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Article

Trends of natural accumulation and detoxification of paralytic shellfish poison in two bivalves from the Northwest Patagonian inland sea

Tendencias de acumulación y detoxificación natural de veneno paralizante de los mariscos en dos bivalvos del mar interior de la Patagonia Noroccidental

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Resumen.- La acumulación de toxinas marinas en filtradores implica serios riesgos a la salud humana y pérdidas económicas. Mientras que el monitoreo directo de la toxicidad de los mariscos permanecerá como una prioridad para proteger la salud humana, la comprensión de la dinámica de acumulación y detoxificación permitiría desarrollar herramientas predictivas para diseñar estrategias de mitigación para la acuicultura y pesca. En este trabajo se estudiaron patrones temporales de acumulación y detoxificación de veneno paralizante de los mariscos (VPM) en dos mitilidos de importancia comercial en el mar interior de la Patagonia Noroccidental: *Mytilus chilensis* y *Aulacomya atra*. Se utilizaron datos de monitoreo, entre 1995 y 1998 recolectados en 13 estaciones durante dos florecimientos de *Alexandrium catenella*. Aplicando modelos lineales generalizados se observó que la concentración de *A. catenella*, tiempo de exposición, salinidad, temperatura y zona, afectaron la concentración de VPM durante la fase de acumulación. Tiempo, salinidad, temperatura y zona afectaron la concentración de VPM durante la fase de detoxificación. La dinámica de acumulación y detoxificación de VPM se estudió a través de un modelo de una caja definido por dos parámetros, 1) el coeficiente de proporcionalidad entre *A. catenella* y VPM y 2) la tasa instantánea de decaimiento de la toxina. A pesar de las limitaciones del los datos utilizados, el modelo logró explicar una fracción significativa de la variación observada en la acumulación y detoxificación de VPM. Sin embargo es necesario validar este modelo contra un set de datos independientes del área de estudio e identificar y cuantificar fuentes de variabilidad, incerteza y sesgo que afecten sus parámetros.

Palabras clave: *Alexandrium catenella*, *Aulacomya atra*, *Mytilus chilensis*, modelamiento, floraciones algales nocivas

Abstract.- The accumulation of marine toxins in aquatic filterers is a recurrent event that imposes serious risks to human health and important economic losses. While direct monitoring of seafood toxicity will remain as a priority for human health protection, a better understanding of toxin accumulation and detoxification dynamics might allow for forecasting tools to design better cost-effective mitigation strategies for bivalve farming and fisheries. In this study we explore monitoring data to extract temporal trends in natural accumulation and detoxification of paralytic shellfish poison (PSP) for two important mytilids from the Northwest Patagonian inland sea: *Mytilus chilensis* and *Aulacomya atra*. The data were collected between 1995 and 1998 in 13 stations, during two *Alexandrium catenella* blooms. The generalized linear models approach applied indicated *A. catenella* concentration, exposure time, salinity, temperature and zone had significant effects upon PSP concentration during the accumulation phase. Time, salinity, temperature and zone had significant effects upon PSP concentration during the detoxification phase. To obtain quantitative descriptors for accumulation and detoxification dynamics, we construct a simplified one-box model, defined by two parameters: 1) the proportionality constant between *A. catenella* concentration and PSP and 2) the instantaneous PSP decay rate. In spite of the limited nature of available data, the proposed model described significantly the observed variation in accumulation and detoxification trends of PSP. It remains. However, an evident need to validate the model against independent data sets from the same area and to identify and quantify sources of variability, uncertainty and bias that may affect model parameters.

Key words: *Alexandrium catenella*, *Aulacomya atra*, *Mytilus chilensis*, modelling, harmful algae blooms

Introduction

Harmful algal blooms (HABs) have been a recurrent problem in southern Chile since 1972 (Guzmán *et al.* 1975, Lembeye *et al.* 1975, Guzmán & Campodonico 1978, Guzmán *et al.* 2002). Most concern has been focused on

Alexandrium catenella (Whedon and Kofoid) Balech, 1985, which is associated with paralytic shellfish poison (PSP). Outbreaks of this species first recorded in the SW Patagonia (Lembeye *et al.* 1975), were not evident in the Northwest Patagonia until the early 1990s (Muñoz *et al.* 1992). Since then, it has been recorded with relatively high frequency in this area, reaching up to the Chiloé Archipelago (Molinet *et al.* 2003, 2006).

Out of the several toxins associated with HABs, PSP is probably the one that attracts the most attention given its consequences for public health and commercial impact on the shellfish industry worldwide (Yu *et al.* 2005). PSP is acquired through the trophic chain, where this toxin is bioaccumulated in aquatic filterers (Bardouil *et al.* 1993, Bricelj *et al.* 2005, Li *et al.* 2005) and eventually, in their predators (Lembeye 1996, Compagnon *et al*. 1998). PSP has caused serious harm to human health in southern Chile, including several human deaths, and severe damage to the economy of local coastal communities, affecting shellfish extraction from natural beds and harvesting from aquaculture farms, particularly those dedicated to mytilids. Although recurrent, these events have only triggered short term monitoring projects, which are the only source of field information about the dynamics of these events in the Northwest Patagonian Inland Sea.

