

FIRST REPORT OF DIARRHEIC SHELLFISH TOXINS IN MOLLUSKS FROM BUENOS AIRES PROVINCE (ARGENTINA) ASSOCIATED WITH *DINOPHYSIS* SPP.: EVIDENCE OF OKADAIC ACID, DINOPHYSISTOXIN-1 AND THEIR ACYL-DERIVATIVES

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Resumen: Primer reporte de toxinas diarreicas de moluscos en bivalvos de la Provincia de Buenos Aires (Argentina) asociado con *Dinophysis* spp.: evidencia de Ácido Okadaico, Dinophysistoxina-1 y sus acyl-derivados. En enero de 2010, los dinoflagelados productores de toxinas *Dinophysis acuminata* y *D. caudata* (10^3 cells·l⁻¹) fueron detectados en Mar Azul durante un monitoreo rutinario de fitoplancton realizado en aguas costeras de la Provincia de Buenos Aires, Argentina. *Mesodesma mactroides* (almeja amarilla) y *Donax hanleyanus* (berberecho) del intermareal de Mar Azul, que son parte de la dieta de los habitantes del lugar y de turistas, dieron resultado positivo para toxinas lipofílicas mediante bioensayo ratón. Este trabajo está focalizado en la detección de Toxinas Diarreicas de Moluscos (DSP) en muestras colectadas durante el evento de toxicidad usando un HPLC-FLD con procedimiento de derivatización precolumna. Los datos evidenciaron contaminación de los moluscos con toxinas DSP y un perfil compuesto por Ácido Okadaico (OA), Dinophysistoxina-1 (DTX-1), Acyl-Dinophysistoxina-1 (Acyl-DTX-1) y Acyl-Ácido Okadaico (Acyl-OA). Las toxinas DSP encontradas en este estudio producen síntomas de diarrea consistentes con los experimentados por los pacientes que habían ingerido moluscos cocinados en enero. Este es el primer reporte de Acyl-derivados en muestras de moluscos procedentes del Atlántico Sudamericano y de OA en muestras de moluscos procedentes de Argentina.

Palabras clave: DSP, Ácido Okadaico, Dinophysistoxina-1, Acyl-derivados, *Dinophysis* spp.

Summary: In January 2010, the toxin-producing dinoflagellates *Dinophysis acuminata* and *D. caudata* (10^3 cells·l⁻¹) were detected in Mar Azul during routine plankton monitoring in Buenos Aires Province coastal waters, Argentina. Wild clams *Mesodesma mactroides* and *Donax hanleyanus* from Mar Azul intertidal beach, which are part of the diet for local inhabitants and tourists, tested positive with the official lipophilic mouse bioassay. This paper focuses on the detection of Diarrhetic Shellfish Poison (DSP) toxins in these samples using a HPLC-FLD pre column derivatization procedure. The data showed that shellfish were contaminated with complex DSP toxin profiles composed of Okadaic Acid (OA), Dinophysistoxin-1 (DTX-1), Acyl-Dinophysistoxin-1 (Acyl-DTX-1) and Acyl-Okadaic Acid (Acyl-OA). The DSP toxins found in this study produce diarrhea symptoms consistent with those experienced by patients who had ingested cooked shellfish in January. This is the first report of Acyl-derivatives in South American Atlantic shellfish samples and of OA in Argentinean shellfish samples.

Key words: DSP, Okadaic Acid, Dinophysistoxin-1, Acyl-derivatives, *Dinophysis* spp.

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INTRODUCTION

The first documented episode of gastrointestinal distress and diarrhea in humans consistent with Diarrhetic Shellfish Poisoning occurred in Argentina was reported from the Gulfs San José and Nuevo, Patagonia Argentina in 2001 (Gayoso *et al.*, 2002). The Diarrhetic Shellfish Poison (DSP) toxin vectors were mussels *Aulacomya ater* Molina and *Mytilus edulis platensis* d'Orbigny, and the dinoflagellate which was present in the plankton, epiphyte on macroalgae and in the stomach contents of the bivalves, was *Prorocentrum lima* (Ehrenberg) Dodge. The only DSP toxin described in that report was Dinophysistoxin-1 (DTX-1).

