [[CrossRef](#)]
70. Okolodkov, Y.B.; Gárate-Lizárraga, I. An annotated checklist of dinoflagellates (Dinophyceae) from the Mexican Pacific. *Acta Bot. Mex.* **2006**, *72*, 1–154. [[CrossRef](#)]
71. Morquecho-Escamilla, L.; Alonso-Rodríguez, R.; Arreola-Lizarraga, J.A.; Reyes-Salinas, A. Factors associated with moderate blooms of *Pyrodinium bahamense* in shallow and restricted subtropical lagoons in the Gulf of California. *Bot. Mar.* **2012**, *66*, 611–623. [[CrossRef](#)]
72. Morquecho-Escamilla, L.; Alonso-Rodríguez, R.; Martínez-Tecuapacho, G.A. Cyst morphology, germination characteristics, and potential toxicity of *Pyrodinium bahamense* in the Gulf of California. *Bot. Mar.* **2014**, *54*, 303–314. [[CrossRef](#)]
73. Garate-Lizárraga, I.; Pérez-Cruz, B.; Díaz-Ortíz, J.A.; Alarcón-Tacuba, M.A.; Alarcón-Romero, M.A.; Chávez-Almazán, L.A.; García-Barbosa, J.L.; Diego-Balderrama, E. Blooms of *Pyrodinium bahamense* var. *compressum* and toxicity in rock oyster at Costa Chica, Guerrero, Mexico. *Oceanides* **2013**, *28*, 37–42. [[CrossRef](#)]
74. Garate-Lizárraga, I.; Pérez-Cruz, B.; Díaz-Ortíz, J.A.; Alarcón-Tacuba, M.A.; Chávez-Almazán, M.A.; Alarcón-Romero, M.A.; López-Silva, S.; Bustillos-Guzmán, J.; Licea-Durán, S. Toxicity and paralytic toxin profile in *Pyrodinium bahamense* var. *compressum* and violet oyster in Bahía de Acapulco, Guerrero, Mexico. *Harmful Algae News* **2012**, *45*, 2–3.
75. Cusick, K.; Duran, G. sxtA4+ and sxtA4- Genotypes Occur Together within Natural *Pyrodinium bahamense* Sub-Populations from the Western Atlantic. *Microorganisms* **2021**, *9*, 1128. [[CrossRef](#)]
76. Usup, G.; Kulis, D.M.; Anderson, D. Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* in laboratory cultures. *Nat. Toxins* **1994**, *2*, 254–264. [[CrossRef](#)]
77. Hummer, C.; Ritscher, M.; Reinhardt, K.; Lucas, B. Analysis of the characteristic PSP profiles of *Pyrodinium bahamense* and several strains of *Alexandrium* by HPLC based on ion-pair chromatographic separation, post-column oxidation, and fluorescence detection. *Chromatographia* **1997**, *45*, 312–316. [[CrossRef](#)]
78. Gedaria, A.I.; Luckas, B.; Reinhardt, K.; Azanza, R.V. Growth response and toxin concentration of cultured *Pyrodinium bahamense* var. *compressum* to varying salinity and temperature conditions. *Toxicon* **2007**, *50*, 519–529. [[CrossRef](#)]
79. Rosales-Loessener, F. The Guatemalan experiences with red tides and paralytic shellfish poisoning. In *Biology, Epidemiology, and Management of Pyrodinium Red Tides, Proceedings of the Management and Training Workshop, Bandar Seri Begawan, Brunei Darussalam, 23–30 May 1989*; Hallegraef, G.M., Maclean, J.L., Eds.; WorldFish: Manila, Philippines, 1989; pp. 49–51.
80. Harada, T.; Oshima, Y.; Kamiya, H.; Yasumoto, T. Confirmation of Paralytic Shellfish Toxins in the Dinoflagellate *Pyrodinium bahamense* var. *compressum* and bivalves in Palau. *Bull. Japan. Soc. Sci. Fish.* **1982**, *48*, 821–825. [[CrossRef](#)]
81. Rosales-Loessner, F.; De Porras, E.; Y Dix, M.W. Toxic shellfish poisoning in Guatemala. In *Red Tides: Biology, Environmental Science, and Toxicology*; Okaichi, T., Anderson, D.M., Nemoto, T., Eds.; Elsevier: New York, NY, USA, 1989; pp. 113–116.
