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Accumulation and biotransformation dynamics of the neurotoxic complex, saxitoxin, in different life stages of *Ostrea chilensis*

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ABSTRACT

The neurotoxic complex saxitoxin, is a group of marine toxins that historically has significantly impacted human health and the ability to utilize marine resources. A steady increase in the distribution and intensity of *Alexandrium catenella* blooms in Chile, and around the world, has caused major ecological and socioeconomic impacts, putting this type of dinoflagellate, and its toxicity, in the spotlight. *Ostrea chilensis* is a commercially and ecologically important resource harvested from wild populations and farmed in centers of southern Chile, where it is exposed to large harmful algal blooms of the type that can cause paralysis in humans. This study contributes to our understanding about the transfer of toxins from *A. catenella* cells to juvenile and adult *Ostrea chilensis* by tracking transformations of the neurotoxic complex until it reaches its most stable molecular form in the intracellular environment of *O. chilensis* tissues. These biotransformations are different in *O. chilensis* juveniles and adults, indicating a differentiated response for these two life stages of this bivalve species. These studies can be used for similar analyses in other ecologically and commercially important species of filter feeding organisms, providing greater understanding of the specific interactions of bivalves in scenarios of toxic dinoflagellate proliferations (e.g. *A. catenella* blooms).

1. Introduction

Harmful algal blooms (HABs) that produce paralytic shellfish toxin (PST) are generated by different dinoflagellate species of the genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium* (Shumway, 1995). The distribution of HABs has increased globally over recent decades (Anderson, 1989; Anderson et al., 1994). *Alexandrium* is a cosmopolitan genus containing species, which produce saxitoxin (STX), a compound grouping around 24 analog derivatives that differ in structure and toxicity (Etheridge, 2010). Chile has not been spared from HABs; the toxic dinoflagellate *Alexandrium catenella* was reported for the first time in 1972 around Magallanes (49°08′S, 74°48′W) (Guzmán et al., 1975) and has gradually moved north. In 2016, an intense *A. catenella* bloom occurred along the southern coast of Chile (39°45′S) (Hernández et al., 2016), where the surf clam, *Mesodesma donacium* from Chiloé Island registered an intoxication peak of 9.4×10^3 µg STX eq/100 g. This coincided with the high concentrations of *A. catenella* in the first 10 m of the water column (2.5×10^5 cells/L). This geographical expansion has negatively impacted southern Chile's public health and economy, affecting both aquaculture and the harvesting of marine organisms from wild populations (Fernández-Reiriz et al., 2008; Navarro et al., 2014).

The toxic composition of dinoflagellate species has been reported to change significantly (Etheridge and Roesler, 2005; Poulton et al., 2005; Montoya et al., 2010). Because the composition and concentration of toxins differ by geographical origin (Etheridge, 2010), it is necessary to study the toxic profiles of these microalgae in each affected region.

Saxitoxin can be accumulated by different taxa of marine animals such as: zooplankton (Doucette et al., 2005), bivalves (Fernandez-Reiriz et al., 2008), crustaceans (Robineau et al., 1991), and gastropods (Ito et al., 2004). All of these can then act as transference vectors to other levels of the trophic chain (Chen et al., 1998; Suzuki et al., 2003). Bivalve and copepod behaviour (Bricelj et al., 2005) and physiology (Bagøien et al., 1996; Frangópolus et al., 2000) have responded negatively to diets containing STX, displaying decreased food intake, metabolic depression, and reduced growth (Shumway et al., 1985; Li et al., 2002; Bricelj et al., 2005; Dam, 2013). The fact that different bivalve

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species respond differently has been linked to evidence a differentiated process of biotransformation and accumulation of toxins present in food for each species; that is, a species-specific response (Bricelj et al., 1991; Choi et al., 2003). Previous studies indicate that bivalves generally contain a lower proportion of N-sulfocarbamoyl toxins (B1, B2, C1, C2, C3, C4) and a higher proportion of carbamoyl toxins (STX, NEO, GTX1, GXT2, GTX3, GTX4), both of which are present in the ingested dinoflagellates (Oshima et al., 1990; Cembella et al., 1993; Choi et al., 2003). Species differ not only by type of toxin biotransformation but also in the distribution of toxins in tissues and depuration kinetics. Choi et al. (2003) exposed two bivalves, *Chlamys nobilis* and *Perna viridis*, to the toxic dinoflagellate, *Alexandrium tamarense*, reporting that a significant fraction of GTX5 was detected in *P. viridis* tissues but none was found in the scallop *C. nobilis*.