Recently, Sar *et al.* (2010) reported another episode of human gastrointestinal illness associated with consumption of cooked wedge clams *Donax hanleyanus* Philippi collected in Villa Gesell (Buenos Aires Province) that gave positives for DSP by mouse bioassay. In that event, the DSP toxin vectors were two bivalve species: wedge clams and mussels *Brachydontes rodriguezii* d'Orbigny. The analyzed phytoplankton community consistently contained the toxigenic species *Dinophysis acuminata* Claparède et Lachmann and *D. caudata* Saville-Kent.

Dinophysis acuminata was originally described as Okadaic Acid (OA) producer based on bloom samples collected at Le Havre, France and Tokyo Bay, Japan by using High Performance Liquid Chromatography with fluorescence detection (HPLC-FLD) (Lee *et al.*, 1989). In 2001 the toxin content of *D. acuminata* from the Galician Rías Bajas, showed OA as the major toxin and a very small peak with retention time corresponding to Dinophysistoxin-2 (DTX-2) (Fernández *et al.*, 2001), then in 2005 *D. acuminata* collected in New Zealand showed a DSP toxin profile including OA, DTX-1, Pectenotoxin 2 (PTX-2) and Pectenotoxin 11 (PTX-11) (MacKenzie *et al.*, 2005). More recently, Hackett *et al.* (2009) established unialgal cultures of *D. acuminata* from Woods Hole, USA, maintained this culture by using a two step feeding system described in (Park *et al.*, 2006). Through chemical analysis of the cell culture extracts by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) and Ultra Performance Liquid Chromatography (UPLC), they reported the detection of OA, diol ester of OA (OA D8), DTX-

1, PTX-2 and hydroxylated PTX-2.

Dinophysis caudata was early described as OA producer, based on picked cells from Johor Strait, Singapore (Holmes *et al.*, 1999) and similarly based on picked cells from the Galician Rías of Vigo and Pontevedra and analyzed by HPLC-FLD and LC-MS (Fernández *et al.*, 2003). Picked cells from Sapián Bay, Panay Islands, Philippines, analyzed by HPLC-FLD determined content of OA and DTX-1 (Marasigan *et al.*, 2001) and later cell extracts from Galician Rías Bajas analyzed by LC-MS/MS have shown that the species may have high levels of PTX-2 (Fernández *et al.*, 2006).

To date DSP toxins in Chile are restricted to the shellfish of the southern regions (García *et al.*, 2010). *Dinophysis acuminata* was mentioned as the most probable source of DTX-1 detected in *Mytilus chilensis* Hupe collected in a Southern Chilean Magellanic fjord (Uribe *et al.*, 2001) and *Dinophysis acuta* Ehrenberg was the species commonly associated with DSP outbreaks in Northern Chilean Patagonian fjords (Lembeye *et al.*, 1993; Zhao *et al.*, 1993). More recently, there are two reports that described strains of *Dinophysis* spp. from Chile that produced only pectenotoxins, one corresponding to *D. acuminata* collected in the North of Chile (Blanco *et al.*, 2007) and the other to *Dinophysis* sp. (Fux *et al.*, 2011).

There is little information about the DSP toxins in the Atlantic coastal waters of South America. *Dinophysis acuminata* was associated with the detection of the diarrhetic toxin OA in mussels from Santa Catarina, Brazil, during the winter of 1995 by HPLC-FLD (Proença *et al.*, 1999) and *Dinophysis acuminata* and *D. caudata* were associated with the detection of diarrhetic toxins by mouse bioassay in clams, wedge clams and mussels during the summers-falls of 1992, 1994 and 1996 from several localities of Uruguay (Méndez & Ferrari, 2002). Extracts of *D. acuminata* and *D. caudata* concentrated by net hauls from Uruguayan waters, analyzed by HPLC-FLD showed OA as the only DSP toxin (Méndez & Ferrari, 2002).

Based on previous results (Sar *et al.*, 2010), we carried out the present study with the purposes of analyzing the phytoplankton composition from Mar Azul between January and April of 2010, checking lipophilic shellfish toxins in bivalve mollusks by mouse bioassay and determining the diarrhetic shellfish toxins profiles by HPLC-FLD.

