HIGH AMOUNT OF DINOPHYSISTOXIN-3 IN *Mytilus chilensis* COLLECTED IN SENO DE RELONCAVÍ, CHILE, DURING MASSIVE HUMAN INTOXICATION ASSOCIATED WITH OUTBREAK OF *Vibrio parahaemolyticus*

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ABSTRACT — This study describes the detection of high amount of 7-*O*-acyl-derivative dinophysistoxin-1 (Dinophysistoxin-3) in filter bivalves collected on February 2005 in the Seno de Reloncaví, Puerto Montt City, Southern Chile, in the same period of time where an intoxication episode was associated with the presence of *Vibrio parahaemolyticus* in shellfish.

The Diarrhetic Shellfish Poisoning (DSP) mouse bioassay of mussel extract samples, performed as described for regulatory testing, were negative to DSP toxins. Therefore, the same mussel samples collected from 8 places of Seno de Reloncaví were then analyzed by the HPLC-FLD method with pre-column derivatization procedure for DSP toxins.

The samples showed mainly 7-*O*-acyl-derivative dinophysistoxin-1 (Dinophysistoxin-3) in concentrations ranging from 190.3 ± 6.8 to 311.1 ± 4.8 ng of DSP toxin/g hepatopancreas and less amounts of Dinophysistoxin-1 ranging from 1.9 ± 1.5 to 11.7 ± 4.6 ng of DSP toxin/g hepatopancreas. After alkaline hydrolysis of the mussel extracts, 279.4 ± 7.2 ng of Dinophysistoxin-1 /g hepatopancreas (mean $\pm$ SEM, N=6) were found in mussel extracts (Zone 8). These data showed that these shellfish samples are contaminated with the ester form 7-*O*-acyl-derivatives of Dinophysistoxin-1, far beyond the safe regulatory limit. This paper also shows a direct relation between lipid content in the mussel tissue extracts and the levels of Dinophysistoxin-3. The 7-*O*-acyl-derivative dinophysistoxin-1 ester was the only compound associated with DSP toxins detected in the shellfish samples, and in view of the fact that metabolic transformation of Dinophysistoxin-3 into Dinophysistoxin-1 in humans has recently been described in the literature, the consumption of shellfish contaminated with 7-*O*-acyl-derivatives dinophysistoxin-1 could be a major reason that explains the diarrhetic symptoms shown by the intoxicated patients.

KEY WORDS: Dinophysistoxin-3, Dinophysistoxin-1, DSP human intoxication, *Vibrio parahaemolyticus*, Filter bivalves

INTRODUCTION

Toxic harmful algal bloom (HAB) arises due to the exponential growth of toxins producing dinoflagellates or diatoms. Blooms are triggered by multiple environmental factors, such as a nutrient increase in the water column, increasing utilization of coastal waters for aquaculture, eutrophication and unusual climatological changes (Hallegraeff 1993). Outbreaks of these

species are a constant threat to public health worldwide. Have a negative impact on the marine ecosystem, and cause severe economic losses to aquaculture, fisheries and tourism (Lagos, 1998). Outbreaks associated with DSP have been reported in the southern Chilean Patagonian fjords since 1970 (Muñoz *et al.*, 1992; Uribe *et al.*, 2001; García *et al.*, 2003, 2004, 2005). Presently, the X Region of Southern Chile, where the Seno de Reloncaví is located, shows the endemic pres-

ence of DSP toxins (Lagos, 1998; Uribe *et al.*, 2001; García *et al.*, 2003, 2004, 2005). Moreover, detection of DTX-3 has also been reported in shellfish samples collected in this Region (García *et al.*, 2004).

Diarrhetic Shellfish Poisoning (DSP), is the syndrome caused by diarrhetic shellfish toxins (DSP toxins), characterized by a toxic symptomatology that develops within 30 min to 6 hr after contaminated bivalve consumption, including diarrhea (60%), nausea (46%), vomits (31%), and abdominal pain (77%). If intoxication level is mild, the syndrome favorably evolves toward total recovery into one to three days illness. No fatalities have been recorded (Yasumoto *et al.*, 1978; Hamano *et al.*, 1986; Aune *et al.*, 1998; García *et al.*, 2003, 2004, 2005).