In spite of its importance to human health and shellfish production, the natural kinetics of PSP accumulation and detoxification and the environmental factors that modify these processes are not well described, particularly in the Northwest Patagonia. A high variability in PSP accumulation rates has been observed in laboratory and field studies, which has been associated with environmental and physiological variables such as: concentration of toxic microalgae (Spencer *et al.* 2001), exposure times, bivalve filtration/ingestion rates (Sekiguchi *et al.* 2001) and concentration of toxins per microalgal individual (Blanco *et al.* 1997) or unit of volume (Moroño *et al.* 2001). Environmental variables such as temperature (Yamamoto *et al.* 2003) and salinity have been described to have some effect on detoxification rates, at least during the so-called rapid phase (Blanco *et al.* 1997).

While direct monitoring of seafood toxicity will remain as the safest approach to protect human health, predictive models of PSP accumulation and detoxification in shellfish under natural conditions may be useful complementary tools. By projecting the onset, magnitude and likely duration of bloom effects upon seafood toxicity, it becomes possible: i) to develop early alert systems and preventive communication programs for consumers and producers; ii) to mitigate production losses by applying anticipated or delayed shellfish harvest strategies, iii) to forecast the extension of closure periods and economical damage to fishermen, farmers and local economies.

From published modeling efforts, linear accumulation models based upon first-order reaction kinetics have shown better fits to observed toxin incorporation curves than non linear models (Moroño *et al.* 2001, Blanco *et al.* 2003). Available PSP detoxification models correspond to linear models (Blanco *et al.* 1997, 2003, Li *et al.* 2005) that have used from two (Blanco *et al.* 1997, Yamamoto *et al.* 2003, Yu *et al.* 2005) to five compartments or organs (Blanco *et al.* 1997, Choi *et al.* 2003, Yamamoto *et al.* 2003, Li *et al.* 2005), with constant detoxification rates, at least in the so-called slow phase of detoxification.

The objective of this study was to assess whether natural trends in PSP accumulation and detoxification rates were observable in the spatially and temporally limited data available for the Northwest Patagonian Inland Sea (Southern Chile). We focused on two historically important shellfish resources, the bivalve mollusk mytilids: blue mussel *Mytilus chilensis* (Hupè, 1854) and ribbed mussel *Aulacomya atra* (Molina, 1972), for which two monitoring efforts were conducted between 1995 and 1998, during two *Alexandrium catenella* blooms. Given this data set contained valuable information about PSP variability in spatial and temporal scales, we developed, applied and tested a simplified one-box predictive model, suitable for characterizing and forecasting natural PSP accumulation and detoxification dynamics in shellfish harvest areas where basic monitoring programs are conducted.

Material and Methods

Study area

Monitoring programs were focused on known shellfish beds in the Northwest Patagonian Inland Sea, between 43°53'S and 45°30'S (Fig. 1). Here the water column is highly stratified, with areas of abrupt bathymetry and changes in the coastal morphology (Pickard 1971). The study area is naturally divided into two basins by the Meninea Constriction (Silva *et al.* 1995, Valle-Levinson & Blanco 2004), which affects the oceanographic characteristics of the water column at both sides of this feature. Because of this natural division and the spatial distribution of available monitoring stations, we classified the latter into two zones (Fig. 1): Northeast (NE), and Southwest (SW). Nonetheless, given *Aulacomya atra* was