Saxitoxin can be divided into three toxic structural groups based on the nature of the lateral chain of each toxic molecule. These are, from most to least toxic: carbamate (R4 = $-CONH_2$); decarbamoyl (R4 = $-OH$), and N-sulfocarbamoyl (R4 = $-CONHSO_3^-$) (Cembella et al., 1993; Krock et al., 2007). Incorporating these toxins into the tissues of organisms produces a series of biotransformations that may differ between life stages within a given species. Toxin absorption by bivalves is often followed by epimerization processes for the β-epimers (C2, GTX3, GTX4), produced primarily by the dinoflagellates. Eventually the β-epimers are transformed into α-epimers (C1, GTX2, GTX1), a more thermostable form. Guéguen et al. (2011) found that when *Crassostrea gigas* was fed with *Aleaxadrium minutum*, toxic dinoflagellate compounds are biotransformed, significantly increasing the proportions of the toxins in the tissues. Li et al. (2012), working with the scallop *Patinopecten yessoensis* and the clam *Saxidomus purpuratus*, intoxicated in the natural environment with PST, found that the biotransformation activity is species-specific. They compared both species and obtained similar toxins, however, the metabolites were different between the species.

Ostrea chilensis is distributed from Chiloé Island to the Guaitecas Islands (Solís, 1967), where ~300 tons are harvested each year from farms and natural populations (Sernapesca, 2015). This suggests that *O. chilensis* could act as a direct toxin transference vector to humans during *A. catenella* toxic events. Given this, it is imperative to identify the types of toxins contained in *A. catenella* strains as well as the intoxication dynamics and toxin transformations of the filter feeding bivalves inhabiting coastal Chile. The objective of this research was to determine the toxic profile of the dinoflagellate, *A. catenella*, isolated from southern Chile (43°6′S, 73°36′W) and to understand how these profiles are bio-transformed in the tissues of juvenile and adult *O. chilensis*.

2. Materials and methods

2.1. Collection sites

In April 2013, juvenile and adult specimens of the bivalve, *O. chilensis* (commonly the Chilean "dredge" or "bluff" oyster), were collected from trays suspended in the water at the Chilean Oyster Farming Center of the Universidad Austral de Chile, Quempillén estuary, southern Chile (41°52′S; 73°46′W). The average oyster shell length was 24.30 ± 0.28 mm (juveniles) and 52.5 ± 0.28 mm (adults). Experimental specimens were maintained under laboratory conditions (8-L aquaria at 14 °C, 30 psμ, with constant aeration) for 2 weeks before any measurements were taken; water was changed every 48 h. During the acclimation period, the oysters were continuously fed a diet of *Isochrysis galbana* (~2 mg L^{-1}) via a peristaltic pump.

2.2. Diets and experimental design

Cultures of the toxic dinoflagellate, *A. catenella* (Coldita strain), were fed to juvenile and adult oysters during the experimental period. *Alexandrium catenella* was isolated in 2011 from Coldita Island, south of Chiloé, Los Lagos Region, by the Fisheries Development Institute (Instituto de Fomento Pesquero, IFOP). These dinoflagellates were cultivated in filtered seawater (0.45 μm) enriched with L1 culture medium (Guillard, 1995) at 14 °C, 30 psμ, under a photoperiod of 14:10 L:D. In turn, a non-toxic microalga, *Isochrysis galbana*, used as part of the experimental diet, was cultivated with f/2 (Guillard, 1975) at 25 °C, 30 psμ, under a photoperiod of 14:10 L:D. Both cultures were used in their exponential growth phase.