MATERIALS AND METHODS

Phytoplankton samples

The phytoplankton samples analyzed in this paper were collected at the Buenos Aires Province coastal waters between early January and middle April of 2010 from Mar Azul (37° 21' 25'' S-57° 01' 49'' W) at about 10 to 20 m from the shoreline, from the surface layer of the water column (0 and 5 meters depth). Qualitative samples were collected with 30 µm net hauls and immediately fixed with 4% formalin; quantitative samples were collected with Van Dorn bottle and fixed with 0.4% formalin.

Microscopic observations were made with a light microscope (LM), Nikon Microphot FX, using phase contrast and with a scanning electron microscope (SEM) Jeol JSM 6360 LV. The microphotographs were taken with the Jeol JSM 6360 LV microscope. The quantitative analyses of phytoplankton were carried out using the Sedgewick-Rafter chamber and the cell counts were made in triplicate. Data were processed with Statistical Software for Windows 7.0. The results were expressed as mean $\pm$ standard deviation.

Qualitative and quantitative samples and permanent slides correlatively labeled were incorporated into the Herbarium, deposited at the División Ficología (LPC index Herbariorum), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata under the numbers LPC 11182, 01/04/2010; LPC 11186, 01/28/2010; LPC 11286, 02/12/2010; LPC11190, 02/26/2010; LPC11194, 03/09/2010; LPC 11198, 03/26/2010 and LPC 11202, 04/12/2010.

Shellfish samples

Shellfish samples were collected at low tide in the same time and area as the phytoplankton samples, along the beach from the intertidal fringe, by digging in the sand. Collected species were wedge clam (*Donax hanleyanus* Philippi) and/or yellow clam (*Mesodesma mactroides* Reeves), depending on which population was accessible. In each sampling, two subsamples of shellfish around 500 g were obtained and frozen at -18 °C for checking DSP toxicity by mouse bioassay in the Departamento de Toxinas Marinas, Regional Mar del Plata of the SENASA and by HPLC-FLD analysis in the Laboratorio Bioquímica de Membrana, Facultad de Medicina, Universidad de

Chile. The sample corresponding to early January was lost.

A sample of cooked wedge clam from a locality neighboring Mar Azul (Villa Gesell, 37° 16' 48'' S-56° 58' 57'' W), ingested by patients who experienced diarrhea, nausea and abdominal cramps, all symptoms consistent with DSP intoxication, was also checked by mouse bioassay and by analytical methods.

Mouse bioassays

The procedure employed for sample preparation prior to mouse bioassay was described in Yasumoto *et al.* (1984) and modified in Fernández *et al.* (2002). The Japanese mouse assay for DSP toxins with modifications is the official method accepted by the Argentinean Officials. Extracts for bioassay were prepared from whole soft tissues of shellfish and mice were intraperitoneal injected with 1 mL Tween 60 1% equivalent to 25 g shellfish tissues. Experimental animals were albino mice strain CF1 of 20 ± 1 g weight. According to the Decision 2002/225/CEE appeared in the Official Journal of the European Communities and followed by the Servicio Nacional de Sanidad y Calidad Agroalimentaria from Argentina, this method uses mouse survival time for determination of DSP toxicity and two of three mouse deaths in less than 24 hours as criterion for a positive test.

Chemical analyses

High Performance Liquid Chromatography with Fluorescence detection (HPLC-FLD)

A portion of 1 g of homogenate prepared from ten individuals of each shellfish sample was extracted twice with 4 mL of methanol-water (8:2 v/v) following Lee *et al.* (1987). The methanolic phase was centrifugated, 2.5 mL of the supernatant diluted with water to a final 26.6% methanol and cleaned up by liquid/liquid partitioning according to García *et al.* (2003, 2010). This eluate was evaporated to dryness under reduced pressure in a Speed Vac Plus (Savant, SC 210A). The dried extraction was resuspended in 100 microliters of methanol.

The alkaline hydrolysis of Acyl-DSP toxins was performed as described in García *et al.* (2004a). Briefly, aliquots of 2.5 mL of 80% methanol extract of each shellfish sample were treated with 2.5 mL of 0.5 N NaOH in 90% methanol solution. The

mixture was kept to 75° for 50 minutes and then the methanol was evaporated. The aqueous layer was acidified with 2.5 mL of 0.5 N HCl, extracted twice with 5 mL diethyl ether, evaporated to dryness and dissolved with 2.5 mL of 80% methanol. The methanolic solution was treated with 1 mL of 0.2% acetic acid, extracted twice with 4 mL of dichloromethane and evaporated to dryness under reduced pressure.