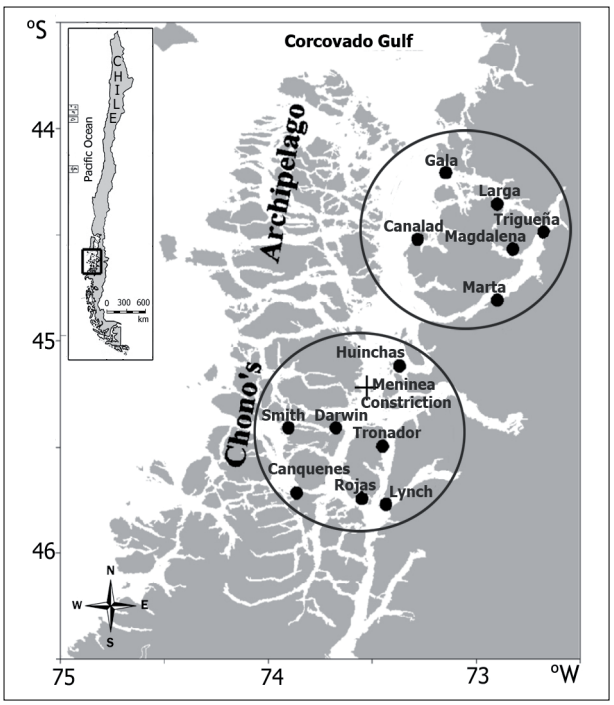

Figure 1. Study area in the inland sea of Aysén Region (Southern Chile) showing the 13 stations sampled during the study period. Filled circles show stations sampled in Northeast and Southwest zones (encircled). Gray cross show Meninea Constriction, an important morphological feature that influences water flows in the study area / Área de estudio en las aguas interiores de la región de Aysén (sur de Chile) mostrando las 13 estaciones durante el periodo de estudio. Los círculos negros muestras las estaciones de muestreo en las zonas Noreste y Suroeste (encerradas en un círculo). La cruz gris muestra la constricción de Meninea, un importante rasgo morfológico que influencia la circulación del agua en el área de estudio

sampled in 4 SW stations and only one NE station, data for this species was modeled only in the SW stations.

Sampling

Mytilus chilensis and *Aulacomya atra* samples of 50-100 individuals by species were taken between October 1995 and May 1998 by scuba diving at 13 monitoring stations, 7 in the Northeast zone and 6 in the Southwest zone (Fig. 1; Table 1). *M. chilensis* was collected in five Northeast stations and in three Southwest stations; on the other hand *A. atra* was collected in four Southwest stations and in one Northeast station. Shellfish were devalved at the field, and kept at 4°C until sent to the laboratory. The samples were analyzed using the mouse bioassay technique (AOAC 1990) at the Regional Center for Environmental Analysis (Universidad Austral de Chile, Puerto Montt, Chile). Toxin concentrations were expressed in the customary unit: µg of saxitoxin-equivalent per 100 g of meat (µg STX eq·100 g^{-1}).

Simultaneous phytoplankton samples and temperature and salinity records were obtained between 0 and 30 m depth at the shellfish sampling sites. The phytoplankton samples were collected by two vertical tows (30 to 0 m depth) by means of a plankton net (30 µm mesh, 35 cm diameter, 1 m long). Temperature and salinity records were obtained from water samples collected at surface, 5, 10, 15, 20 and 30 m depth, using a water analyzer YSI 30.

Phytoplankton samples were observed under a microscope (Olympus® BH2) with phase contrast. Abundance estimates were obtained by counting five 1 ml aliquots in a Sedgewick-Rafter cell, using a magnification of 100x and/or 200x. The results were expressed in number of cells per liter after scaling the readings by the tow volume, calculated as a product between the net mouth section and the towed distance.

Data analysis

To evaluate the effects of explanatory variables upon PSP concentration, we applied a generalized linear model approach, GLM (McCullagh & Nelder 1989), assuming a Poisson distribution for errors respect to the untransformed response variable. For such purpose, we first divided the available data from the two blooms into two phases "accumulation" and "detoxification". The accumulation phase considered the period between the first sampling event when *Alexandrium catenella* was recorded (within a year) and the one when the maximum PSP concentration was reached. The detoxification phase was defined as the period between the sampling event when the maximum PSP was reached and the one where PSP dropped below the 80 µg STX eq·100 g^{-1} safety threshold.

A deviance analysis was used to evaluate the relative contribution of covariates to explain variability in PSP concentration in the accumulation and detoxification GLM models. The significance of each explanatory variable was determined using a χ^2 test (Venables & Ripley 1998). Tested covarites for the accumulation phase GLM model were i) exposure index, ii) average water column salinity; iii) average water column temperature, and iv) zone, which was obtained from a previous non parametric analysis (Kruskal-Wallis), which grouped sample stations in two zones (Southwest and Northeast). Tested covariates for the detoxification phase GLM were: i) time since maximum PSP was reached, ii) average water column temperature, and iii) average water column salinity. The exposure index used in the accumulation phase model represented the integral of *A. catenella* concentrations across time and was defined as:

Table 1. Number of samples of *Mytilus chilensis* and *Aulacomya atra* collected in the Northeast and Southwest zones for the paralytic shellfish poisoning analysis in the monitoring program between 1995 and 1998 in the northwestern Patagonia / Número de Muestras de *Mytilus chilensis* y *Aulacomya atra* colectadas en las zonas Noreste y Suroeste de la Patagonia Noroccidental, para análisis de veneno paralizante de los mariscos en el programa de monitoreo entre 1995 y 1998

Zone	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Oct	Nov	Dec
Mytilus chilensis												
Northeast	1995									5	5	5
	1996	5	5	5	5	5	5		5	5	5	5
	1997			4	4	4	4	4	4	4	4	4
	1998	4	4	4	4	4						
Southwest	1995									3	3	3
	1996	3	3	3	3	3	3		3	3	3	3
	1997			1	1	1	1	1	1	1	1	1
	1998	1	1	1	1	1						
Aulacomya atra												
Northeast	1995									1	1	1
	1996	1	1	1	1	1	1		1	1	1	1
	1997			1	1	1	1	1	1	1	1	1
	1998	1	1	1	1	1						
Southwest	1995									4	4	4
	1996	4	4	4	4	4	4		4	4	4	4
	1997			3	3	3	3	3	3	3	3	3
	1998	3	3	3	3	3						

$$I_{\exp} = \frac{\Delta t * \bar{x}}{1000}$$

Where $\bar{x}$ is the geometric mean of *Alexandrium catenella* density (cells·l-3) and Δt is the time elapsed (in days) between consecutive samplings.