During the experimental period, juvenile and adult oysters were exposed to a mixed toxic diet (70% *A. catenella* + 30% *I. galbana*) for 30 days. In the case of juvenile oysters, five replicates were set up containing 25 juveniles each (in 8-L aquaria). Another five replicates, also with 25 juveniles each, were set up and fed the control diet (100% *I. galbana*). Both diets were delivered through peristaltic pumps, providing 1.48 mg/day/oyster, or 4% of the average dry weight of the soft tissues of the experimental specimens. On each sampling day (0, 5, 10, 30), three specimens were removed from each replicate for PST analysis.

In the case of adult oysters, four replicates (15 specimens each) were set up (in 8-L aquaria) for the experimental group exposed to the toxic diet (70% *A. catenella* + 30% *I. galbana*). Likewise, the control group consisted of four replicates (15 oysters each, in 8-L aquaria); however, these were fed the non-toxic diet (100% *Isochrysis galbana*). Specimens in both groups were fed daily 7.5 mg/day/oyster, or 1% of the average dry weight of the soft tissues of the experimental adult oysters. Experimental specimens were sampled on days 0, 5, 10, 20, and 30, in order to identify toxin profiles and estimate toxicity. The latter is expressed as μg STX eq/100 g soft tissue according to the level of toxicity of the STX derivatives caused in a mouse bioassay (Quilliam, 2007).

To estimate the weight and organic content of the experimental diets, known volumes were filtered through previously dried, burned, and weighed Whatman GF/C filters (47-mm in diameter). These filters were washed with an isotonic solution of ammonium formate, dried for 24 h at 100 °C, and weighed to obtain the dry weight of the sample. They were then burned at 500 °C for 3 h to estimate the organic and inorganic fractions present in the diets (Navarro et al., 2016).

2.3. Toxin extraction from cells and mollusks

A. catenella cells and *O. chilensis* soft-tissue from seeds and adults were sampled for the profile and quantification of PSTs.

A volume of the *A. catenella* cultures (equivalent 5×10^5 cells) in the early growth stage was centrifuged at 3000 rpm for 20 min. Eight samples were obtained. Once the supernatant was removed, each cell-pellet was re-suspended in 300–500 μL of 0.05 M acetic acid (HOAc) and homogenized by vortex for 1 min. The homogenates were filtered through a 0.45-μm nylon filter (13 mm) and transferred to 1.5-mL vials. A volume of 5 μL was injected into the LC–FLD.

The *O. chilensis* flesh (n = 3) was ground by hand or blender. The homogenates were individually transferred to 15-mL plastic centrifuge tubes and homogenized in equal volumes by weight of HCl 0.1 N. They were mixed for 1 min and centrifuged at 6000 rpm for 10 min. The samples were filtered (0.45-μm nylon filter, 13 mm) prior to LC–FLD analysis (5 μL injected).

2.4. LC–FLD analyses

The identification and quantification of PST toxins were carried out by LC-FLD following the method of Franco and Fernández-Vila (1993). The presence of GTXs, dcGTXs, and Cs toxins in the standards and samples was confirmed using a Shimadzu LC-10ADvp equipped with column oven CTO 10-10Avp with an Inertsil ODS-3 C18 column (150 × 4.6 mm ID, 5 μm) (GL Sciences Inc., Japan) at 35 °C. STX, NeoSTX, and dcSTX were separated with a LiChrosphere 100 RP-18 column (12.5 × 4 mm ID, 5 μm) (Merck).

The first eluent for the separation of STX, NeoSTX, and dcSTX

consisted of 1 mL min^{-1} 94% 1 mM sodium octane sulphonate, 10 mM ammonium phosphate, and 6% acetonitrile aqueous solution adjusted to pH 7.2. The second eluent for the separation of GTXs and dcGTXs consisted of 0.8 mL min^{-1} 1.5 mM sodium octane sulphonate and 10 mM ammonium phosphate aqueous solution adjusted to pH 7.0. To separate C-toxins, a third eluent was used: 0.8 mL min^{-1} 2 mM tetrabutyl ammonium phosphate and 10 mM H_3PO_4 aqueous solution adjusted to pH 6.5. All the aqueous solutions were pH-adjusted with 0.5 M ammonium hydroxide (NH_4OH).