Clean and dry extracts and standards were derivatized with 9-anthryl-diazomethane (ADAM, Molecular Probe, USA) freshly prepared solution of 0.1% ADAM in 100 µl of acetone and 400 µl of methanol. The mixture was kept to 25° for 60 minutes and then evaporated to dryness. Residues were diluted in 200 µl of dichloromethane/hexano (1:1 v:v).

The derivatized samples were cleaned up on a solid-phase extraction device, silica column Sep-Pak cartridge (Water CO.) (García *et al.*, 2003) and analyzed by HPLC using isocratic conditions with mobile phase of acetonitrile/methanol/water (8:1:1 v/v), and column with reversed phase, Supelcosil LC (C18, 4 × 250 mm, 5 µm). The chromatographic separation was performed on a Liquid Chromatograph System equipped with a pump (Shimadzu LC-6A), a Rheodyne injector (7725i Rheodyne) and in-line fluorescence detector (Jasco FP-2020 Plus). The fluorescence detector was set for 365 nm excitation and 415 nm emissions. Peaks in the resulting chromatograms were identified by comparison with the retention times of toxins analytical standards. Certified reference materials containing Dinophysistoxin-1 (CRM-DSP-Mus-b) and Okadaic acid (NCR-CRM-OA-c) were obtained from National Research Council Canada. The detection limits for the DSP toxins as ADAM esters were 0.02 ng of DSP toxins detected in the chromatogram meaning a signal that it was the double amount of the base signal noise in the recorded equipment (García *et al.*, 2010).

RESULTS AND DISCUSSION

Temporal distribution and density of the species of *Dinophysis* in Mar Azul

The concentration of *Dinophysis* cells was quite low in Buenos Aires coastal waters in the years 2008 and 2009 and the occurrence from one

year to the next varied considerably (unpublished data). Nevertheless, in early January 2010, there occurred an unusual proliferation of *Dinophysis acuminata* (Figure 1A) that reached a maximum number of cells in Mar Azul, 26,000 cells·l⁻¹, with a 2.41% relative contribution to the total phytoplankton (Table 1). In later January the cell concentrations of *Dinophysis acuminata* declined to 9,000 cells·l⁻¹, *Dinophysis caudata* (Figure 1B) appeared in lower cell concentrations than *D. acuminata*, 1,000 cells·l⁻¹, and the phytoplankton assemblage was dominated by the diatoms *Asterionellopsis glacialis* (Castracane) Round and *Rhizosolenia imbricata* Brightwell, as well as additional nanoplanktonic centric diatoms and phytoflagellates of indeterminate identity (Table 1).

From February to early March the density of *Dinophysis acuminata* tended to diminish, varying between 8,675 and 333 cells·l⁻¹ (Figure 2) and the relative contribution of both species into the total phytoplankton assemblage also decreased (0.43 to 0.01% respectively) (Table 1). The opposite situation was found in reference to the cell concentration of the total phytoplankton population that increased between 2.0·10⁶ cells·l⁻¹ to 5.1·10⁶ cells·l⁻¹ and *Dinophysis caudata* was only found in qualitative samples in this period (Table 1). In later March the abundance of *Dinophysis acuminata* increased again to 1,330 cells·l⁻¹ and *D. caudata* appeared at concentration

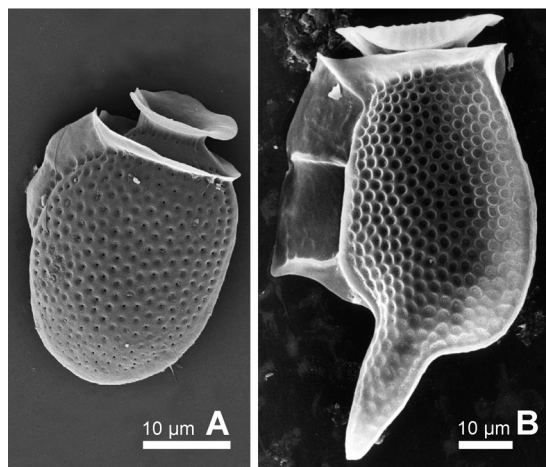


Fig. 1. A: SEM micrography of *Dinophysis acuminata*. **B:** SEM micrography of *Dinophysis caudata*.

