Microalgal blooms: A global issue with negative impact in Chile*

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Ecological and health problems posed by microalgal blooms (red tides) occurring in the Southern part of Chile are reviewed. Out of the six human illnesses provoked by microalgal toxins, paralytic shellfish poisoning is the most important, because of its high mortality rate and the high levels of phycotoxins found in contaminated molluscs. Saxitoxin and its analogues bind to a receptor in the voltage-gated sodium channel of neural membranes. The most important toxin-producer microalgae are ***Alexandrium catenella*** *and* ***Dinophysis acuta****. Phycotoxins become concentrated by filter-feeding shellfish, like* ***Mytilus chilensis****. Highly sensitive methods available for detection of microalgal toxins are analyzed.*

Key-terms*: **Alexandrium catenella**, microalgal blooms, paralytic shellfish poisoning, phycotoxins, red tides, saxitoxin, toxins detection*

INTRODUCTION

The ever-increasing number of toxic microalgae species coupled with high incidence of outbreaks of these species presents a constant threat to public health worldwide. Blooms of toxic or harmful microalgae, commonly called "red tides", represent a significant and expanding threat to human health and fisheries resources throughout the world (White, 1988; Smayda, 1992; Hallegraeff, 1993). The impact of these phenomena ranges from illness and death of human consumers of shellfish or fish that have accumulated algal toxins to ecosystem alteration and massive mortality of fish, seabirds and marine mammals (Geraci *et al*, 1989; Anderson, 1989; Anderson & White, 1989; Smayda, 1992; Hallegraeff, 1993).

Microalgae play an important role in the marine biological system. With their photosynthetic ability, they are the major producers of biomass and organic compounds in the ocean. In most cases, the proliferation of plankton algae, up to a million of cells per liter, is therefore beneficial for aquaculture and wild fisheries. Nevertheless, in some circumstances, algal blooms produce negative effects, causing severe economic losses to aquaculture, fisheries and tourism, with major environmental and health problems.

Among the 5,000 to 10,000 species of microalgae described in the literature (Sournia *et al*, 1991; Shimizu, 1993), about 320 species had developed in an intensity enough to change the color of sea waters (red tides). Furthermore, around 45 species, including cyanophytes in inland waters, have produced harmful algal blooms due to their capacity to produce potent toxins (phycotoxins), that through shellfish, fish and just freshwater can reach human beings

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who thus become intoxicated (Smayda, 1992; Hallegraeff, 1993; Hawkins *et al*, 1985; Carmichael & Falconer, 1993; Falconer, 1996).

It is not clear why these microorganisms produce phycotoxins. They are secondary metabolites, known as natural products with no explicit role in the internal economy of the organism that produce them, but with very specific and potent activities on fish, birds and mammals. If their producers (diatoms and dinoflagellates) use them as a way to compete for space, fight predation, or defend themselves from the overgrowth of other organisms, are still open questions. On the other hand, this is a very old phenomenon with which mankind has been dealing with ever since. The first known written mention of a harmful algal bloom is described in the Old Testament, in the Bible: "... all the water that were in the river were turned to blood. And the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river" (Exodus 7: 20-21).

SYNDROMES PROVOKED BY MICROALGAL POISONING

Until now, six human illnesses associated with microalgae toxins have been described (Hallegraeff, 1993; Yasumoto & Murata, 1993; Yasumoto *et al*, 1995; Falconer, 1996) (Table I):

- Paralytic Shellfish Poisoning (PSP)
- Diarrheic Shellfish Poisoning (DSP)
- Amnesic Shellfish Poisoning (ASP)
- Neurotoxic Shellfish Poisoning (NSP)
- Ciguatera Poisoning (CP)
- Cyanobacterial Poisoning (CNP)

Cyanobacterial poisoning is not a harmful marine issue, but some species of cyanobacteria also known as freshwater blue-green algae are producers of extremely toxic phycotoxins often associated with poisoning in humans and animals (Carmichael & Falconer, 1993; Falconer, 1996; Sivonen, 1996; Carmichael, 1996; Lagos *et al*, 1997). Freshwater cyanobacteria may accumulate in surface water supplies as blooms and

Table I

Syndromes associated to microalgal poisoning

Syndrome	**Paralytic Shellfish Poisoning**	**Diarrheic Shellfish Poisoning**	**Amnesic Shellfish Poisoning**	**Neutotoxic Shellfish Poisoning**	**Ciguatera Poisoning**	**Cyanobacterial Poisoning**
Causative microalga	*Alexandrium sp.* *G. catenatum* *P. bahamense*	*Dinophysis sp.* *P. lima*	*Pseudo-nitzchia sp.* *pungens f. multiseries australis: seriata, pseudodelicatissima*	*G. breve* *G. cf breve*	*G. toxicus*	Cyanobacterias
Vectors	Shellfish	Shellfish	Shellfish	Shellfish	Fish	Freshwater Haemodialysis
Toxins	Saxitoxin and analogous	Okadaic acid DTX1 DTX2 DTX3	Domoic acid and isomers	Brevetoxins	Ciguatoxins Maitotoxin Gambiertoxins	Anatoxin-a Anatoxin-a(s) Microcystins Nodularin Cylindrospermopsin PSP toxins
Detection	HPLC Mouse assay Binding assay Cytotoxic assay	HPLC PP2A assay Mouse assay	HPLC Binding assay CE-UV	HPLC ELISA Mouse assay	HPLC ELISA	HPLC ELISA Immunoassay PP2A assay
Mortality	13 %	0 %	3 %	0 %	< 1 %	?