Two Series-I LabAlliance pumps were used for delivering the post-column reagents. The oxidation reaction was performed in a Teflon coil (10 m, 0.5 mm ID) at 65 °C using 0.5 mL min^{-1} of 7.0 mM periodic acid and 50 mM H_3PO_4 aqueous solution adjusted to pH 9.0 with 5 M sodium hydroxide (NaOH). The effluent was acidified with 0.5 mL min^{-1} 0.5 M acetic acid.

The fluorescent toxin derivatives were detected with a spectrofluorometric detector Shimadzu RF-551 equipped with a 150-W xenon lamp (Ushio), a 12-μL flow cell, with excitation wavelength set at 330 nm and emission at 390 nm (15 ± 5 nm band width), and a signal/noise ratio of 3.

The following working solutions were used to generate a four-point calibration curve with a 5-μL injection volume: concentrations from 0.5 to 2.4 ng μL^{-1} (DL 0.011 μg mL^{-1}) for STX, NeoSTX, and dcSTX; concentrations from 0.1 to 5.0 ng μL^{-1} (DL 0.016 μg mL^{-1}) for GTX 1–4, GTX 2–3, dcGTX 2–3, and GTX5 and concentrations from 0.6 to 5.0 ng μL^{-1} (DL 0.018 μg mL^{-1}) for C 1–2. The samples were quantitatively analyzed by comparing their retention times and fluorescence emission maxima with PST standards. Data acquisition and processing were performed with Class VP software.

Certified toxin standards - commercially available and provided by NRC (Canada) - were used to calibrate the LC–FLD as well as to quantify and/or identify the toxins detected in the samples. The PST groups included in the sample analyses comprised: saxitoxin (STX); neosaxitoxin (NeoSTX); decarbamoyl saxitoxin (dcSTX); gonyautoxin (GTX)-1, 2, 3, 4, 5; decarbamoyl gonyautoxin (dcGTX)-2, 3 and *N*-sulfocarbamoyl gonyautoxin (C)-1, 2.

2.5. Statistical analysis

A descriptive analysis of the toxins present in the dinoflagellate *A. catenella* determined the percentage of toxins present in this strain. To determine the temporal differences in toxin biotransformation, a non-parametric approach was used based on a permutational multivariate analysis of variance (PERMANOVA). For both adults and juveniles, the analysis considered exposure time to *A. catenella* as a fixed factor. On the other hand, the different toxins were evaluated as random factors. To determine the contribution of each toxin over time, a similarity percentage analysis (SIMPER) was used. SIMPER calculates the average dissimilarity between all pairs of samples and then evaluates the relative dissimilarity contributed by each toxin, with a cut-off level of 60% contribution. All analyses were performed using RStudio, Vegan library. The significance was accepted at $p < 0.05$.

3. Results

3.1. Paralytic shellfish toxins in Alexandrium catenella

A total of seven toxins were identified in eight samples of *A. catenella*, with a mean concentration of 22.5 ± 3.4 fmol/cel. The N-sulfocarbamoyl group, represented by C2 (11.9 fmol/cel; 53%) and C1 (1.1 fmol/cel; 4.9%), dominated the toxin profile, followed by the gonyautoxin group represented by GTX4 (4.6 fmol/cel; 20%), GTX3 (1.3 fmol/cel; 6%), and GTX2 (0.2 fmol/cel; 0.88%). The decarbamoyl group was also present, with dcGTX2 (3.2 fmol/cel) and dcGTX3 (0.1 fmol/cel) constituting 14.6% (Fig. 1).