In order to provide a simple model suitable for interpreting observed results and for forecasting accumulation and detoxification dynamics of PSP concentrations given this or similar datasets, we constructed a simple one-box model, defined by the first order linear differential equation (Schwarzenbach *et al.* 1993):

$$\frac{dy}{dt} = J(t) - K(t)$$

Where y corresponds to PSP concentration in the shellfish, $J(t)$ corresponds to the intake rate, and $K(t)$ to the gross detoxification rate. We assumed $J(t)$ to be a constant proportion of *A. catenella* concentration in the water, which we expressed as:

$$J(t) = q \cdot A_0 \cdot e^{g \cdot t}$$

Where q is a proportionality constant, A_0 is the *A. catenella* concentration at the beginning of the time interval t and g is the instantaneous rate of change in *A. catenella* concentration within the same interval.

The PSP concentration-dependent detoxification rate was expressed as a simple exponential decay rate, defined by the equation:

$$K(t) = y_0(1 - e^{-k \cdot t})$$

Where y_0 is the PSP concentration in the mussel at the beginning of the time interval t, and k is the instantaneous decay rate, assumed to be constant and time-independent.

After integration over a time interval t, the previous equations are combined into the general model:

$$y_t = \begin{cases} y_0 \cdot e^{-k \cdot t} + q \cdot A_0 \cdot \dfrac{e^{g \cdot t} - e^{-k \cdot t}}{g + k} & \text{for } g \neq 0 \\ y_t = y_0 \cdot e^{-k \cdot t} & \text{for } g = 0 \end{cases}$$

Under this simplified one-box model, the steady state (maximum) concentration of PSP in the shellfish becomes dependent on the concentration of *Alexandrium catenella*,

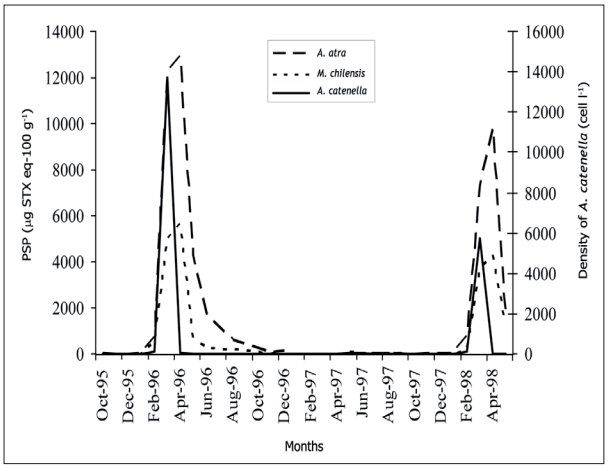

Figure 2. Blooms of *Alexandrium catenella* recorded in the study area between 1996 and 1998 and their associated paralytic shellfish poison (PSP) in *Aulacomya atra* and *Mytilus chilensis* / Floraciones de *Alexandrium catenella* registradas en el área de estudio entre 1996 y 1998 y su asociación con el veneno paralítico de los mariscos (VPM) en *Aulacomya atra* y *Mytilus chilensis*

its rate of change and the constant detoxification rate, following the equation,

$$Y_{\max} = \frac{J_t}{g_t + k}$$

For the present application the parameter *g* was computed directly from *Alexandrium catenella* concentration data, assuming a monotonous exponential growth or decay within each time interval. Parameters *k* and *q* were, on the other hand, estimated by maximum likelihood non linear regression (Littel *et al.* 1996).

Results

During the studied period two algal blooms of *Alexandrium catenella* were observed in a large part of the study area (Molinet *et al.* 2003), reaching rising from a few cells to around 30000 cell l^{-1} in some stations during spring-summer (Fig 2). During these blooms (Fig. 2), PSP was found in both *Aulacomya atra* and *Mytilus chilensis* at concentrations between 35 and 28,000 µg STX eq·100 g^{-1}. No significant differences were observed between the toxicities of *M. chilensis* and *A. atra* (U1, 0.05= 231, $P <$ 0.23). Nonetheless, for practical purposes, toxicity results were analyzed separately for each species.

Temperature showed a clear seasonal pattern in the study area (Fig. 3A), with higher temperatures in the northeast zone, particularly during spring and summer, when *Alexandrium catenella* blooms were reported. While temperature reached up to 15ºC in the NE zone during summer, it remained below 12°C in the SW zone (Fig. 3A). On the other hand, salinity was higher in the southwest zone, showing also a narrow range of variation, between 27 and 31 psu, during the entire study period (Fig. 3B).