concentrate on the surface as blue-green scum. They produce toxins which are classified according to their mode of action into hepatotoxins (*e.g.*, microcystins, nodularin), neurotoxins (*e.g.*, anatoxins), PSP toxins (*e.g.*, saxitoxins and analogous) (Jachim & Gentile, 1968; Mahmood & Carmichael, 1986; Humpage *et al*, 1994; Negri & Jones, 1995; Onodera *et al*, 1997; Lagos *et al*, 1997), skin irritants and other toxins (*e.g.*, cylindrospermopsin) (Ohtani *et al*, 1992; Sivone, 1996). In Chile, cyanobacteria blooms with presence of microcystine L-R and massive fish death have already been reported (Peñaloza *et al*, 1990; Gaete *et al*, 1994).

The global distribution of harmful algal blooms is shown in Figure 1. This figure, updated to 1997, shows the presence of the four major poisoning syndromes related to marine toxins. Two of them are present in Chile (PSP and DSP), a third one (ASP) is potentially a threat to Chile, since the diatom *Nitzschia pseudoseriata* (*Pseudonitzschia australes* Frenguelli), one of the postulated causative organisms that produce domoic acid, has been described frequently in phytoplankton sampling in Chilean waters (Lembeye *et al*, 1975; Muñoz *et al*, 1992; Hallegraeff, 1993). PSP and DSP are well documented in Chile (Zao *et al*, 1993; Uribe, 1993; Lembeye, 1992; Lagos *et al*, 1996; Compagnon *et al*, 1998) and virtually worldwide (Hallegraeff, 1993; Yasumoto & Murata, 1993; Proenca *et al*, 1997).

Due to the extension of the topic, this review will be focused on determination of PSP toxins and its presence in the Southern part of Chile.

PARALYTIC SHELLFISH POISONING

This poisoning poses the most serious threat to public health and the shellfish industry in Chile, because of its high mortality rate (Table II) and the highest

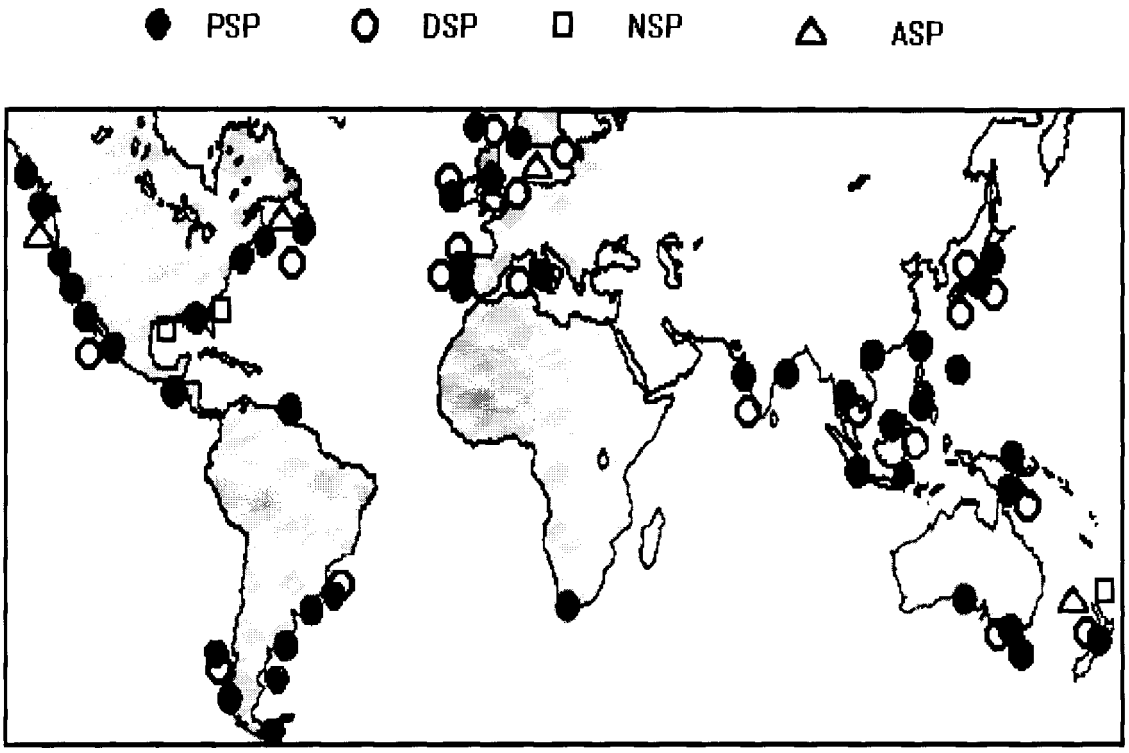


Fig 1. Global distribution of four human illnesses associated with marine toxic algal blooms: Paralytic Shellfish Poisoning (PSP), Diarrheic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP) and Neurotoxic Shellfish Poisoning (NSP).