Table 1. Density of the total phytoplankton assemblage and relative density of the potentially toxigenic species of dinoflagellates and of the more abundant species, from Mar Azul, in the samplings from January to April.

Date	01/04/10	01/28/10	02/12/10	02/26/10	03/09/10	03/26/10	04/12/10
Dinoflagellates							
<i>Dinophysis acuminata</i>	$2.60 \cdot 10^4$	$9.00 \cdot 10^3$	$8.67 \cdot 10^3$	$2.33 \cdot 10^3$	$3.33 \cdot 10^2$	$1.33 \cdot 10^3$	
<i>Dinophysis caudata</i>		$1.00 \cdot 10^3$		qs	qs	$3.33 \cdot 10^2$	qs
<i>Gymnodinium catenatum</i> (cyst)		$8.33 \cdot 10^3$					
<i>Scrippsiella</i> sp.				$1.64 \cdot 10^4$			
Undetermined dinoflagellates					$6.60 \cdot 10^4$		
Diatoms							
<i>Asterionellopsis glacialis</i>	$2.83 \cdot 10^5$	$2.11 \cdot 10^6$	$1.32 \cdot 10^6$		$4.75 \cdot 10^6$		$4.17 \cdot 10^4$
<i>Actinocyclus</i> sp.						$1.25 \cdot 10^4$	$1.67 \cdot 10^4$
<i>Bacteriastrum</i> sp.				$3.30 \cdot 10^4$			
<i>Cerataulina pelagica</i>				$1.33 \cdot 10^5$			$4.17 \cdot 10^4$
<i>Chaetoceros subtilis</i> var. <i>abnormis</i>				$1.70 \cdot 10^5$			$3.33 \cdot 10^4$
<i>Chaetoceros</i> sp.				$1.17 \cdot 10^5$	$3.28 \cdot 10^4$		
<i>Delphineis</i> sp.				$5.00 \cdot 10^4$			
<i>Detonula pumila</i>				$8.33 \cdot 10^4$			
<i>Guinardia delicatula</i>							$3.33 \cdot 10^4$
<i>Leptocylindrus danicus</i>		$6.67 \cdot 10^4$	$1.33 \cdot 10^5$	$3.25 \cdot 10^5$		$1.25 \cdot 10^4$	
<i>Leptocylindrus minimus</i>			$1.90 \cdot 10^5$	$1.70 \cdot 10^5$			$8.33 \cdot 10^4$
<i>Paralia</i> sp.					$5.80 \cdot 10^4$		
<i>Phaeodactylum tricornutum</i>					$3.30 \cdot 10^4$		
<i>Rhizosolenia imbricata</i>		$7.42 \cdot 10^5$					
<i>Skeletonema</i> sp.	$5.00 \cdot 10^4$			$9.50 \cdot 10^5$			$1.67 \cdot 10^4$
<i>Thalassionema</i> sp.	$3.33 \cdot 10^4$	$5.00 \cdot 10^4$					
<i>Thalassiosira gravida</i>				$3.75 \cdot 10^5$			
<i>Thalassiosira</i> spp.							$3.33 \cdot 10^5$
Undetermined centric diatoms	$5.01 \cdot 10^5$	$6.00 \cdot 10^5$	$2.10 \cdot 10^5$	$2.83 \cdot 10^5$	$1.25 \cdot 10^5$	$9.57 \cdot 10^4$	$5.42 \cdot 10^5$
Undetermined pennate diatoms					$1.67 \cdot 10^4$		
Phytoflagellates							
Undetermined phytoflagellates	$1.17 \cdot 10^5$	$1.00 \cdot 10^5$	$1.67 \cdot 10^4$			$2.08 \cdot 10^4$	$1.00 \cdot 10^5$
Undetermined silicoflagellates						$5.42 \cdot 10^4$	
Total phytoplankton	$1.08 \cdot 10^6$	$4.14 \cdot 10^6$	$2.00 \cdot 10^6$	$2.78 \cdot 10^6$	$5.14 \cdot 10^6$	$2.43 \cdot 10^5$	$1.26 \cdot 10^6$
Relative contribution <i>D. acuminata</i>	2.41%	0.22%	0.43%	0.10%	0.01%	0.55%	
Relative contribution <i>D. caudata</i>		0.02%				0.12%	