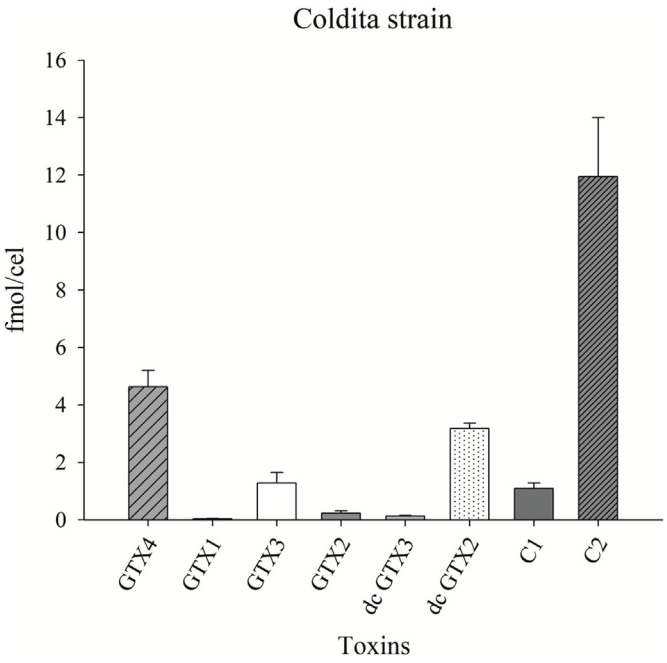

Fig. 1. Toxic profile of *Alexandrium catenella*.

3.2. Adult oysters

In terms of toxicity, the intoxication kinetics of adults were characterized by a rapid accumulation in the first five days (102.10 μg STX eq/100 g soft tissue, st), exceeding the safety limit of 80 μg STX eq/100 g st. The toxin profile was dominated by the GTX group, with concentrations of 65.02 μg STX eq/100 g st for GTX1 (63.69%), 5.22 μg STX eq/100 g st for GTX2 (5.11%), 18.74 μg STX eq/100g st for GTX3 (18.36%), and 12.96 μg STX eq/100 g st for GTX4 (12.69%). Day 5 showed a low concentration of C2 (0.15 μg STX eq/100 g st, 0.15%).

On day 10, the toxicological analysis revealed concentrations of 87.55 μg STX eq/100 g st for GTX1 (60.86%), 2.39 μg STX eq/100 g st for GTX2 (1.66%), 10.32 μg STX eq/100 g st for GTX3 (7.18%), 26.60 μg STX eq/100 g st for GTX4 (18.49%) and the saxitoxin group (16.80 μg STX eq/100g st) represented 11.68% of the total toxins (143.87 μg STX eq/100 g st). The concentration of the N-sulfocarbamoyl group was lower, with C2 concentrations of 0.2 μg STX eq/100 g st (0.14%).

On day 20, toxin concentrations were variable, and STX was the most abundant (93.48 μg STX eq/100 g, 57.03%). In the GTX group, concentrations were 15.76 μg STX eq/100 g st for GTX1 (9.62%), 6.31 μg STX eq/100 g st for GTX2 (3.85%), 25.35 μg STX eq/100 g st for GTX3 (15.46%), and 13.49 μg STX eq/100 g for GTX4 (8.23%). On day 20, the N-sulfocarbamoyl group was also observed, represented by C1 (0.73 μg STX eq/100 g st, 0.45%) and C2 (8.80 μg STX eq/100 g st, 5.37%). The total toxin concentration on day 20 was 163.92 μg STX eq/100 g.

The toxin composition in the tissues of adult *O. chilensis* specimens on day 30 reached 170.88 μg STX eq/100 g (Fig. 2). The GTX group dominated: GTX1 reached 57.03 μg STX eq/100 g (33.38%); GTX2 3.18 μg STX eq/100 g (1.86%); GTX3 22.88 μg STX eq/100 g (13.39%) and GTX4 35.81 μg STX eq/100 g (20.96%). A concentration of 31.51 μg STX eq/100 g, or 18.44% of the total toxins was recorded in the STX group. The N-sulfocarbamoyl group was present in lower concentrations, with C1 reaching only 1.30 μg STX eq/100 g (0.76%) and C2 at 19.15 μg STX eq/100 g (11.21%) (Fig. 2).

The PERMANOVA analysis indicated significant differences in the proportion of toxins accumulated with exposure times to *A. catenella*, ($F_{4.14}$ = 1.780, P = 0.032). During the first five days of exposure, the