The DSP toxins are 38 carbons lipophylic polyethers, among which stand out Okadaic Acid (OA), Dinophysistoxin-1 (DTX-1) and Dinophysistoxin-2 (DTX-2), produced by dinoflagellates of the genre *Dinophysis* *sp* and *Prorocentrum* *sp* (Yasumoto *et al.*, 1979; Lee *et al.*, 1989; Yasumoto and Murata, 1993). The DSP toxins are accumulated by filter bivalves which agglomerate 80% of toxins in their digestive gland. Depending on the bivalvular refill and natural rate of depuration, the DSP toxins can be metabolically modified inside the bivalves. Due to their chemical stability and lipophylic nature, normal shellfish cooking or steaming does not reduce the toxic effect of DSP toxins (Hu *et al.*, 1992a, 1992b; Gestal-Otero, 2000). In mussel tissues DTX-1 is esterified in the hydroxyl group of Carbon 7. This enzymatic modification is done using fatty acids ranging from tetradecanoic acid (C14:0) to docosahexaenoic acid (C22:6w3), palmitic acid being the most common fatty acid found in DTX-1, of which 90% is linked to the 7-*O*-acyl-derivative of dinophysistoxin-1 named Dinophysistoxin-3 (DTX-3) (Yasumoto *et al.*, 1985).

The *Vibrio parahaemolyticus* is a gram-negative, halophylic bacterium that inhabits warm estuarine waters worldwide and causes gastroenteritis linked to consumption of contaminated raw or undercooked seafood. In addition to diarrhea, its intoxication symptoms include abdominal cramp (82%), chills (44%), myalgias (36%), self-reported fever (34%), headache (32%) and vomiting (29%). Diarrhea may contain mucus (21%) or blood (7%). The mean incubation infection period is 15 hr (ranging from 4 to 90 hr) and the mean duration of illness is 5 days (ranging from 1 to 13 days) (McLaughlin *et al.*, 2005).

The *Vibrio parahaemolyticus* has been monitored by the Chilean Health Authority after the Summer

2000 outbreaks that intoxicated 1,500 patients. Since, other outbreak occurred from January to March 2004 in Puerto Montt, regulations have been enforced to obligate compulsory notification of this disease to the National Health Authority (González-Escalona *et al.*, 2005). There are no reports of *V. parahaemolyticus* infection before 2004 in this X Region. Analysis of clinical samples showed the presence of the *V. parahaemolyticus* O3:K6 pandemic clonal group. However, the analysis of shellfish samples during the epidemics of 2004 and 2005 showed that only 53% of the total shellfish samples analyzed were shown to be *V. parahaemolyticus*-positive. Moreover, from 50 positive shellfish samples, only three contained detectable levels of the pandemic clone (Fuenzalida *et al.*, 2006). In the 2005 summer season, 3,693 people were intoxicated by shellfish consumption with clinical diagnostics reported in this X Region (Olea *et al.*, 2005).

Because of the symptom similarities between DSP toxins and enteropatogens intoxication, this type of poisoning is frequently associated clinically with that produced by *Vibrio parahaemolyticus* and *Bacillus cereus*, which are usually found in bivalves.

In this paper we report the presence of 7-*O*-acyl-derivative-dinophysistoxin-1 (DTX-3) as the only DSP toxin compound detected in shellfish consumed by humans intoxicated with diarrhetic symptoms. These patients were clinically associated with the presence of *Vibrio parahaemolyticus* in Puerto Montt City, Seno de Reloncaví, Southern Chile.