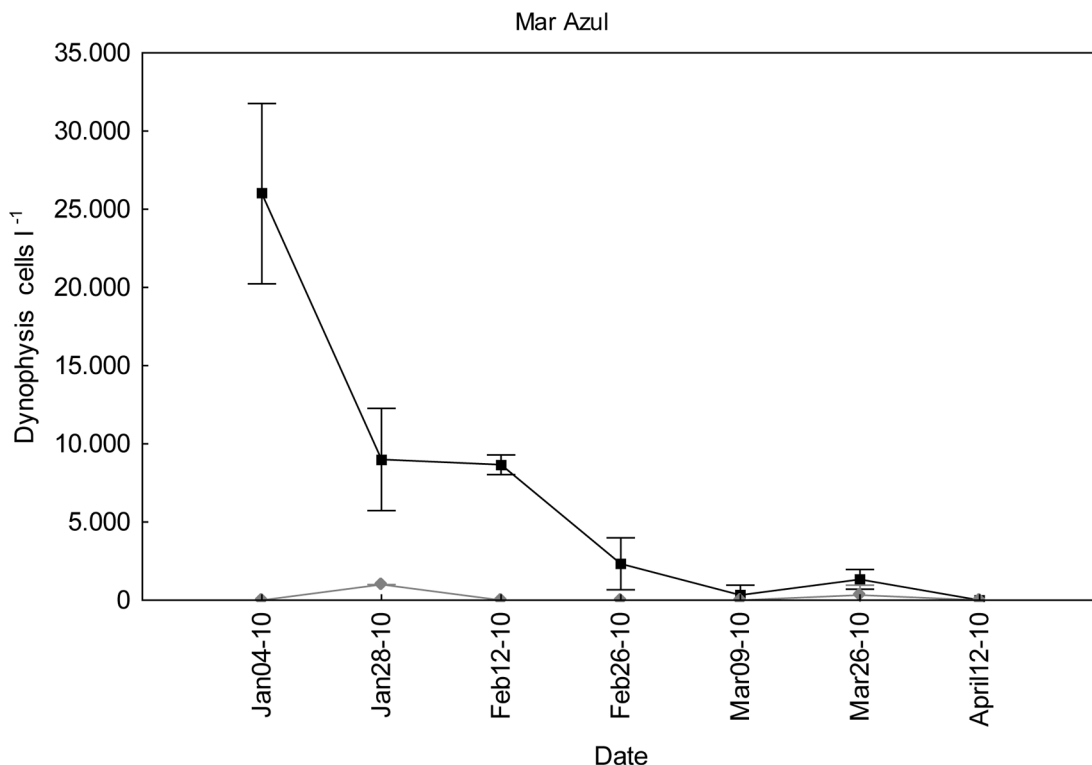


Fig. 2. Temporal distribution and density of the species of *Dinophysis* in Mar Azul. ■ *Dinophysis acuminata*, ◆ *Dinophysis caudata*.

of 333 cells·l⁻¹ (Figure 2). The relative contributions of both species to the total phytoplankton also increased (0.55% and 0.12% respectively), the density of the total phytoplankton was the lowest of all the sampled periods, 2.4·10⁵ cells·l⁻¹ and the phytoplankton assemblage was dominated by indeterminate phytoflagellates and nanoplanktonic centric diatoms. In April both toxigenic species disappeared or only scattered cells persisted in qualitative samples.

Shellfish extracts toxicity tested by mouse bioassay

From all the shellfish samples tested for DSP toxicity using the mouse bioassay, only one, dated in 12 April 2010, tested negative with one of three mice died. In general, mice presented prostration and weakness as common symptoms in most of the samples. Details about survival times post inoculation and death rate in relation with cells concentrations of *Dinophysis* spp. in the

phytoplankton are shown in Table 2.

HPLC-FLD analysis

All shellfish sample extracts including the one that tested negative by mouse bioassay, were analyzed by HPLC-FLD as described above. The search of each DSP toxin was performed on all sample extracts derivatized with ADAM.