Table II

Human illness and fatalities associated to paralytic shellfish poisoning (by A. *catenella*) in Chile since 1972. (Source: Ministerio de Salud, S.S.R. Magallanes).

Date	Locality	Illness	Death	Bivalve sp.	M. Bioassay
XII Region					MU
22 Oct 1972	Bahia Bell	3	3	A. ater	96,000
20 Feb 1981	Seno Unión	26	2	A. ater	W/Q
16 Apr 1989	Estero Núñez	8	0	A. ater	1,555
29 Mar 1991	Bahía Nash	95	2	M. chilensis	9,855
18 Nov 1991	Seno Unión	125	2	A. ater	W/Q
26 Dic 1991	Seno Nevado	2	2	A. ater	11,448
13 Jan 1992	Bahía Wodsworth	14	6	M. chilensis	12,636
13 Jan 1992	Estero Asia	5	0	M. chilensis	70,157
16 Feb 1992	Km 49 Sur	3	0	M. chilensis	7,560
01 Mar 1992	Caleta La Olla	1	1	M. chilensis	W/Q
06 May 1992	Paso Nuevo	3	0	A. ater	W/Q
18 Jul 1992	Isla Vancouver	1	0	A. ater	W/Q
20 Jul 1992	Paso Schoal	3	0	A. ater	6,795
27 Dic 1992	Puerto Williams	6	1	M. chilensis	14,544
02 Jan 1994	San Juan	8	0	M. chilensis	10,800
02 Jan 1994	Los Ñires	1	0	M. chilensis	4,104
02 Jan 1994	Punta Arenas	2	1	M. chilensis	2,610
03 Jan 1994	Punta Arenas	1	0	M. chilensis	2,412
04 Feb 1994	Punta Arenas	1	0	M. chilensis	W/Q
11 Apr 1994	Bahía G. Grande	1	0	A. ater	W/Q
27 Jun 1994	Seno Ringdove	1	1	A. ater	8,415
02 Jan 1995	Chabunco	4	0	M. chilensis	1,896
09 Feb 1995	Seno Profundo	1	0	A. ater	W/Q
06 Jan 1997	Isla Isabel	2	0	A. ater	1,627
10 Jan 1997	Fuerte Bulnes	1	0	A. ater	12,330
23 Jan 1997	Santa María	1	0	A. ater	2,602
Total XII Region		319	20		
XI Region					HPLC µg/100g
05 Jun 1995	Isla Toto	8	1	M. chilensis	38,554
28 Nov 1995	Puerto Aguirre	1	1	A. ater	W/Q
24 Feb 1996		1	1	W/Q	
Total XI Region		10	3		
TOTAL		329	23		

W/Q = without quantitation

[1]mportant: 3 more fatalities occurred in XI Region during Spring 1987-Summer 1998 period.

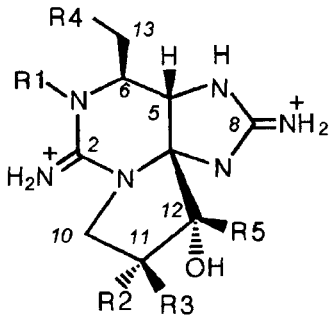
toxicity found in its molluscs (Lagos *et al*, 1996; Compagnon *et al*, 1998).

Paralytic shellfish poisoning toxins act by reversibly binding to a receptor on the voltage-gated sodium channel, blocking neuronal transmission (Kao, 1966; Henderson *et al*, 1973; Strichartz, 1984; Moczydlowski *et al*, 1984, 1986; Guo *et al*, 1987; Hall *et al*, 1990; Long *et al*, 1990; Strichartz *et al*, 1995). Saxitoxin, was the first one known and the most studied component of PSP toxins; it is hydrophilic (positively charged at neutral pH), low mol wt (298 Da) and a non-protein toxin. Due to its high affinity to its binding site, it has been a very useful tool to quantify the sodium channel density, to purify and study the sodium channel properties. Until know,

it has been described more than 26 analogues of Saxitoxin which occur naturally (Fig 2) (Harada *et al*, 1982; Oshima *et al*, 1989; Oshima, 1995; Onodera *et al*, 1997).

The organisms that are recognized as primary sources of PSP toxins include three morphologically distinct genera of dinoflagellates, *Alexandrium sp.*, *Pyrodinium sp.* and *Gymnodinium sp.* (Hall *et al*, 1990; Hallegraeff, 1993; Yasumoto *et al*, 1995; Oshima, 1995), as well as four species of blue-green alga, *Aphanizomenon flos-aquae, Anabaena circinalis, Lyngbya wollei and Cylindrospermopsis raciborskii* (Mahmood & Carmichael, 1986; Humpage *et al*, 1994; Negri & Jones, 1995; Falconer, 1996; Sivonen, 1996; Onodera *et al*, 1997; Lagos *et al*, 1997).