MATERIALS AND METHODS

Reagents

Okadaic acid (OA) and Dinophysistoxin-1 (DTX-1), standard toxins, were obtained from SIGMA (Sigma Chemical Co, St, Louis, Mo, USA); 9-antryldiazomethane (ADAM) was purchased from Molecular Probes (USA); deoxycholic acid (DOCA) was purchased from SIGMA (Sigma Chemical Co, St, Louis, Mo, USA); HPLC grade solvents (acetonitrile, diethyl ether, acetone, methanol, dichloromethane, chloroform) were purchased from Merck (MERCK, Darmstadt, Germany); the SEP-PAK® Cartridges for solid phase extraction of Silica and C-18 were purchased from Waters Corporation (Division of MILLIPORE, Milford, Ma. USA); dichloromethane and hexane, used for extraction and clean-up (Mallinckrodt, USA); glass distillation was used when solvent quality did not meet the requirements of purity specified by standard operation procedures (SOP); water of high purity grade

was obtained by elution through an ion exchange cartridge, and then by boiling for 2 hr with nitrogen bubbling.

Mouse Bioassay of DSP toxins

The Bioassay was done according to Yasumoto *et al.* (1980). Twenty-gram samples of homogenized hepatopancreas are extracted thrice with 100 ml of acetone. The extracts are filtered, then the filtrate is collected and the solvent removed by rotary evaporation. The residue consists of up to 20 ml of water and the suspension extracted thrice with 50 ml of diethyl-ether. The combined organic layers are backwashed twice with small quantities of water and evaporated to dryness. As in the original procedure described above, the residue is resuspended in 1% Tween 60 solution to a concentration of 5 g hepatopancreas/ml Tween 60 prior to intraperitoneal injection into each of three mice (CF-1) weighing 18 g. Initially, 1 mouse unit (MU) was defined as the minimum dose of toxin required to kill a 16 – 20 g mouse within 24 hr.

Mussel extraction and analytical High-Performance liquid chromatography

The mussel extracts were obtained from shellfish samples collected on February 2005 from the X Region of Chile. Two grams of the digestive glands were removed from *Mytilus chilensis* (Blue mussel), then homogenized and extracted twice with 3 ml of chilled 80% methanol, under mechanical stirring using a tissue tearer (BioHomogenizer M 133/2280, Biospec Products, Inc., Bartlesville, OK, USA). Then the methanolic phase was centrifuged at $1,500 \times g$ for 5 min. and 2.5 ml of the supernatant diluted with water to a final 26.66% methanol. 5 ml from this dilution were then transferred to a 250 mg C-18 SEP PAK® cartridge. The system was washed with 5 ml of 50% methanol. Then 5 ml of pure methanol were added, in order to elute the DSP toxins, and this eluted fraction was evaporated to dryness under reduced pressure in a Speed Vac Plus (Savant, SC 210A, Farmingdale, NY, USA). The clean and dry extracts were used for derivatization with ADAM.

The HPLC chemical analysis was performed on a Shimadzu Liquid Chromatograph System equipped with a pump (Shimadzu LC-6A), a Rheodyne injector (7725i Rheodyne, Cotati, Ca. USA) and a Fluorescence detector (Shimadzu RF-535). 10 µl of sampled toxins derivatives were injected on a reverse phase column Supelcosil LC-18 (5 µm; 25 cm × 4 mm) (Supelco, Bellefonte, PA, USA). An isocratically

mobile phase of CH₃CN/CH₃OH/H₂O 8:1:1 (v/v) with a flow rate of 1 ml/min was run at room temperature. The excitation and emission wavelengths were set at 365 and 415 nm respectively. Peaks in the resulting chromatograms were identified by comparison with the retention times of each DSP phycotoxin analytical standard.

Alkaline hydrolysis esterifies DSP toxins

The hydrolysis of the extracts was done according to García *et al.* (2004). In this case, 2.5 ml of 0.5 N NaOH in 90% methanol solution were added to a 2.5 ml aliquot of the 80% methanol extract of each shellfish sample. The mixture was heated for 50 min at 75°C. After evaporating the methanol from the reaction mixture, the aqueous layer was acidified with 0.5 N HCl and then extracted twice with 5 ml of diethyl ether. After evaporating the solvent, the extracts were dissolved in 2.5 ml of 80% methanol and extracted twice with 2.5 ml of hexane. Additionally, 1 ml of 0.2% acetic acid was added to the methanolic solution, with the resulting toxin solution extracted with 4.0 ml of dichloromethane. This eluted fraction was evaporated to dryness under reduced pressure in a Speed Vac Plus (Savant, SC 210A, Farmingdale, NY) and then derivatized.