The chromatographic runs performed by HPLC-FLD analysis of the sample yellow clam (03/26/10) as examples of fluorescent chromatograms performed in this study, are shown in Figure 3. In the top chromatogram (labeled Methanol Sample) the peaks displayed correspond to OA and DTX-1 respectively. The second chromatogram (labeled Hydrolyzed Sample), that corresponds to an extract hydrolyzed with NaOH, shows the same peaks (OA and DTX-1) but an increase in signal was observed due to the hydrolysis of the Acyl-derivatives. The third chromatogram (labeled STD) shows

Table 2. Results obtained by mouse bioassay on whole flesh of shellfish samples collected in period 01/25-28/10 – 04/12/10 from Atlantic coast of Buenos Aires Province, Argentina. Yellow clam: *Mesodesma mactroides*, wedge clam: *Donax hanleyanus*.

Sample date of collection / Species	Station	Cells l ⁻¹ of Dinophysis spp.	Bioassay result	Death rate	Survival time post inoculation
01/28/10 Wedge clam	Mar Azul	<i>D. acuminata</i> 9·10 ³ <i>D. caudata</i> 1·10 ³	Positive	3:3 (100%)	Between 1h and 1h 15min.
01/25/10 to 01/28/10 Cooked wedge clam	Villa Gesell	<i>D. acuminata</i> 9·10 ³ <i>D. caudata</i> 1·10 ³	Positive	3:3 (100%)	Between 1h and 1h 5min.
02/12/10 Wedge clam	Mar Azul	<i>D. acuminata</i> 8.7·10 ³	Positive	2:3 (66%)	Between 1h 55min and 2h 10min.
02/26/10 a) Yellow clam b) Wedge clam	Mar Azul	<i>D. acuminata</i> 2.33·10 ³	a and b Positive	a) 3:3 (100%) b) 3:3 (100%)	a) Between 4 h and 12 h b) Between 8h and 18h
03/09/10 Wedge clam	Mar Azul	<i>D. acuminata</i> 3.33·10 ²	Positive	3:3 (100%)	Between 5h 40 min and 21 h
03/26/10 Yellow clam	Mar Azul	<i>D. acuminata</i> 1.33·10 ³ <i>D. caudata</i> 3.3·10 ²	Positive	3:3 (100%)	Between 10 and 20 h
04/12/10 Wedge clam	Mar Azul	Absents	Negative	1:3 (33%)	Less than 24 h

the analytical standards of OA and DTX-1. In the bottom of Figure 3, the last chromatogram (labeled DOCA) shows the run internal standard which corresponded to Deoxicholic acid derivatized with ADAM. The alkaline hydrolysis revealed the chemical transformation of Acyl-DTX-1 into DTX-1 and the Acyl-OA into OA. In fact, this is the only way to detect the Acyl derivatives (Acyl-DTX-1 and Acyl-OA) by HPLC-FLD. This procedure was repeated with every sample extract and the results are shown in Table 3.

The sample that tested negative by mouse bioassay showed no peaks corresponding to OA or DTX-1 for the extract not hydrolyzed with NaOH and showed small peaks corresponding to OA and DTX-1 for the hydrolyzed extract.

During routine phytoplankton monitoring carried out in Buenos Aires Province coastal waters, some

shellfish samples tested positive with the official DSP mouse bioassay when *D. acuminata* and *D. caudata* were detected in parallel water samples (Sar *et al.*, 2010). As an extension of this work the present study allowed us to determine in shellfish sample extracts that the DSP toxin profiles are composed of OA, DTX-1, Acyl-OA and Acyl-DTX-1. This is the first report of Acyl-derivatives compounds in the South American Atlantic Ocean and of OA in Argentinean waters. Acyl derivatives were previously reported in shellfish extracts from the Southern Chile by García *et al.* (2004b; 2006; 2010). The metabolic transformation of OA and DTX-1 in their respective Acyl-derivatives is a biochemical way of self protection from these toxic compounds that are chemically lipophilic and very hard to eliminate as has been recently reviewed by Rossignoli *et al.* (2011).