Toxic algal blooms have been detected in the three Southernmost regions of the country, with *Dinophysis acuta* and



	R1	*R2*	*R3*	*R4*	*R5*
STX	H	H	H	$COONH_2$	OH
neoSTX	OH	H	H	$COONH_2$	OH
dcSTX	H	H	H	OH	OH
dcneoSTX	OH	H	H	OH	OH
doSTX	H	H	H	H	OH
GTX1	OH	OSO_3^-	H	$COONH_2$	OH
GTX2	H	OSO_3^-	H	$COONH_2$	OH
GTX3	H	H	OSO_3^-	$COONH_2$	OH
GTX4	OH	H	OSO_3^-	$COONH_2$	OH
GTX5	H	H	H	$COONHSO_3^-$	OH
GTX6	OH	H	H	$COONHSO_3^-$	OH
dcGTX1	OH	OSO_3^-	H	OH	OH
dcGTX2	H	OSO_3^-	H	OH	OH
dcGTX3	H	H	OSO_3^-	OH	OH
dcGTX4	OH	H	OSO_3^-	OH	OH
doGTX2	H	OSO_3^-	H	H	OH
doGTX3	H	H	OSO_3^-	H	OH
C1	H	OSO_3^-	H	$COONHSO_3^-$	OH
C2	H	H	OSO_3^-	$COONHSO_3^-$	OH
C3	OH	OSO_3^-	H	$COONHSO_3^-$	OH
C4	OH	H	OSO_3^-	$COONHSO_3^-$	OH
1	H	H	OSO_3^-	$COOCH_3$	H
2	H	H	OSO_3^-	$COOCH_3$	OH
3	H	OSO_3^-	H	$COOCH_3$	OH
4	H	H	H	H	H
5	H	H	H	$COOCH_3$	OH
6	H	H	H	$COOCH_3$	H

Fig 2. Chemical structures of paralytic shellfish toxins.

Alexandrium catenella being the most important toxic species (Guzmán *et al*, 1975a,b; Muñoz *et al*, 1992; Lembeye, 1992; Lagos *et al*, 1996; Compagnon *et al*, 1998).

Paralytic shellfish poisoning has been recognized for over a century as a clinical entity in this far part of South America. In 1908, Segers documented the intoxication and periodic mass poisoning of native people due to mussels consumption in the proximity of Ushuaia (Beagle Channel) in 1886. This is the earliest report of PSP in this area. Since then, the prevention of human intoxication due to the ingestion of toxic shellfish has been an important issue of mutual interest to public health and fishery authorities of the country.

Alexandrium catenella produces PSP toxins which contains saxitoxin and its derivatives. This dinoflagellate is mainly found in the Southernmost regions of Chile, a distance covered by 1,200 km long between 43°45'00"-56°00'00" Lat S (Fig 3) (Lagos *et al*, 1996). Since 1972, toxic blooms of *A. catenella* had resulted in the death of 26 persons in Chile (Table II), all of them intoxicated by the consumption of shellfish harvested in the XI and XII Regions (Lembeye, 1992; Montebruno, 1993; meeting presentations of officials belonging to *Servicios Regionales de Salud)*. Also, the highest PSP contaminated shellfish had been reported in this part of South America (Benavides *et al*, 1995; Compagnon *et al*, 1998). Since 1993, the appearance of PSP is considered endemic in this part of the world (Lembeye, 1992; Uribe, 1993; Lagos *et al*, 1996; Compagnon *et al*, 1998).

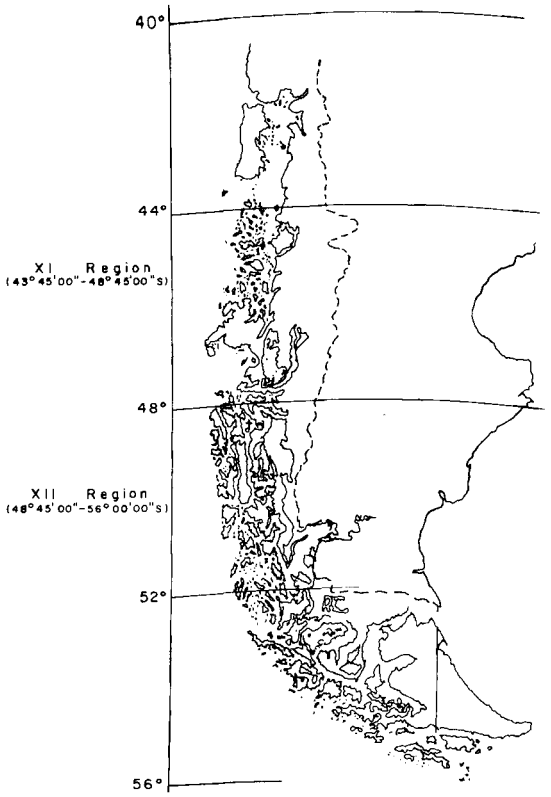


Fig 3. Geographical location of the two Southern regions of Chile, where paralytic shellfish poisoning is occurring. All samples were collected from these regions.

