Effects of the toxic dinoflagellate Gymnodinium catenatum on uptake and fate of paralytic shellfish poisons in the Pacific giant lions-paw scallop Nodipecten subnodosus

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Abstract Juvenile Pacific giant lions-paw scallops Nodipecten subnodosus were fed the toxic dinoflagellate Gymnodinium catenatum, a producer of paralytic shellfish poison (PSP), supplied with Isochrysis galbana (a nontoxic microalgae). Short-term (<24 h) experiments were performed to determine clearance and ingestion rates of G. catenatum. Kinetics of PSP was examined in longer-term experiments (>2 days). At high food concentrations, juvenile scallops showed production of pseudofeces, partial shell valve closure, and reduction in feeding. According to HPLC analysis, the only toxins present in the dinoflagellate G. catenatum and in the scallops were the gonyautoxins (GTXs), except in the labial palps and digestive gland, where trace amounts of saxitoxin (STX) were present in scallops. These tissues could play an important role in toxin biotransformation. The ranking of toxin concentration in tissues was: digestive gland > labial palps > intestine > gills > mantle > adductor muscle, where the total contribution of viscera was more than 80% of the total toxin body burden. Juvenile scallops exhibited no apparent detrimental physiological responses during the long-term feeding experiment. The dinoflagellate may contribute nutrients to the scallop, in addition to the microalgae I. galbana. The dinoflagellate may enhance cell uptake and byssus production. Once PSP accumulated during the first 12 days, it was slowly eliminated. The limited capacity for accumulating toxins in the adductor muscle favors domestic marketing of scallops.

Introduction

Paralytic shellfish poison (PSP) comprise a suite of at least 18 natural neurotoxic tetrahydropurine derivatives produced by several species of free-living marine planktonic microalgae, principally dinoflagellates, including Alexandrium spp., Pyrodinium bahamense var. compressum, and Gymnodinium catenatum (Cembella et al. 1993; Bricelj and Shumway 1998). PSP produced by toxigenic dinoflagellates move through marine food chains and accumulate in a variety of marine organisms, including bivalves, crabs, fish, gastropods, starfish, polychaetes, and zooplankton (Noguchi et al. 1985; Kvitek and Beitler 1988; Bricelj and Shumway 1998). Incidents of human illness and death from PSP are principally associated with ingestion of toxic mussels, clams, oysters, and scallops, which as filter feeders, acquire these toxins from the water column or resuspended benthic material. These bivalves, in concentrating toxins in their tissues, become vectors and are potentially toxic to other animals (Hall et al. 1990; Bricelj and Shumway 1998; Lagos 2003). Worldwide outbreaks of dinoflagellate...
poisoning are a constant threat to public health, have negative impacts in marine ecology, and cause serious economic losses to aquaculture, fisheries, and tourist industries (Lagos 1998). Among dinoflagellates known to produce PSP, G. catenatum is the only unarmored species (Oshima et al. 1993); described for the first time in the Gulf of California, Mexico by Graham (1943). G. catenatum is widespread in temperate and tropical waters (Balech 1964; Yuki and Yoshimatsu 1987; Ikeda et al. 1989; Franca and Almeida 1998; La Barbera-Sanchez 2001; Hallegraeff et al. 1989; Viquez and Hargraves 1995; Fukuyo et al. 1993).

Toxin levels in bivalves are a function of the cell density, duration of exposure, and toxicity of the phytoplankton species and their relative abundance (Bricelj and Shumway 1998). Likewise, differences in the dynamics of toxin uptake and detoxification mechanisms, anatomical localization, and physiological breakdown or transformation determine the persistence of toxins in tissues of bivalves (Bricelj et al. 1990; Cembella et al. 1993). Field studies have provided useful information concerning in situ concentrations of toxins in shellfish, but the kinetics of dinoflagellate toxins in bivalves can only be approximated in the field because there is no control of environmental variables, such as water temperature and dissolved inorganic nutrients, phytoplankton concentration, and cellular toxin content. Under controlled laboratory conditions, precise determination of physiological feeding parameters leading to the accumulation of toxins in shellfish tissues, can be achieved, and bivalves can be more closely monitored for detrimental effects caused by exposure (Shumway and Cucci 1987; Bricelj et al. 1990; Bauder et al. 2001).