PSP accumulation in shellfish

Toxin concentrations in *Mytilus chilensis* varied between 35 and 22,000 µg STX eq·100 g^{-1}, and significant differences in PSP concentrations were found between zones (Table 2), with higher PSP levels in Southwest stations (Fig. 4A). In *Aulacomya atra*, PSP concentrations ranged from 35 to 28,000 µg STX eq·100 g^{-1}, although PSP concentration in the only Northeast station tended to be lower than in samples collected in Southwest stations (Fig. 4B).

Alexandrium catenella exposure index was the strongest explanatory variable for PSP concentration in both *Mytilus chilensis* and *Aulacomya atra*, accouting for *circa* 50% and 70% of explained deviance, respectively (Table 2). There was also a significant zone effect, which explained 4% of total deviance in *M. chilensis*. Although temperature and salinity explained < 1% of total deviance, their contributions were still significant in both species (*P*

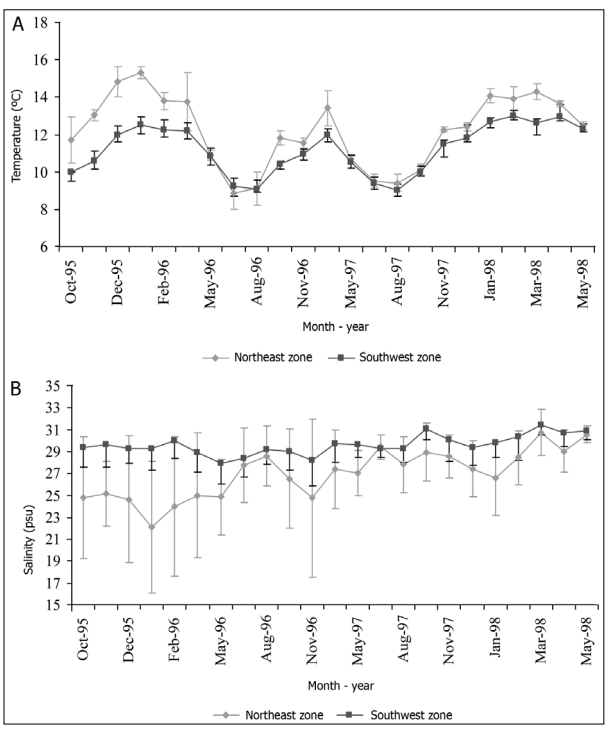

Figure 3. Temporal variability of: A) Sea water temperature and B) Salinity recorded where shellfish were collected in the Northeast and Southwest zone during the entire study period / Variabilidad temporal de: A) Temperatura del agua y B) Salinidad registrada donde los mariscos fueron colectados en las zonas Noreste y Suroeste durante el periodo de estudio

Table 2. Results from the sequential deviance analysis for the response variable PSP accumulation in *Mytilus chilensis* and *Aulacomya atra*, during the accumulation phase. Predictive variables were: exposure index, temperature, salinity and zone. Zone effect not considered for *A. atra* due to low number of stations (1) sampled in the NE zone. NULL represents the saturated model, *i.e.*, the most complex model given the current distribution and link function / Resultados del análisis de desvianza secuencial para la variable respuesta: concentración de VPM en *Mytilus chilensis* y *Aulacomya atra* durante la fase de acumulación. Las variables predictoras fueron el índice de exposición, temperatura, salinidad y zona. El efecto de la zona no fue considerado para *A. atra* dado el bajo número de estaciones (1) muestreadas en la zona NE. NULL representa el modelo saturado, es decir, el modelo más complejo dada la distribución y la función de vinculación

Predictive variables	Df	Deviance	Residual Df	Residual Deviance	*P*(>|Chi|)
NULL, *M. chilensis*			111	550902	
Exposure index	1	368093	110	182809	0.0001
Zone	1	22483	109	160326	0.0001
Temperature	1	3477	108	156849	0.0001
Salinity	1	5649	107	151201	0.0001
NULL, *A. atra*			49	467043	
Exposure index	1	348721	48	118321	0.0001
Temperature	1	4935	47	113386	0.0001
Salinity	1	1730	46	111656	0.0001

< 0.05), and their inclusion produced more informative GLM models (ΔAIC > 2) in both species (Table 2).

Shellfish detoxification

PSP Detoxification data for *Mytilus chilensis* and *Aulacomya atra* were principally obtained from the 1996 bloom. This was because the 1998 monitoring program was suspended in May, loosing the chance to track detoxification dynamics for the second *A. catenella* bloom, observed between January and March, 1998. GLM analysis indicated that time elapsed since maximum PSP was reached, was the most important explanatory variable for detoxification in both species (41% and 66% respectively) (Table 3). As observed for toxin accumulation temperature and salinity were significant but their contributions were very small (< 1%). Salinity was highly variable during detoxification periods (Fig. 3B), but also showed a consistent difference between zones.