Derivatization of DSP phycotoxins with ADAM

The derivatization of standards and extracts of toxin samples were carried out according to García *et al.* (2003). Briefly, the solid mussel extract residues or standards were treated with a freshly prepared solution of 0.1% ADAM (in 100 µl of acetone and 400 µl of methanol) (Lee *et al.*, 1987). After 1 hr at 25°C in the dark, samples were evaporated to dryness and the residues diluted in 200 µl CH₂Cl₂/hexane, 1:1 (v/v) and then transferred into a 500 mg Silica gel SEP PAK® cartridge. Each system was washed successively with 5 ml of CH₂Cl₂/hexane, 1:1 (v/v) and 5 ml CH₂Cl₂ and finally eluted with 5 ml of CH₂Cl₂/methanol, 1:1 (v/v). The last fractions were evaporated to dryness, each one dissolved in 1 ml methanol, and then 10 µl samples injected and analyzed by HPLC with fluorescence on line detection (HPLC-FLD).

Analysis of *Vibrio parahaemolyticus*

Feces samples collected from patients were forwarded to the Instituto de Salud Pública laboratory and tested for *Vibrio parahaemolyticus*. Enrichment, isolation, enumeration, screening and confirmation were performed as described in the Bacteriological Analyti-

cal Manual of the US Food and Drug Administration (Kaiser and DePaola, 2004).

Collection and analysis of phytoplankton

Phytoplankton samples were collected using a Rutner net with a 10.4 cm mouth opening and mesh size of 20 μm . Vertical hauls were carried out from 15 meters depth. The phytoplankton sample (water sample) were examined directly in the living state and in samples preserved with Lugol's iodine solution.

Toxic species were first identified using a Zeiss inverted microscope and then counted according to the standard Utermöhl technique (Utermöhl 1958). The result of the counting was expressed by liter, thus establishing the numerical density of toxic species and total phytoplankton. The taxonomic analyses were also performed by direct observation with a contrast phase microscope Unilux-12.

Analyses of lipid content

The shellfish tissues were homogenized with 2.0 g of sodium sulfate anhydrous and then extracted with 180 ml dichloromethane for 8 hr under reflux with Soxhlet extraction apparatus. This extract was reduced to a 1 ml volume for Kuderna-Danish concentrators at 60°C. Then 20 μl of the sample were weighed using an analytical balance. The data of the lipid content analysis correspond to eight samples collected in each monitoring site (black dots in the map, Fig. 1) and are expressed in Fig. 4 and Table 1, as Average $\pm$ Standard Deviation, $n=8$.

RESULTS

A total number of 35 patients were intoxicated, ten children (8.9 ± 1.6 years; mean $\pm$ SEM, $N=10$) and twenty five adults (39.3 ± 3.5 years; mean $\pm$ SEM, $N=25$). They arrived at the local Emergency Room of Puerto Montt Hospital in January 2005, exhibiting symptoms of digestive intoxication. They reportedly had eaten Chilean blue mussels (*Mytilus chilensis*) two hours before, in a place near Angelmo in the Seno de Reloncaví ($41^\circ 28' \text{ L.S.} - 72^\circ 56' \text{ L.W.}$). Patient's symptoms were as follows: nausea (35%), vomiting (26%), abdominal pain (85%) and diarrhea (75%). The most frequent complaints were associated with intense abdominal pain and fluid diarrhea.