Pacific giant lions-paw scallop Nodicephucten subnodosus (Mollusca: Pectinidae) is a suspension-feeding bivalve mollusc occurring from Laguna Ojo de Liebre, on the west coast of the Baja California Peninsula of Mexico to the southern coast of Peru (Keen 1972). In Baja California Sur, Mexico, a local commercial fishery for this scallop is of interest to aquaculturalists. The economic value of this scallop is related to its large size and weight (>20 cm and >1,500 g total wet weight), appealing flavor, and its large adductor muscle. The physiological mechanisms of uptake and elimination of toxins have not been studied. Understanding uptake and detoxification rates of toxins is important to the industry, as well as serving as a model for toxin kinetics, eventually leading to a better understanding of pectinid bivalve physiology. In a laboratory environment during short- and long-term feeding experiments, this study examined rates of feeding, physiological response, and PSP kinetics in juvenile giant lions-paw scallops that consume toxigenic cells of G. catenatum as part of its diet.

Materials and methods

Algal culture and source of scallops

The dinoflagellate G. catenatum (Strain GCCV-6) was obtained from the microalgal collection at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Baja California Sur, Mexico and cultured in GSe medium (Blackburn et al. 1989) with filtered (0.45 μm) seawater. Dinoflagellates were grown in monoaflagal cultures in 20-l glass flasks on a 16:8 h light:dark photocycle at 21°C under 70-W fluorescent lamps and anaerobic conditions.

During acclimation (14–21 days), juvenile scallops were fed a mixture of microalgae (Chaetoceros calcitrans, C. gracilis, and Isochrysis galbana; 1:1:1). C. calcitrans and C. gracilis were cultured in 20-l plastic bags in F/2 growth medium at 22°C with constant illumination at 32 psu. I. galbana (Strain ISG-1) was grown in MA-F/2 medium at 22°C at 32 psu in 20-l plastic bags under constant illumination. I. galbana was also used as the non-toxic diet for the control. G. catenatum cultures were harvested in the late exponential growth phase and the others in the stationary growth phase. At harvest, to estimate concentrations, cells were counted in Sedgewick-Rafter counting chambers under an optical microscope (400×) for the dinoflagellates and an electronic particle counter (Coulter Multi-sizer) for I. galbana. Cultivated juvenile scallops were collected from suspended cultures (Lodeiros et al. 1998) at Rancho Bueno, Baja California Sur, Mexico (24°32′N, 111°42′W) and transported to CIBNOR. Twenty scallops were placed in each 40-l plastic tank containing filtered (1 μm) seawater collected directly from the sea. The water was maintained at 22°C at 35 psu and constantly aerated with air stones. The entire volume of water in the tanks was exchanged every 2 days.

Feeding experiments

The juvenile scallops were used to examine effects on feeding activity after exposure to G. catenatum and PSP kinetics, using slight modifications of an experimental procedure by Bauder et al. (2001). Short-term (<24 h) experiments were used to determine clearance rates (CRs) and ingestion rates (IRs). Toxin kinetics and post-ingestion activity were examined in long-term experiments (>2 days). Before the experiments,
specimens were placed in filtered (1-µm) seawater without food for 24 h to clear their digestive tract.