TOXINS DETECTION

Although the first toxic bloom of *A. catenella* was documented in the Magellan Strait (XII Region) in 1972 (Guzmán *et al*, 1975a,b), is well known that PSP is also present in the XI Region since 1992 (Muñoz *et al*, 1992). In both cases, until 1996, all the monitoring of PSP in survey programs and during outbreaks has been done using the mouse bioassay to estimate the total toxicity in shellfish (Sommer & Meyer, 1937). This bioassay has several drawbacks: lack of specificity and precision, inconvenience and difficulty of maintaining large stock of animals, no information about toxin composition and significant errors when testing samples of low toxicity. Thus, an alternative method using a post-column derivatization incorporated with high performance liquid chromatography (HPLC) and fluorescence detection was introduced (Oshima *et al*, 1989; Oshima, 1995). With this new analytical tool, the PSP toxin profiles of Chilean shellfish and dinoflagellates had been known for the first time (Lagos *et al*, 1996; Compagnon *et al*, 1998).

A schematic illustration of the PSP toxins analyzer used in our laboratory is shown in Figure 4. The radiochemical liquid scintillation counting flow-through monitor coupled on-line also allows us to follow the radioactive toxins (β emitters like ^{3}H or ^{14}C) in real time display of both

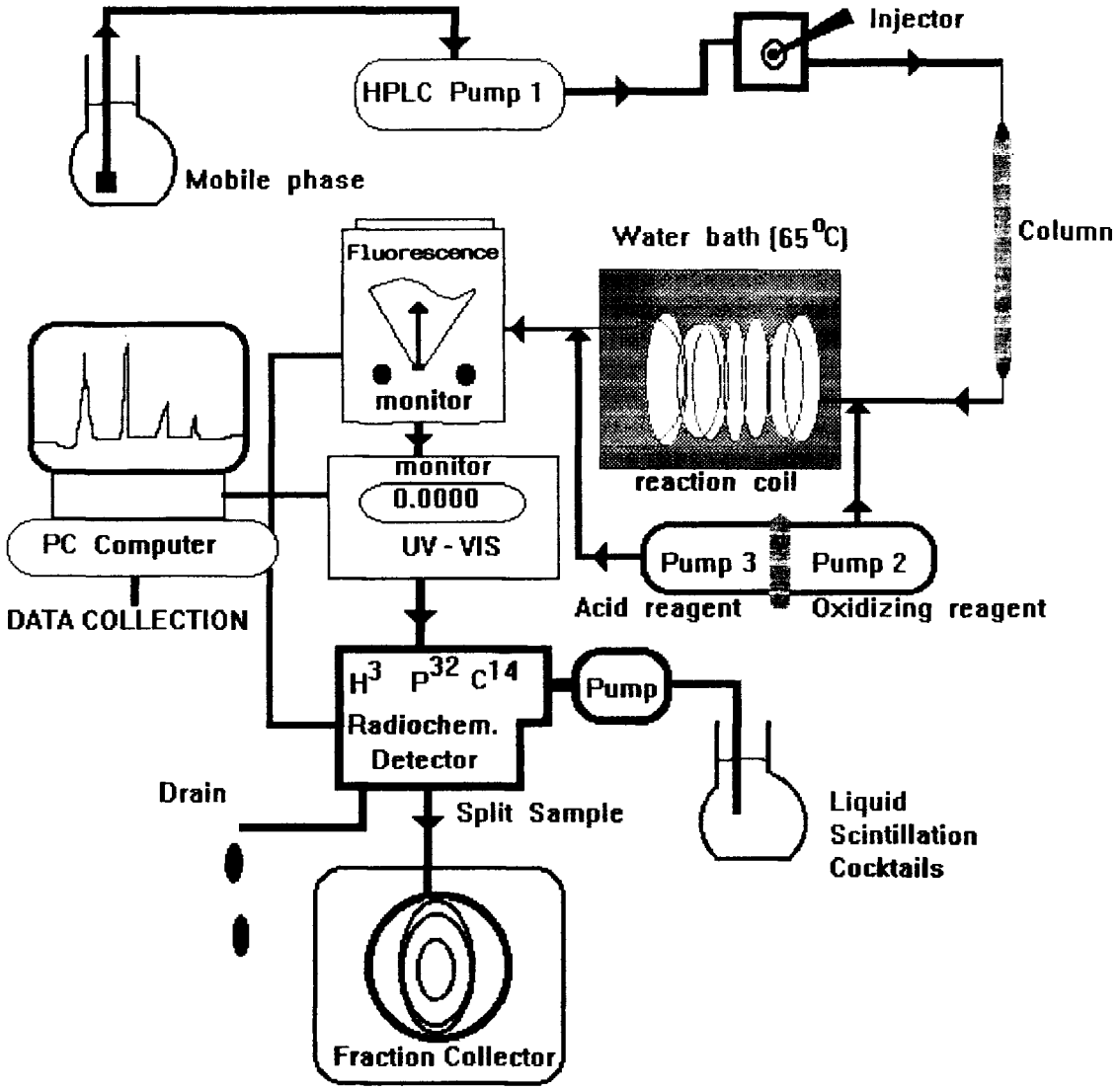


Fig 4. Schematic illustration of PSP toxins analyzer with fluorescent and radiochemical detection on line.