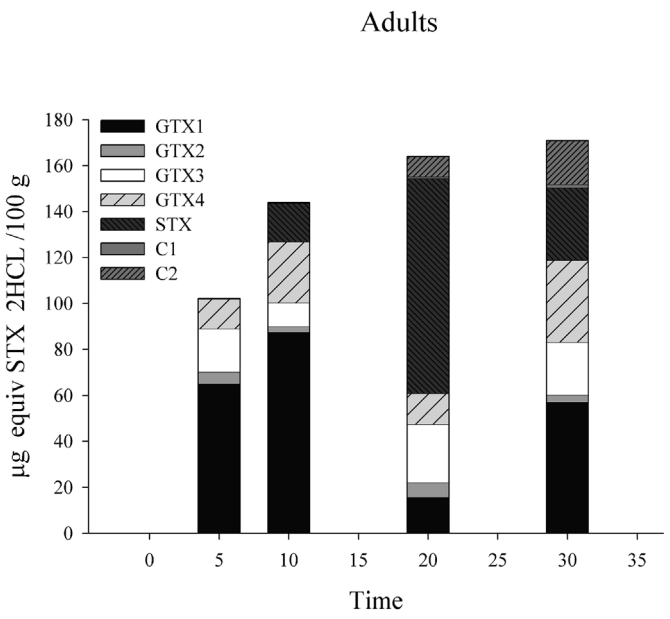

Fig. 2. Toxic profile of *Ostrea chilensis* adults exposed for 30 days to a toxic diet containing *Alexandrium catenella*.

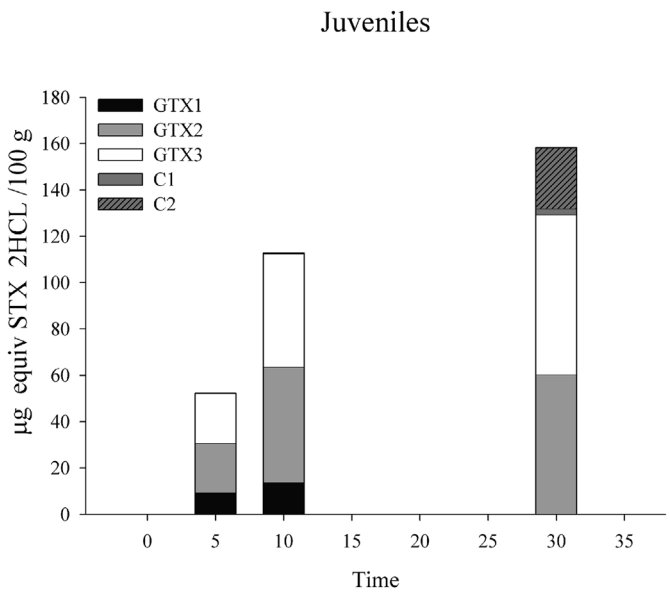

Fig. 3. Toxic profile of *Ostrea chilensis* juveniles exposed for 30 days to a toxic diet containing *Alexandrium catenella*.

Table 1
Summary of similarity percentage (SIMPER). Results show the contribution of toxins at different exposure times (60% cut-off) for *Ostrea chilensis* adults.

Toxins	Mean contribution to overall dissimilarity	Cumulative sum	*P*-value
	Day 0 - Day 5		
GTX1	0.43	0.47	0.05
GTX3	0.18	0.66	0.03
	Day 5 - Day 10		
GTX1	0.40	0.53	0.08
GTX4	0.12	0.68	0.38
STX	0.12	0.83	0.73
	Day 10 - Day 20		
STX	0.26	0.36	0.17
GTX1	0.23	0.68	0.65
GTX4	0.09	0.80	0.77
GTX3	0.08	0.91	0.70
	Day 20 - Day 30		
STX	0.17	0.35	0.51
GTX1	0.15	0.64	0.86
GTX4	0.09	0.82	0.74
GTX3	0.04	0.90	1.00
C2	0.04	0.97	0.63
GTX2	0.01	1.00	0.96
C1	0.00	1.00	0.84
Overall dissimilarity in exposure times for *O. chilensis* adults			0.98

SIMPER analysis indicated that GTX1 (43%) and GTX3 (18%) contributed, on average, the most (98%) to the variation in toxin accumulation with exposure time to *A. catenella* (Table 1).