Short-term feeding experiments

Clearance rates and IRs were measured from the depletion of algal cells over time in closed systems. The scallops were fed different concentrations of the toxic dinoflagellate under controlled conditions for 7 h to scallops of two different shell heights: 2.77 ± 0.21 and 5.17 ± 0.53 cm. Scallops were divided into three replicate pools for each cell concentration, each containing five specimens. Each pool was placed in 1.5-l plastic containers. Scallops were exposed to four concentrations of *G. catenatum* (200, 450, 850, and 1,650 cells ml⁻¹) for each shell height size, with a standard concentration of *I. galbana* (1.5 × 10⁴ cells ml⁻¹) in a final volume of 1.5 l for each container at 35 psu. *I. galbana* was also used as the control diet (3.5 × 10⁴ cells ml⁻¹). Two containers with algal suspensions, but without scallops were used as an additional control, and two containers with scallops, but without food were also used as controls. The algae were kept in suspension with an air stone and in the dark to reduce cell growth during the experiment. Samples of the suspension (5 ml) were taken from each container at the beginning of exposure, 15 min, 1 h, 2 1/2 h, and 7 h for counting under the optical microscope. Scallops were sacrificed at 7 h to determine total wet tissue weight. CRs were estimated according to the equation: $CR = \ln \left( \frac{C_0}{C_1} \right) \times \frac{V}{t} \times n^{-1}$ (Coughland 1969), where $C_0$ and $C_1$ are the initial and final cell density; $V$ is the volume of the suspension; $t$ is the time interval; and $n$ is the number of scallops. IRs were determined according to Bauder et al. (2001), where $IR = CR \times cell density$. Data were analyzed by one-way ANOVA and the Tukey post hoc test. Significant differences were set at $P < 0.05$.

Long-term feeding experiments

Two separate experiments were conducted to determine PSP uptake and depuration in juvenile scallops: (1) scallops were exposed for 2 days to test for compartmentalization of toxins in tissues, (2) scallops were exposed to *G. catenatum* cells for 12 days to measure toxin uptake, followed by 12 days feeding on non-toxic *I. galbana* cells to determine toxin depuration.

Compartmentalization of toxins

To study compartmentalization of toxins, the 2-day feeding experiment was conducted on larger juvenile scallops (SH = 5.9 ± 0.39 cm). In total, 21 scallops were exposed to *G. catenatum* (1 × 10⁶ cells scallop⁻¹ day⁻¹) in a closed aquarium system containing a constant concentration of *I. galbana* (16 × 10³ cells scallop⁻¹ day⁻¹) in natural sea water at 35 psu and 22°C. All scallops were removed and divided into three pools of seven scallops at the end of the 2-day exposure. From each scallop, the mantle, gills, adductor muscle, digestive gland-stomach complex, intestine, and labial palps were dissected. Tissues were weighed and stored at −80°C.

Toxin uptake and depuration

For toxin uptake, juvenile scallops (SH = 5.9 ± 0.39 cm) were held for 12 days in closed 20-l containers containing 201 filtered (1 µm) natural sea water at 35 psu and 22°C with 20 specimens in each container. Cultures of *G. catenatum* were grown in a staggered sequence and harvested daily, metered from a stock container into the aquarium with a drip method at a cell density of approximating 3 × 10⁵ cells scallop⁻¹ day⁻¹ and supplied with a diet biomass of *I. galbana* of 5 × 10⁷ cells scallop⁻¹ day⁻¹. During the 12-day depuration, scallops were fed a constant concentration of *I. galbana* at 1 × 10⁶ cell scallop⁻¹ day⁻¹, while controls were fed this diet for 24 days. The entire volume of water in each tank was exchanged daily to prevent fouling and re-ingestion of fecal material. Replicate (n = 3) samples of three pooled scallops were removed every four days (days 1, 4, 8, 12, 16, 20, and 24) from the tanks for measurement of toxins and replaced with marked scallops to maintain a constant biomass in the container. For each pool, the mantle, gills, adductor muscle, and digestive gland-stomach complex were dissected. Tissues were weighed and stored at −80°C. Algal cell concentrations in the tanks were determined by optical microscopy every four days. Feces were assayed for toxins.

Analysis of toxins

Algal toxins

Two liters of *G. catenatum* culture were harvested for analysis by extracting the biomass by centrifugation at 5,000g, then suspended in 30–50 ml 0.1 N HCl, and finally homogenized with glass beads (5 mm dia.). Three successive washes by stirring was done (30 s vortex: 30 s ice) and the supernatants were pooled. Examination under an optical microscope of the remaining cell debris after homogenization revealed that the cells
had been completely disrupted. Toxins were stored at −80°C.

**Scallop toxins**

Scallop specimens were removed from the culture containers after each experiment. About 5–10 g of each dissected tissue was homogenized in equal volumes by weight of 0.1 N HCl with a plastic homogenizing rod. The cell suspension or homogenized tissue was heated to 85°C for 5 min. After cooling the vial to room temperature, pH was adjusted to 3.4–3.6, and the extract volume was measured. The extract was centrifuged for 10 min at 10,000 g, and the supernatant was prepared for the HPLC. The tissues were stored at −80°C.