Model performance

Values Predicted by the one-box model showed a reasonable (Figs. 5, 6) and significant ($P < 0.001$) fit to observed PSP concentrations during 1996 and 1998, where both model parameters were found to be significantly different from zero for each species and zone (northeast and southwest). Within this general context, observed PSP values for *Mytilus chilensis* tended to be higher than predicted ones in the NE (Fig. 5A), showing a much better fit for the SW zone, particularly for the first PSP outbreak during

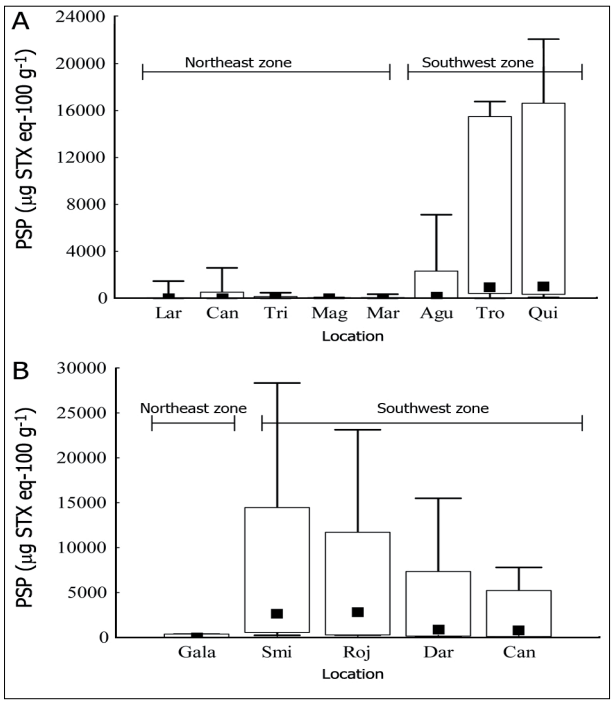

Figure 4. Paralytic shellfish poison recorded in stations of the Northeast and Southwest zones of the study area in A) *Mytilus chilensis* and B) *Aulacomya atra* samples. Gala, Lar: Larga Island, Can: Canalad, Tri: Trigueña, Mag: Magdalena, Mar: Marta, Agu: Huichas, Tro: Tronador, Qui: Quitralco, Dar: Darwin, Roj: Rojas, Smi: Smith, and Caq: Canquenes / Veneno paralizante de los mariscos registrado en las estaciones de las zonas Noreste y Suroeste del área de estudio en muestras de A) *Mytilus chilensis* y B) *Aulacomya atra*. Gala, Lar: Larga Island, Can: Canalad, Tri: Trigueña, Mag: Magdalena, Mar: Marta, Agu: Huichas, Tro: Tronador, Qui: Quitralco, Dar: Darwin, Roj: Rojas, Smi: Smith y Caq: Canquenes

Table 3. Results of the sequential deviance analysis for the response variable PSP concentration in *Mytilus chilensis* and *Aulacomya atra*, during the detoxification phase. Predictor variables used were: elapsed days, temperature, salinity and zone. Zone effect not considered for *A. atra* due to low number of stations (1) sampled in the NE zone. NULL represents the saturated model, *i.e.*, the most complex model given the current distribution and link function / Resultados del análisis de desvianza secuencial para la variable respuesta: concentración de VPM en *Mytilus chilensis* y *Aulacomya atra* durante la fase de detoxificación. Las variables predictoras fueron el índice de exposición, temperatura, salinidad y zona. El efecto de la zona no fue considerado para *A. atra* dado el bajo número de estaciones (1) muestreadas en la zona NE. NULL representa el modelo saturado, es decir, el modelo más complejo dada la distribución y la función de vinculación

	Df	Deviance	Residual Df	Residual Deviance	$P(>\|Chi\|)$
NULL, *M. chilensis*			69	496621	
Elapsed time	1	197757	68	298863	0.0001
Zone	1	186254	67	112609	0.0001
Temperature	1	35757	66	76853	0.0001
Salinity	1	3180	65	73672	0.0001
NULL, *A. atra*	44	370144			
Elapsed time	1	244183	43	125961	0.0001
Salinity	1	7096	42	118865	0.0001
Temperature	1	8	41	118857	0.0044

1996 (Fig. 5B). The PSP intake proportionality constant (model parameter q) reached lower values in the NE and higher values in the SW for both species (Table 4). Both minimum and maximum q values were observed in *M. chilensis*. The instantaneous decay rate, model parameter k, was estimated to be highest for *M. chilensis* in the NE zone, while it was similar for all the other cases (Table 4).