3.3. Juvenile oysters

Toxin accumulation in *O. chilensis* seeds increased from day 0 to day 30, with variations in toxin concentrations and profiles. On day 5, the seeds reached 52.22 µg STX eq/100 g, when only the gonyautoxin (GTX) group was present with concentrations of 9.41 µg STX eq/100 g for GTX1 (18.02%), 21.25 µg STX eq/100 g for GTX2 (40.70%), and 21.56 µg STX eq/100 g for GTX3 (41.28%). On day 10, the safety limit for human consumption (80 µg de STX eq/100 g st) was surpassed, reaching concentrations of 112.75 µg STX eq/100 g (Fig. 3), with increased concentrations in the GTX group. Recorded concentrations of GTX were 13.75 µg STX eq/100 g for GTX1 (12.20%); 49.84 µg STX eq/100 g for GTX2 (44.21%) and 48.79 µg STX eq/100 g for GTX3 (43.28%). The N-sulfocarbamoyl group was present in low concentrations, with 0.21 µg STX eq/100 g for C1 (0.19%) and 0.15 µg STX eq/100 g for C2 (0.13%).

On day 30, toxin concentrations reached 158.25 µg STX eq/100 g and were still dominated by the gonyautoxin group; however, concentrations of the N-sulfocarbamoyl (C1-C2) group also increased. The profile results showed concentrations of 60.23 µg STX eq/100 g for GTX2 (38.06%), 69.04 µg STX eq/100 g for GTX3 (43.62%), 2.53 µg STX eq/100 g for C1 (1.60%), and 26.44 µg STX eq/100 g for C2 (16.71%).

The PERMANOVA analysis revealed significant differences between *A. catenella* exposure times for *O. chilensis* juveniles ($F_{3.11} = 5.98$, $P = 0.03$). The SIMPER analysis showed a dissimilarity percentage of 99% between the different toxins. The toxins with the highest contributions were GTX2 (40%) and GTX3 (39%) between exposure days 0 and 5 (Table 2).

4. Discussion

The toxin profile for cells of the dinoflagellate, *A. catenella* from the

Table 2
Summary of similarity percentage (SIMPER). Results show the contribution of toxins at different exposure times (60% cut-off) for *Ostrea chilensis* juveniles.

Toxins	Mean contribution to overall dissimilarity	Cumulative sum	*P*-value
	Day 0 - Day 5		
GTX2	0.40	0.40	0.01
GTX3	0.39	0.80	0.02
	Day 5 - Day 10		
GTX2	0.22	0.37	0.78
GTX3	0.22	0.74	0.89
GTX1	0.15	1.00	0.38
C1	0.00	0.99	0.95
C2	0.00	1.00	0.95
	Day 10 - Day 30		
GTX3	0.19	0.34	0.96
GTX2	0.18	0.66	0.96
C2	0.10	0.84	0.33
GTX1	0.08	0.98	0.68
C1	0.01	1	0.08
Overall dissimilarity in exposure times for *O. chilensis* juveniles			0.99

present study resembled those reported by Krock et al. (2007) and Montoya et al. (2010); all showed the main toxic components to be types C2 and GTX4. However, dcGTX2 and dcGTX3 were also present in the profiles studied herein. These toxic components belong to an uncommon toxic group in *A. catenella* strains, being present in lower percentages than the strain's other toxic components. This could be because, as explained by Montoya et al. (2010), the toxic composition of *Alexandrium* strains can vary despite a lack of genetic differences. This suggests that genetic recombination during the encystment stage allows the toxin composition to vary between *A. catenella* cells (Montoya et al., 2010).

Our results showed that the both the *A. catenella* strain and *O. chilensis* transformed toxins from one type to another. According to Krock et al. (2007), this would due to epimerization processes and metabolic transformations. Transformations in *O. chilensis* tissues are differentiated between development stages, resulting in very different toxic profiles for juvenile versus adult oysters. The compositions of these profiles changed with duration of exposure to the toxic diet but were always different from the toxic profile of the dinoflagellate. Likewise, Blanco et al. (2003) observed changes in the toxic profile of tissues from the bivalve, *Mytilus galloprovincialis*. Such changes in the conformation of toxic profiles are due to epimerization processes. These spontaneous transformations most likely occur in response to the intracellular environment of the bivalve, which differs from the interior environment of the *A. catenella* cell (Blanco et al., 2003). The β epimers (C2, GTX3, GTX4), primarily produced by dinoflagellates, are usually transformed to their thermodynamically more stable α epimers (C1, GTX2, GTX1) after being consumed by bivalves (Oshima, 1995; Bricelj and Shumway, 1998). This is clear from comparisons of the profile of *A. catenella* with those of the juvenile and adult oysters, both of which show the presence of GTX1 despite its absence from the Coldita strain profile. This indicates the occurrence of epimerization processes in the bivalve tissues, process also observed in other bivalves of southern Chile, such as *Aulacomya ater* (Krock et al., 2007).