After the intoxication crisis, a monitoring program for *Vibrio parahaemolyticus* was activated in this area. Search for *Vibrio parahaemolyticus* was negative for all mussel samples tested during this period (Insti-

tuto de Salud Pública de Chile). Samples also tested negative for the DSP toxins mouse bioassay (Servicio Salud Puerto Montt). The mouse bioassay is one of the customary methods to detect DSP toxins in shellfish extract (Yasumoto *et al.*, 1978).

The plankton sample, collected by horizontal dragging in the Seno de Reloncaví area, showed the presence of *Dinophysis acuta*, a worldwide dinoflagellate DSP toxin producer, also present in Chilean coastal waters (Hernández, 2005. Personal Communication). Fig. 1 shows a map of Seno de Reloncaví in which the DSP- contaminated Blue mussel samples were collected (Zone 1: $41^\circ 41.5' \text{ S} - 72^\circ 37' \text{ W}$; Zone 2: $41^\circ 25' \text{ S} - 72^\circ 18' \text{ W}$; Zone 3: $42^\circ 17' \text{ S} - 72^\circ 49' \text{ W}$; Zone 4: $41^\circ 36.5' \text{ S} - 72^\circ 25' \text{ W}$; Zone 5: $41^\circ 30.00' \text{ S} - 72^\circ 55' \text{ W}$; Zone 6: $41^\circ 31' \text{ S} - 72^\circ 52' \text{ W}$; Zone 7: $42^\circ 26' \text{ S} - 72^\circ 15' \text{ W}$; Zone 3: $41^\circ 17' \text{ S} - 73^\circ 46' \text{ W}$). The mussel sample extracts testing negative for the DSP toxin mouse bioassay were analyzed by HPLC-FLD according to the derivatization conditions described by García *et al.* (2003). Fig. 2A shows the chromatographic run of mussel extract esterified with ADAM, a fluorescent chromophorus, and as detected by HPLC-FLD. Fig. 2A shows the typical mussel sample extract chromatogram, here with only a small amount of DTX-1 toxin detected (range, 1.9 ± 1.5 to 11.7 ± 4.6 ng/g hepatopancreas; mean $\pm$ SEM, $N=6$). The DTX-1 toxin yielded a single peak showing a retention time of 11:15 min. When the mussel extracts were treated by an alkaline hydrolysis procedure, the amount of DTX-1 increased to 274.7 ± 7.4 ng/g hepatopancreas (mean $\pm$ SEM, $N=6$), showing the chemical transformation between 7-*O*-acyl-derivatives of dinophysistoxin-1 (DTX-3) into DTX-1 (Fig. 2B). Contents of DTX-1 and DTX-3 in the extracts of mussel samples collected in eight places of the Seno de Reloncaví are shown in (Table I). The levels of total DTX-1 equivalent, ranged from 160.2 ± 13.2 to 320.4 ± 5.6 ng/g hepatopancreas (mean $\pm$ SEM, $N=6$). When DTX-1 standard is spiked into dichloromethane-clean extracts the average recoveries were 97% when compared with standard solutions. Fig. 2C shows the DTX-1 analytical standard chromatogram of the spiked sample.

Fig. 3 shows the DTX-3 percentage as a function of the total content of DTX-1- equivalent (ng/g hepatopancreas) in the blue mussel extracts analyzed. The percentage of Dinophysistoxin-3 ranged from 96 to 99%, meaning that DTX-1 was in its esterified form in percentage above 96% in the blue mussel samples analyzed.

Fig. 4 shows the amount of DTX-3 (ng/g hepato-

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pancreas) as a function of the total lipid content of the blue mussel digestive gland dry mass extracts. All samples showed a direct relation between the high levels of total lipid contents and the level of DTX-3. The DTX-3 toxin contents are higher in zones with high lipid contents of 23.6 to 24.7% (mean $\pm$ SEM, N=6), showing a clear affinity of the toxin for a lipidic environment.

DISCUSSION

Data collected in this paper suggest that the massive human intoxications produced by consumption of bivalves are mainly associated to DTX-1 which originates from its esterified form of 7-*O*-acyl-derivatives of dinophysistoxin-1 (DTX-3) instead of epidemiologic association with *Vibrio parahaemolyticus* as the preliminary diagnostics done by the Physicians in the Emergency Room.