Discussion

PSP concentration during the accumulation phase was significantly higher in the Southwest zone for *Mytilus chilensis,* whereas *Aulacomya atra* showed the highest concentration of PSP in SW zone in this study. PSP concentrations were not only smaller, but also more variable in the NE zone, where lower densities and shorter duration of *Alexandrium catenella* outbreaks were recorded during the study period. The NE zone is characterized by a strongly stratified water column, influenced by several river discharges (Valle-Levinson *et al.* 2001, Cáceres & Valle-Levinson 2004), which causes high variability in salinity and may induce a relatively large net superficial transport out of this system. This net transport could explain lower densities and shorter duration of *A. catenella* blooms recorded in the NE zone, where lower PSP concentrations were measured in both bivalves. On the other hand the southwest zone is characterized by a more homogeneous water column and a more complex flow pattern conditioned by the Meninea Constriction (Silva *et al.* 1995, 1998, Valle-Levinson & Blanco 2004), which could increase retention and consequently higher accumulation of *A. catenella* in SW waters and PSP in bivalves.

PSP accumulation was less variable in *Aulacomya atra* than in *Mytilus chilensis*, possibly because of the different habitats occupied by each species. *M. chilensis* was collected principally in intertidal habitats, while *A. atra* was collected only in subtidal habitats. These sampling differences are representative of vertical segregation patterns in the study area (Viviani 1979), which produce important differences in exposure, ingestion rates and overall bioenergetics between them. Such differences might explain higher toxin concentrations registered for the subtidal *A. atra*, which is coincident with that proposed by Bricelj & Shumway (1998) for subtidal *vs* intertidal populations.

As found in other mytilids, such as *Mytilus edulis* (Lassus *et al.* 1993) and *M. californianus* (Bricelj & Shumway 1998), natural detoxification processes in *M. chilensis* and *A. atra* appeared to be mainly related to elapsed time (after the bloom) and maximum PSP concentration acquired by the shellfish. Minor, but still significant, effects from zone, temperature and salinity indicated likely environmental effects upon metabolic, ventilation or ingestion rates modified the general response patterns, during the study period.

Natural detoxification in *Mytilus chilensis* and *Aulacomya atra* followed an exponential decay curve, similar in shape to those observed by other authors (Bricelj & Shumway 1998, Blanco *et al.* 2003). Detoxification

Table 4. PSP intake proportionality constant (model parameter q) and instantaneous decay rate (model parameter k) estimated for *Mytilus chilensis* and *Aulacomya atra* in the NE and SW zone / Constante de proporcionalidad para la incorporación de VPM (parámetro del modelo q) y tasa de decaimiento instantánea (parámetro del modelo k), estimada para *Mytilus chilensis* y *Aulacomya atra* en la zonas NE y SW

Species	Location	Parameter	Estimate	Standard Error
M. chilensis	Northeast	*q*	0.047	6.29E-05
		k	0.044	5.15E-05
	Southwest	*q*	0.270	2.22E-02
		k	0.020	2.76E-03
A. atra	Southwest	*q*	0.187	1.31E-02
		k	0.020	2.24E-03

periods we observed in *A. atra* were, however, longer than those observed for the same species in Península Valdés, Argentina (Andrinolo *et al.* 1999). This difference may be explained by the lower water temperature and, mainly, by the maximum PSP concentrations recorded in our data (~30,000 µg STX eq·100 g^{-1}), much higher than the 631 µg STX eq·100 g^{-1} reported by Andrinolo *et al.* (1999).

Given the limited spatial and temporal coverage of the available data, we focused on developing a parsimonious modeling approach, which captures the main two results from our data analysis: i) PSP accumulation is a function of cell concentration plus time, while ii) PSP detoxification is a function of time (plus acquired PSP concentration). Thus, the model intake function, *J(t)*, was defined to be directly proportional to *A. catenella* concentration in the water, where a constant proportionality coefficient (*q*) is estimated for each species and zone to account for major physiological or trophic differences between areas. While the potential effects of temperature, salinity, food supply or other factors upon *q* remain to be investigated, they could be incorporated explicitly in future expansions of the model. The second term of the model, *K(t)*, reflects gross detoxification, and was also made dependent upon a single parameter (*k*), also assumed to be constant for each species and zone. This parameter may be expected to be highly dependent upon metabolic rates and, therefore, upon temperature. A predictive sub-model for parameter *k*, based upon available bioenergetics models for *A. atra* and *M. chilensis* (Navarro & Winter 1982, Navarro *et al.* 2006) would deserve future consideration.

Blanco *et al.* (2003) used a similar approach to ours for modeling kinetics of accumulation and transformation of PSP (GTX_1, GTX_2, GTX_3, GTX_4) in *M. galloprovincialis*, under laboratory conditions. Our approach could be compared with their first model, which assumes i) there is no transformation between toxins; (ii) the uptake and detoxification rates are the same for all toxins and (iii) the detoxification follows first-order kinetics. Although they concluded that their model was not able to describe the experimental data of most toxins, we obtained a reasonable fit between predicted and observed values, particularly in the case of the southwest zone. However, temporal frequency of data collection in this study seemed to be insufficient for a full understanding of natural variability in accumulation and detoxification kinetics which seems to occur at temporal scales smaller than a week.