radioactive and fluorescent signals. Using this method, a high sensitivity with detection limits of 100-200 fmol was attained. Figures 5A and 5B show typical chromatograms of PSP toxins. These correspond to pure toxins isolated and purified from Chilean highly contaminated shellfish using preparative liquid chromatography. Identification and purity determination of these toxins were done by HPLC with on-line fluorescence detection (HPLC-FDL) and HPLC coupled mass spectrometry (HPLC-MS). For routine analysis of shellfish or dinoflagellates samples, standard calibrated solutions of PSP toxins as reference material were prepared (FONDECYT 1961122).

HPLC-FDL is an analytical method that has the ability to quantify each toxin in crude samples of small size (20 µl), which yield a single toxin concentration. It is also a powerful tool in the research field, specially for analysis of toxins production by dinoflagellates from phytoplankton collected in sea waters or in cultured samples. The high sensitivity of the system enable us to elucidate the complete toxin profiles of *A. catenella* clones in culture

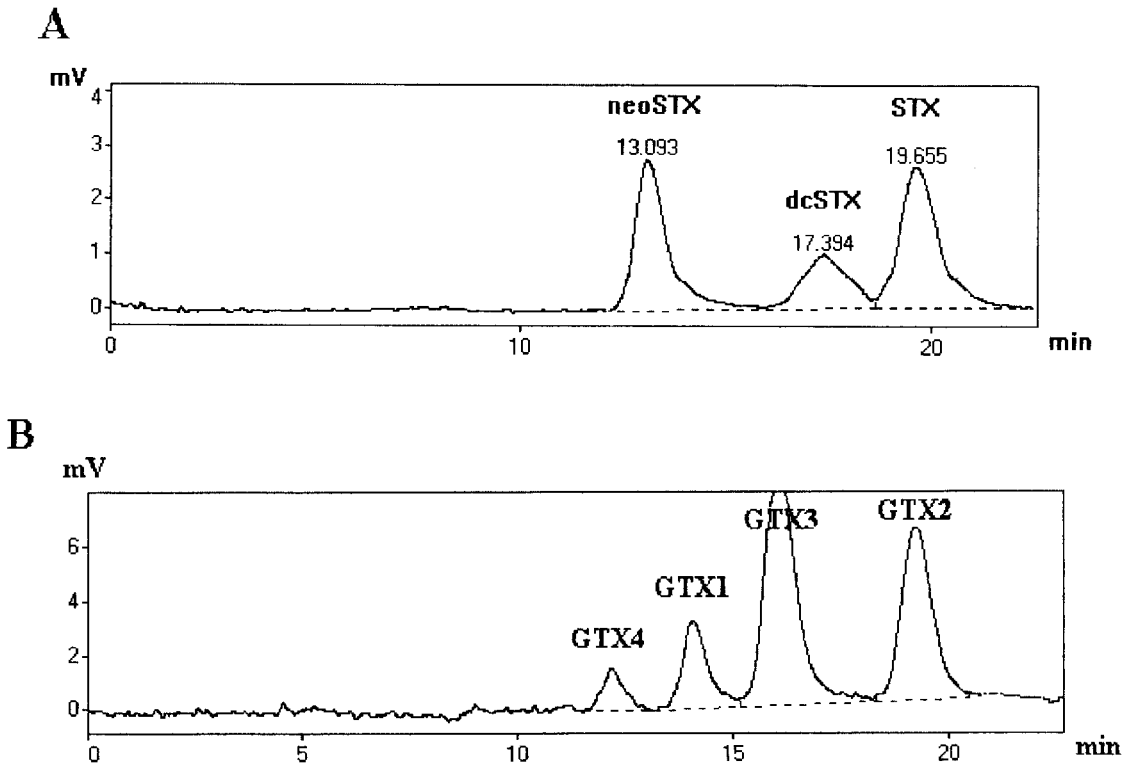


Fig 5. Chromatograms of PSP toxin mixtures in low concentration. **A.** Mixture of STXs group: neoSTX (9.7 pmol), dcSTX (2.7 pmol) and STX (4.5 pmol). **B.** Mixture of GTXs group: GTX4 (1.2 pmol), GTX1 (2.8 pmol), GTX3 (2.1 pmol) and GTX2 (2.3 pmol).

collected in both regions and also, for the first time, to measure the amount of PSP toxins/cell produced by these clones of *A. catenella* (Fig 6).

Pure toxins of known concentration are essential for this type of analysis. Most of the PSP toxins are not commercially available. Our laboratory is the only one in South America and one of the few worldwide to have them. Toxin concentrations of samples were resolved by comparing the peaks areas of each toxin with those of the standards (Oshima, 1995; Lagos *et al*, 1996; Compagnon *et al*, 1998).