The *O*-acyl-derivative dinophysistoxin-1 was not

detected by the customary mouse bioassay method, and consequently the shellfish was authorized for sale at the local market. The fact that all of the victims consumed blue mussels after heat cooking reduced the possibility of bacterial contamination but not of intoxication with DSP-toxins, which are heat-stable.

Since the *O*-acyl-derivative dinophysistoxin-1 (DTX-3) was the major toxic component found in shellfish sample extracts, a possible explanation for the diarrheic symptoms and the intoxication syndrome showed by the patients would be the metabolic transformation of *O*-acyl-derivative dinophysistoxin-1 (DTX-3) into Dinophysistoxin-1 (DTX-1) inside the patients digestive tract (García *et al.*, 2005).

The negative results for enteropathogens analysis in patient fecal samples and the negative mouse bioassay for DSP toxins strengthens this hypothesis. The metabolic transformation of DTX-3 into DTX-1 should occur in the digestive tract, mainly in the stomachs of poisoned patients. Enzymes such as lipases and

Fig. 1. Map of Seno de Reloncaví in Puerto Montt city, X Region, Chile. The dot ●○ show the littoral places where the DSP-contaminated shellfish were collected by the monitoring program after the intoxication.

esterases, which normally digest daily nutrients and xenobiotics, can hydrolyze DTX-3 by converting it into DTX-1, the most potent DSP toxin (Rivas *et al.*, 2000). DTX-1 was responsible for the intoxication

syndrome suffered by the patients. The fast appearance of the diarrhetic intoxication symptoms, less than 2 hr after shellfish consumption reported by the intoxicated patients, also strengthens the hypothesis of DSP intox-

Fig. 2. Chromatograms of DSP toxin standards and blue mussel extract samples. A.- Blue mussel extract without alkaline hydrolysis. B.- Blue mussel extract after 0.5 N NaOH treatment (alkaline hydrolysis) . C.- Dinophysistoxin-1 analytical standard (DTX-1).

Table 1. Content of Dinophysistoxin-1 and 7-*O*-acyl-derivatives of Dinophysistoxins-1 esters in 8 zones in Seno Reloncavi, X Región. The data is expressed as Average $\pm$ Standard Deviation, n=8.

Origin of Sample Blue mussels	Free DTX -1 (ng/g)	DTX -3 (ng/g)	Total DTX-1 equivalent (ng/g)	OA esters %
Zone 1	6,1 $\pm$ 1,8	220,5 $\pm$ 5,0	226,6 $\pm$ 3,9	98
Zone 2	2,8 $\pm$ 1,7	275,9 $\pm$ 7,0	278,7 $\pm$ 6,1	98
Zone 3	9,4 $\pm$ 2,4	311,1 $\pm$ 4,8	320,4 $\pm$ 5,6	97
Zone 4	1,9 $\pm$ 1,5	158,2 $\pm$ 13,1	160,2 $\pm$ 13,2	96
Zone 5	11,7 $\pm$ 4,6	270,5 $\pm$ 7,0	282,2 $\pm$ 10,5	96
Zone 6	11,1 $\pm$ 2,1	199,4 $\pm$ 3,7	210,5 $\pm$ 4,2	99
Zone 7	2,8 $\pm$ 1,7	190,3 $\pm$ 6,8	193,1 $\pm$ 6,8	97
Zone 8	4,7 $\pm$ 3,0	274,7 $\pm$ 7,4	279,4 $\pm$ 7,2	99

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ication, since enteropathogens need incubation periods of at least of 12 hr to produce diarrhetic symptoms (McLaughlin *et al.*, 2005).