**HPLC analysis**

Paralytic shellfish poison was measured under the conditions described previously, using post-column derivatization HPLC with the fluorescent online detection method (Oshima 1995a; Andrinolo et al. 1999). Briefly, 20 μl pretreated sample was injected into a silica-base reverse phase column (Supelcosil 5 μm C-8, 0.46 × 15 cm, SUPELCO, Bellefonte, PA, USA, for separation of the toxins of the saxitoxin (STX) group: STX, neosaxitoxin (neoSTX), and gonyautoxins (GTXs). The following mobile phase was used: 2 mM 1-heptenesulfonic acid in 30 mM ammonium phosphate buffer pH 7.1:acetonitrile (100:3) at a flow rate of 0.7 ml min⁻¹. The column eluted fractions were mixed with 7 mM periodic acid in 10 mM buffered potassium phosphate at pH 9.0 at 0.4 ml min⁻¹, heated to 65°C by passing through a coil of Teflon tubing (0.5 mm i.d., 10 m long), and then mixed with 500 mM acetic acid at a flow rate of 0.3 ml min⁻¹ just before entering the monitor. The fluoromonitor was set to excite at 330 nm and emission at 390 nm. For this HPLC procedure, a Shimadzu LC-10AD liquid chromatograph with a Shimadzu RF-551 spectrofluorometric detector was used. The oxidizing reagent and the acid were pumped by a dual-head pump (model SP-D-2502, Nihon Seimitsu Kagaku, Japan). Data acquisition and data processing were performed with Shimadzu CLASS-CR-10 software. Identification of PSP was made by comparing chromatograms obtained from the sample extracts with those resulting after injection of standard solutions. Quantification of PSP content was made with the factor response (peak area/toxin concentration) with injection of known quantities of toxin standards. As an external standard, pure toxin solutions calibrated by combustion analysis nitrogen measurements and HPLC-MS were used (Lagos 1998; García et al. 2004).

**Results**

**Feeding physiology: clearance and IRs**

Small juvenile *N. subnodosus* (SH = 2.7 cm) exposed to concentrations >450 cells ml⁻¹ *G. catenatum* showed partial shell valve closure, low feeding activity, and production of pseudofeces. There was no significant difference in CRs for different concentrations of *G. catenatum*, which ranged from 0.4–0.81 scallop⁻¹ h⁻¹ (Fig. 1). The specimens did not finish food that was offered once during the 7-h experiment (Fig. 2). There was a significant difference in IRs for different concentrations of *G. catenatum* (*P < 0.05*) (Fig. 2). Partial closing was followed by gradual reopening over 7 h. At lower concentrations, active feeding of toxigenic dinoflagellates occurred without any apparent negative response.

In large juvenile scallops (SH = 5.17 cm), at initial concentrations of 850 and 1,650 cells ml⁻¹, ingestion of cells decreased during the first hour, then returned to normal rates, and the scallops finished filtering all the food over the next two hours, with only small amounts of pseudofeces. There were no significant differences in CRs for the concentrations of *G. catenatum* tested, which ranged from 2.7–3.41 scallop⁻¹ h⁻¹ (Fig. 1). Also, there were no significant differences in IRs (Fig. 2). For both sizes of control scallops fed an initial *I. galbana* concentration of 3.5 × 10⁷ cells l⁻¹, clearance and IRs were significantly higher during the first hour of the experiment, and this was sufficient for ingesting their once-only meal at the rate of 2.31 scallop⁻¹ h⁻¹ for the small scallops and 7.71 scallop⁻¹ h⁻¹ for the large scallops. No deaths occurred.

![Fig. 1](image-url)
during the experiment. We confirmed that dinoflagellate concentrations that do not repel the toxigenic cells and do not produce pseudofeces were below 450 cells ml\(^{-1}\) for the small scallops and 1,650 cells ml\(^{-1}\) for the large scallops (2.7 and 5.1 cm, respectively).