82. Usup, G.; Cheah, M.Y.; Ng, B.K.; Leaw, C.P.; Ahmad, A. Toxin profile and relative toxicity of three paralytic shellfish poisoning toxin-producing dinoflagellates from Malaysia. *Malays. Appl. Biol.* **2006**, *35*, 41–45.
83. Montojo, U.M.; Sakamoto, S.; Cayme, M.F.; Gatdula, N.C.; Furio, E.F.; Relox, J.R., Jr.; Sato, S.; Fukuyo, Y.; Kodama, M. Remarkable difference in toxin accumulation of paralytic shellfish poisoning toxins among bivalve species exposed to *Pyrodinium bahamense* var. *compressum* bloom in Masinloc bay, Philippines. *Toxicon* **2006**, *48*, 85–92. [[CrossRef](#)]
84. Banguera-Hinestroza, E.; Eikrem, W.; Mansour, H.; Solberg, I.; Cúrdia, J.; Holtermann, K.; Edvardsen, B.; Kaartvedt, S. Seasonality and toxin production of *Pyrodinium bahamense* in a Red Sea lagoon. *Harmful Algae* **2016**, *55*, 163–171. [[CrossRef](#)] [[PubMed](#)]
85. Poot-Delgado, C.A.; Núñez-Vázquez, E.J.; Ruiz-Ibáñez, J.A. Intoxicaciones humanas por consumo de peces botete (Tetraodontidae) en Campeche, México. In *Proceedings of the Memorias del XVIII Congreso Nacional de Ciencia y Tecnología del Mar, San Carlos, Mexico, 7–9 September 2011*.
86. Abbott, J.P.; Flewelling, L.J.; Landsberg, J.H. Saxitoxin monitoring in three species of Florida puffer fish. *Harmful Algae* **2009**, *8*, 343–348. [[CrossRef](#)]
87. Andersen, P.; Throndsen, J. Estimating cell numbers. In *Manual on Harmful Marine Microalgae*; Monographs on Oceanographic Methodology no. 11; Hallegraef, G.M., Anderson, D.M., Cembella, A.D., Eds.; UNESCO Publishing: Place de Fontenoy, Paris, 2004; pp. 99–130.



88. Reguera, B.; Méndez, S.; Alonso, R. Quantitative analysis of microalgae: General considerations. In *Guide for Designing and Implementing a Plan to Monitor Toxin-Producing Microalgae*, 2nd ed.; Reguera, B., Alonso, R., Moreira, A., Méndez, S., Dechraoui-Bottein, M.Y., Eds.; UNESCO: Place de Fontenoy, Paris, 2016; p. 66.
89. UNEP. *Standard Chemical Methods for Marine Environmental Monitoring. Reference Methods for Marine Pollution Studies 50*; United Nations Environment Programme: Nairobi, Kenya, 1991; p. 52.
90. Utermöhl, H. Zurvervollkommung der quantitative. Phytoplankton-Methodik. *Verh. Internat. Verein. Theor. Angew. Limnol.* **1958**, *9*, 1–38.
91. Fritz, L.; Triemer, R.E. A rapid simple technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. *J. Phycol.* **1985**, *21*, 662–664. [[CrossRef](#)]
92. Laycock, M.V.; Donovan, M.A.; Easy, D.J. Sensitivity of lateral flow tests to mixtures of saxitoxins and applications to shellfish and phytoplankton monitoring. *Toxicon* **2010**, *55*, 597–605. [[CrossRef](#)]
93. Yu, R.C.; Hummert, C.; Luckas, B.; Qian, P.Y.; Zhou, M.J. Modified HPLC method for analysis of PSP toxins in algae and shellfish from China. *Chromatographia* **1998**, *48*, 671–676. [[CrossRef](#)]
94. European Food Safety Authority. Marine biotoxins in shellfish—Saxitoxin group. *EFSA J.* **2009**, *1019*, 1–76.