While no model is expected to replace direct monitoring of seafood to assure human health protection, predictive models might still be helpful in scheduling shellfish harvest

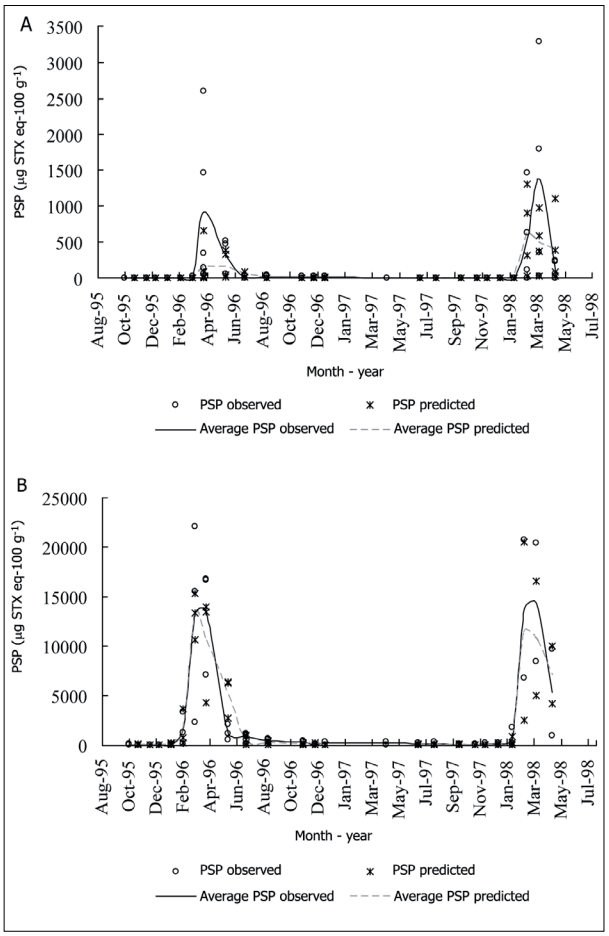

Figure 5. Paralytic shellfish poison accumulation-detoxification observed and predicted for our one-box model for *Mytilus chilensis* in A) Northeast zone and B) Southwest zone of the study area / Acumulación y detoxificación de veneno paralizante de los mariscos observado y predicho a través de nuestro modelo de una caja para *Mytilus chilensis* en las zonas A) Noreste B) Suroeste del área de estudio

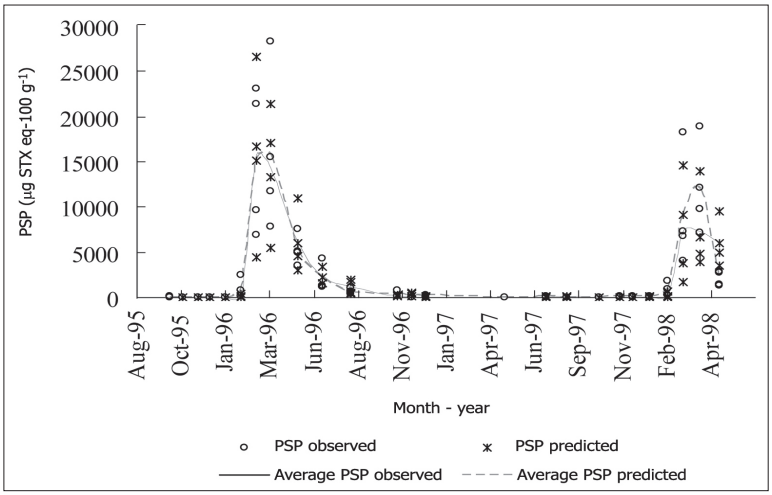

Figure 6. Paralytic shellfish poison accumulation-detoxification observed and predicted for our one-box model for *Aulacomya atra* in the Southwest zone of the study area / Acumulación y detoxificación de veneno paralizante de los mariscos observado y predicho a través de nuestro modelo de una caja para *Aulacomya atra* en la zona Suroeste del área de estudio

strategies, forecasting economical damage and triggering early alert systems in areas affected by HABs. While the proposed model showed to be useful as a descriptive tool for our data set, its application as a predictive tool requires of its validation against independent data sets from the same exploitation/management areas. A better designed, more intense and longer-term or permanent monitoring program would increase model accuracy and a realistic estimation of forecasts uncertainty. We expect the proposed model may be also useful as a descriptive or predictive tool for other data-poor monitoring scenarios. Such model transference would require of area- and species-specific estimates for both empirical parameters q and k. As before, the accuracy of the model would be a function of the quality of the data.

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