In turn, some toxin-reducing transformations are characterized by a decrease in the N−OH toxin group (GTX1, GTX4) and an increase in the N−H toxin group (GTX2, GTX3, STX). These reactions occur by enzymatic desulfonation (i.e. sulfotransferases), natural reductants, (i.e. glutathione) (Krock et al., 2007), or by the enzymatic activity of bacteria commonly associated with mollusk tissues (Kotaki et al., 1985; Smith et al., 2001).

Results show that N-desulfonation of C1-2 was apparent in the toxic profile of *O. chilensis* juveniles, where concentrations of their analogues GTX2-3 were higher than those of the other toxic groups. In the case of adult oysters, an apparent desulfonation was also observed, resulting in the presence of the toxins GTX2-3. The STX detection of in this same experimental group, suggests that additionally to the N-desulfonation, a desulfation took place in the carbon C11 of the C1-2 precursors. Similar biotransformation has been reported for the other Chilean mollusks (Krock et al., 2007; Alvarez et al., 2009) and the epimerization and reduction process can also occur when experimentally exposing bivalve species to toxic diets (Jaime et al., 2007).

Recently, several new analogue STX's has been discovered that are bio-transformed by mussels tissues, metabolites detected using hydrophilic interaction liquid chromatography-mass spectrometry techniques (Dell' Aversano et al., 2008; Ding et al., 2017). The precursors of these analogues are C2, GTX2-3 and GTX1-4, the same toxins detected in this study. These metabolites found in mussel tissues suggest that the biotransformation that occur in bivalves do not always use the same pathways and they can take different routes depending on the species. However, the sensitivity of the technique used in this study could not detect if *O. chilensis* is able to generate these metabolites. The sample preparation method of PSTs used in this study also doesn't allow precise determination of the epimerization processes. So, it is not clear whether epimerization occurs within intact live dinoflagellate cells and mollusks tissue, or if it is exclusively a sample preparation effect.

Our study showed that the biotransformation processes of paralyzing toxins could be differentiated between the development stages of *O. chilensis*. In the juvenile toxic profiles, the most toxic group, STX, was absent; however, in adult specimens of the same species, this group was present from day 10 through to the end of the experiment. According to these results, *O. chilensis* is able to accumulate and biotransform the toxins produced by *A. catenella*, thereby acting as a vector towards higher trophic levels. *Ostrea chilensis* juveniles exposed to the toxic diet displayed significant mortality, indicating that the development stage of this species plays an essential role in the selection of more tolerant individuals after exposure to a paralytic toxin-producing dinoflagellate bloom. Navarro et al. (2016) showed that the toxic diet has dramatic negative effects on feeding and metabolism of the juvenile individuals of *Ostrea chilensis*, with high reduction of the lipid storage and growth. Mortality was also increased in individuals fed with the contaminated diet. This study supports the conclusion that the toxic dinoflagellate *A. catenella* restricts the energy acquisition in the juvenile *O. chilensis*, corroborating the high sensitivity of early development stages to the toxic conglomerate produced by *A. catenella*, even though the biotransformation of this stage did not produce the most lethal toxins (e.g., saxitoxin). This research allowed us to understand the accumulation and biotransformation dynamics of the toxins using *A. catenella* cells and the tissues of the studied bivalves. Indeed, we were able to observe the biotransformation dynamics of the neurotoxic complex, saxitoxin, during its interaction with the intracellular environment of *O. chilensis* tissues. Such studies can be used for similar analyses in other ecologically and commercially important species of filtering organisms, providing greater understanding of the specific interactions of bivalves in scenarios of toxic dinoflagellate proliferations (e.g. *A. catenella* blooms) and how these could affect the production of filtering bivalve crops.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.01.014.

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