TOXINS PROFILES

Using the above method, the analysis of mussel *Mytilus chilensis* samples -collected in 1994 in the XII Region and in 1994-95 in the XI Region- allowed us to determine and compare the average profile of both regions (Lagos *et al*, 1996). After the analysis of more than 100 samples, we found a distinctive toxin profile for each region. Figure 7 shows both average profiles (top and center). They display eleven of the most known PSP toxins already described in other mussel samples worldwide (Oshima *et al*, 1989; Cembella *et al*, 1987; Oshima, 1995). Nevertheless, both average profiles undoubtedly showed different amounts of each toxin.

The samples from XII Region (nearby the Magellan Strait, 52°00'00"-56°00'00") showed the GTX1/GTX4 epimeres as the most abundant ones. In contrast, samples from XI Region showed the GTX2/GTX3 epimeres as the major ones. The presence of GTXS was also unique in these samples and in the saxitoxins group (STXs), the STX content was higher than neoSTX. Both profiles showed the gonyautoxins group as the most abundant in the extract samples, being in both cases around 80% of

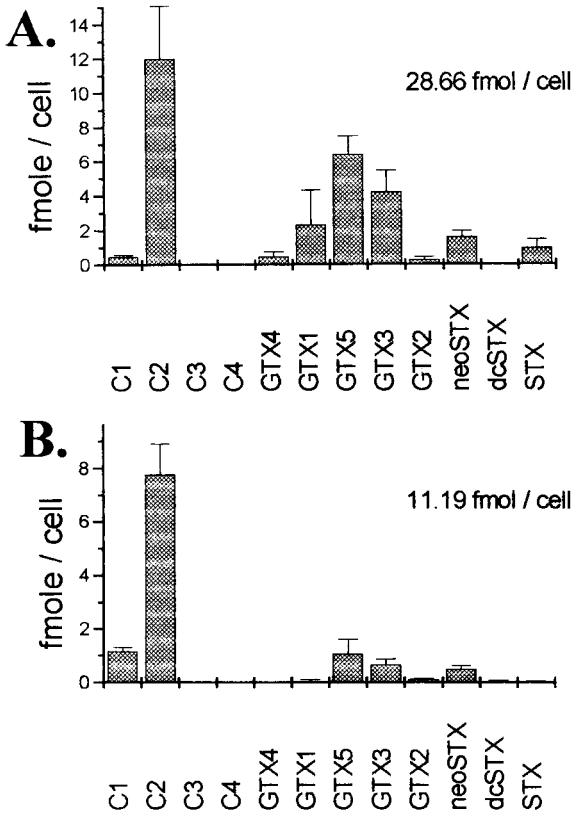


Fig 6. PSP toxins produced by A. *catenella* clones in culture (femtomole/cell; Means ± SD; N=4). A. Clone AAC7 from XI Region. B. Clone MAC8 from XII Region. Data from MI Bahamonde's Thesis as Marine Biologist, performed in our laboratory (June 1997).

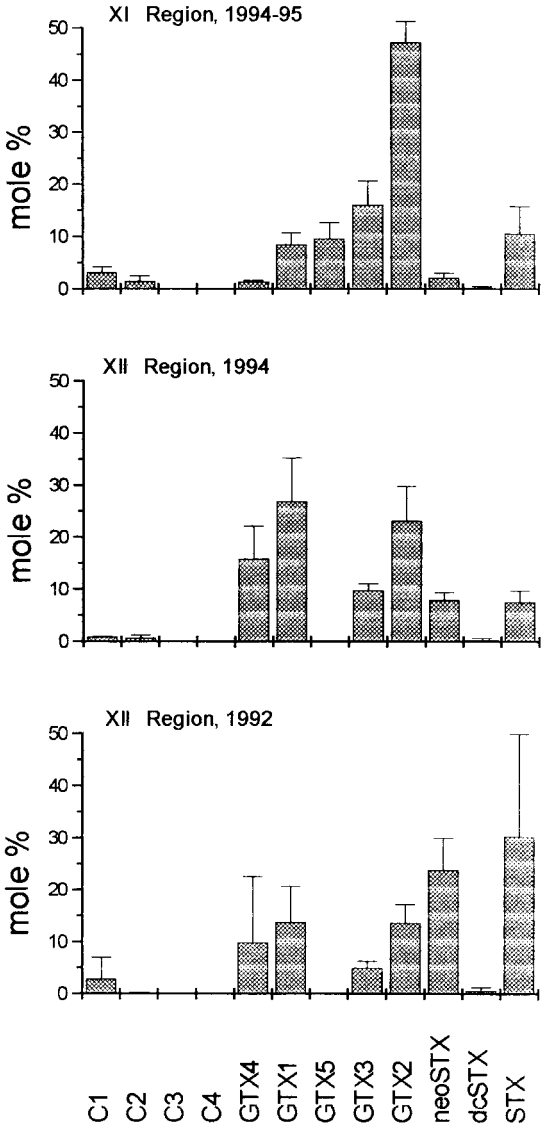


Fig 7. Average PSP toxin composition (mole %) found in *Mytilus chilensis* extracts of samples collected in 1994-95 from XI Region (top), in 1994 from XII Region (center) and in 1992 from XII Region (bottom).

the total content. In the Chilean mussel samples, gonyautoxins (GTXS) are the toxins that account for the high toxicity of the shellfish in both regions (Lagos *et al*, 1996; Compagnon *et al*, 1998).