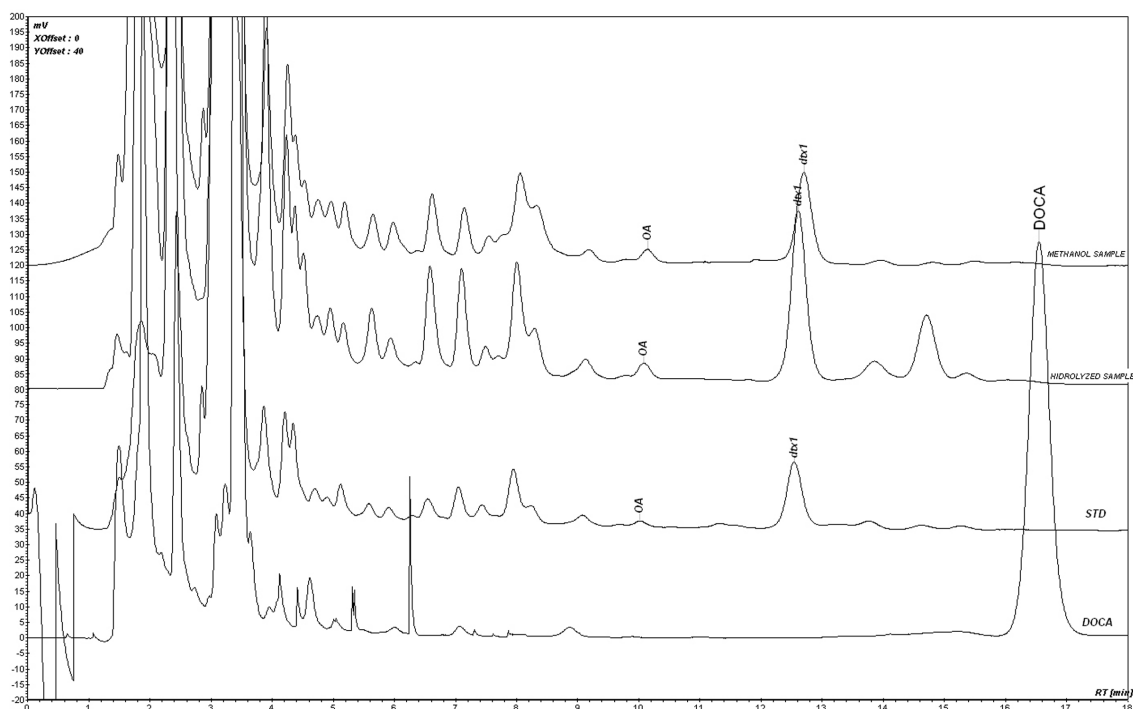


Fig. 3. Chromatographic runs performed by HPLC-FLD analysis of the yellow clam sample (03/26/10) derivatized with ADAM. OA, Okadaic acid; DTX-1, Dinophysistoxin-1; DOCA, Deoxicholic acid; STD, analytical standards.

Table 3. DSP toxins presence in bivalve samples. Ten individuals of each shellfish sample (whole flesh) were used to obtain homogenate. Yellow clam: *Mesodesma mactroides*, wedge clam: *Donax hanleyanus*. Nd: no detectable.

Sample collection date / Species	Cells l ⁻¹ of <i>Dinophysis</i> spp.	OA	DTX-1	Acyl-DTX-1	Acyl-OA
01/28/10 Wedge clam	<i>D. acuminata</i> 9·10 ³ <i>D. caudata</i> 1·10 ³	positive	nd	nd	positive
01/25/10 to 01/28/10 Cooked wedge clam	<i>D. acuminata</i> 9·10 ³ <i>D. caudata</i> 1·10 ³	nd	nd	positive	positive
02/12/10 Wedge clam	<i>D. acuminata</i> 8.7·10 ³	positive	nd	nd	positive
02/26/10 Yellow clam	<i>D. acuminata</i> 2.33·10 ³	positive	positive	nd	positive
03/09/10 Wedge clam	<i>D. acuminata</i> 3.33·10 ²	positive	positive	nd	positive
03/26/10 Yellow clam	<i>D. acuminata</i> 1.33·10 ³ <i>D. caudata</i> 3.3·10 ²	positive	positive	nd	positive
04/12/10 Wedge clam	Absents	nd	nd	positive	positive

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