The presence of DTX-3 in Chilean shellfish is not unusual in samples collected in this area. Moreover, all samples measured by our laboratory in the last five years have shown DTX-1 after alkaline hydrolysis treatment of the shellfish extract, with this DTX-1 coming from the 7-*O*-acyl-derivatives of dinophysistoxin-1, making DTX-1 the major DSP toxin in Chilean shellfish samples (Uribe *et al.*, 2001; Lagos, 2002; García *et al.*, 2004). The first report of DSP toxins in Chilean shellfish samples described DTX-1 as the major DSP toxin in Chilean mussel samples (Zhao *et al.*, 1993).

Contaminated mollusks can modify DSP toxins through enzymatic processes, allowing their esterification with fatty acids. This metabolic transformation generates products such as the 7-*O*-acyl-derivatives of dinophysistoxins (Suzuki *et al.*, 1999; Vale and Sampayo *et al.*, 2002). DSP esterifies toxins do not inhibit Protein Phosphatase activity and therefore show

low toxicity in mamms. The covalent binding generates a steric impediment to the carboxyl group, which is a determinant for the inhibition of Protein Phosphatase (Takai *et al.*, 1992). The DTX-1 to 7-*O*-acyl-DTX-1 biotransformation is likely to be a route for DTX-1 detoxification in mussels, since DTX-3 does not inhibit protein phosphatases and for that reason exhibits very low toxicity (García *et al.*, 2005). The levels of total DTX-1 equivalent, ranging from 160.2 ± 13.2 to 320.4 ± 5.6 ng/g hepatopancreas (mean $\pm$ SEM, N=6), are over the international safety limit of 200 ng/g of DSP toxins / gram of hepatopancreas. Moreover, the contaminated shellfish showed DTX-3 amounts of nearly 200 ng/g hepatopancreas (between 158.2 ± 13.1 to 311.1 ± 4.8 ng/g hepatopancreas; mean $\pm$ SEM, N=6).

The seasonal variations in the filter bivalve fatty acid composition of the triacylglycerol are well documented (Napolitano and Ackman 1993). The saturated fatty acid composition increases significantly in summer, where the total lipid content of the digestive gland is above 24% of the dry mass during the period of

Fig. 3. Content of 7-*O*-acyl-derivative-dinophysistoxin-1 (DTX-3). Percentages are show as a function of the total content of DTX-1 equivalent (ng/g hepatopancreas).

energy storage, such as the spring – summer period (Pazos *et al.*, 2003). The lipophilic character of the DTX-3 molecule together with the relatively high lipid content of the digestive gland (De Zwaan and Marhiers, 1992) indicates that DTX-3 may have an affinity for lipid-rich cellular and intracellular components such as membranes and lipid droplets (Svensson and Forlan, 2004). The degree of bioaccumulation depends mainly on the hydrophobicity of the contaminant together with the lipid content of the organism. (Svensson, 2002).

In conclusion, since: a) the victims consumed blue mussels after heat cooking, reducing the possibility of bacterial contamination, b) the negative result for the presence of enteropathogens in patient fecal samples, c) the diarrhetic symptoms developed in no more than 2 hr after the shellfish was eaten, and d) the *O*-acyl-derivative dinophysistoxin-1 (DTX-3) was the major toxic component found in shellfish sample extracts, this study suggests that the intoxication symptoms showed by the 35 patients described above are

more likely to be associated with to the presence of DSP toxins than with the enteropathogen *V. parahaemolyticus* in the blue mussel filter bivalve (*M. chilensis*). This finding also suggests that during the summer period in the Seno de Reloncaví, Puerto Montt, the monitoring of DSP toxin content in shellfish samples should be enforced. Furthermore, the shellfish extracts should be treated by alkaline hydrolysis in order to detect DTX-3 as 7-*O*-acyl-DTX-1, since the DTX-3 to DTX-1 biotransformation in humans has been reported and shown to be responsible for massive intoxication by 7-*O*-acyl-DTX-1 in contaminated shellfish consumption (García *et al.*, 2005).

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Fig. 4. Lipid content (%) in mussel tissue and amount of DTX-1 equivalents in hepatopancreas of blue mussel samples collected in Seno de Reloncaví. The data is expressed as Average $\pm$ Standard Deviation, $n=8$.

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