We have found evidence that the toxin profiles in filter-feeding shellfish like *Mytilus chilensis*, are mainly a reflection of the toxin profiles of the dinoflagellates occurring in each region, and subsequently mark the event outbreak of diverse strains or clones of *A. catenella* in Chilean waters. Similar evidence had been found in Japan (Oshima *et al*, 1989; Oshima, 1995) and in the Eastern coast of Canada (Cembella *et al*, 1987; Bricelj *et al*, 1991).

At the bottom of Figure 7, an average toxin profile of six mussel samples collected in 1992 is shown. These samples were kept frozen by Dr Luis Vergara, former chief of *Departamento Programas sobre el Ambiente, Servicio de Salud Regional de Magallanes*. This profile shows a total different PSP toxins composition. Here, the most abundant toxins were STX and neoSTX, which are the most toxic of PSP toxins. These two PSP toxins show, by mousse bioassay, specific toxicities of 2,483 and 2,295 MU/pmol, respectively (Oshima, 1995).

The average percentage of toxicity (MU %) by group of PSP toxins of these samples is shown in Figure 8. Definitely, the predominant one corresponds to the STXs group (STX, neoSTX and dcSTX), which represents 64% of the total toxicity of the samples. This feature explains the high mortality rate that occurred during the Spring-Summer period (from November 1991 to March 1992). During this outbreak, 10 out of 150 intoxicated patients died (Table II). During that season, Chile had the highest registered number of intoxicated people and fatalities associated to a PSP outbreak of our history. Moreover, in the same Summer of 1992, an exceptional bloom of *A. catenella* was reported in the North-Eastern shore of Beagle Channel (55°00'00" S), where mussel samples collected on January 20 reached a maximum of 127,200 µg STX eq/ 100 g, by mousse bioassay. This is the highest mussel toxicity reported in the literature (Benavides *et al*, 1995).

We believe that in the sediment of our Southern fjords in XI and XII Regions, different strains of *Alexandrium* sp. exist. They survive as dormant *Alexandrium* cysts, and every year –during the Southern Spring-Summer season– these cysts come back as germinative cells, producing outbreaks. This results in the contamination of the complete food chain of the fjord, starting with the filter-feeding shellfish (Compagnon *et al*, 1998).

The fjord system in the Southern part of Chile, most unspoiled land and distant from cities with high population densities, presents one of the highest levels of natural bioaccumulation of PSP toxins by shellfish in the world (Compagnon *et al*, 1998). The presence of *A. catenella* is endemic in both regions and, every Spring-Summer season, outbreaks of different intensity and toxicity arise, one in the Spring and other in late Summer (Dr Luis Vergara, personal communication; Lagos *et al*, 1986; Compagnon *et al*, 1998).

Traditionally, only filter-feeding molluscs that concentrate these toxic algae are considered in monitoring programs for shellfish poisons. However, increasing attention is being paid to higher order consumers, such as carnivorous gastropods and crustaceans, due to the their accumulation capacity of PSP toxins and to be frequently consumed by humans (Shumway, 1995; Compagnon *et al*, 1998). The carnivorous gastropods *Concholepas concholepas (loco)* and *Argobuccinum ranelliformes (caracol del sur)*, which are popular domestic and commercial sea foods in Chile, had been reported to be highly contaminated for months after an *A. catenella* outbreak (Compagnon *et al*, 1998).

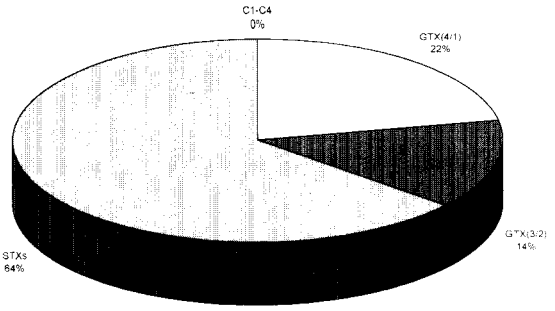


Fig 8. Toxicity contribution by groups of PSP toxins (MU obtained from average toxin composition and each toxin specific toxicity of 6 samples collected in 1992. Value 0% in group of N-sulfocarbamoyl-11-hydroxysulfate toxins (C1-C4 0.03 MU %) means that it contributes less than 1% to total sample toxicity.

In the past, in Chile, the principal impediments to analyze PSP toxins in native molluscs, phytoplankton (sea water sample) and dinoflagellates in culture, were the lack of a simple, automatic, reproducible method with sufficient sensitivity to detect picogram quantities and the analytical standards required for such analysis. Both impediments have been overcome by our laboratory.

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