Toxin analysis of *G. catenatum*

Figure 3 shows the representative chromatograms of the HPLC-FLD run. The PSP standard mixture (Fig. 3a) shows three peaks: gonyautoxin group (GTX), neosaxitoxin (neoSTX), and saxitoxin (STX). In *G. catenatum* cells, we did not detect neoSTX or STX under our culture conditions. The GTXs were detected at 2–5 pg STX eq cells\(^{-1}\) (Fig. 3).

Tissue compartmentalization of toxins in *N. subnodosus*

Pooled samples of shellfish meat from the scallops were obtained from experiments and submitted for HPLC analysis. PSP profiles found in *N. subnodosus* are mainly a reflection of the toxin profile of the dinoflagellate because PSP in all the tissues were also only represented by the mixture of GTXs, except in the digestive gland and labial palps, where in some cases, trace amounts of STX were detected (Fig. 3c). Concentration of toxin was highest in the digestive gland, followed by labial palps, intestine, gills, mantle, and adductor muscle. Viscera contained >80% of the toxin body burden (Fig. 4).
Toxin uptake kinetics and depuration

With these clearance and ingestion rate data, we used large scallops (5.9 ± 0.39 cm) for the long-term feeding experiment with *G. catenatum* cell density of approximately $3 \times 10^5$ cells scallop$^{-1}$ day$^{-1}$ and *I. galbana* at $5 \times 10^7$ cells scallop$^{-1}$ day$^{-1}$. Most PSP was found in the digestive gland and, after uptake of toxin for 12 days, ranking of toxin load in tissues was: digestive gland > mantle > gills > adductor muscle. These scallops achieved a remarkably high maximum toxin body burden ($600 \mu g STX eq$ 100 g$^{-1}$ wet weight) during the 12 days of exposure to the toxic dinoflagellate, where GTXs were the most abundant toxin. After ingesting toxic dinoflagellates for 12 days, feces contained 50 to 100 $\mu g STX eq$ 100 g$^{-1}$. Depuration rates varied among tissues (Fig. 5). For example, after the first day, toxins in the digestive gland were reduced by 60% and the adductor muscle was toxin-free after 8 days. Most tissues remained toxic after 12 days. Total body burden remained $>110 \mu g STX eq$ 100 g$^{-1}$. Shellfish harvesting is not permitted at levels $>80 \mu g STX eq$ 100 g$^{-1}$. Scallop fed a mixture of *G. catenatum* and *I. galbana* had byssus production, while scallops fed only *I. galbana* did not show this healthy physiological response. Minimal pseudofeces production occurred during this experiment. Throughout the experiment, there were no signs of illness or mortality attributable to PSP loading.

Discussion

Feeding physiology

Based on feeding responses of juvenile scallops, the results suggest that giant lions-paw scallops are not affected by exposure to PSP toxigenic cells at moderate cell concentrations. CRs of toxin from the dinoflagellate *G. catenatum* were greatly reduced, compared to the nontoxic control diet, and may be partly attributable to concentration-dependent effects because dinoflagellates were added at relatively high cell density. At concentrations greater than 450 cells ml$^{-1}$ for small scallops (SH = 2.7 cm) and 1,650 cells ml$^{-1}$ for large scallops (SH = 5.1 cm), feeding behavior may be one of the principal factors controlling feeding rates in *N. subnodosus*, such as production of pseudofeces,
partial shell valve closure, and reduced feeding rates. Reduced feeding rates have been reported for a number of bivalves exposed to toxic dinoflagellates (Dupuy and Sparkes 1967; Shumway and Cucci 1987; Wildish and Saulnier 1993). Pseudofeces production is a good indicator that the bivalve can regulate particle ingestion. These mechanisms could permit bivalves to maximize energy gain, avoid toxic or dangerous food, and satisfy nutritional requirements by selecting cells based on their morphological characteristics and probably by biochemical aspects (Shumway et al. 1997). Scallops fed *G. catenatum* reduce their filtration rates in the first hours, but several hours later, filtration rates are normal and no detrimental effects were observed. It may partly be the result of behavioral adaptations to the first response and/or a period of acclimation to a new feeding regimen.

During the 12 days that scallops were fed a mixture of *G. catenatum* (5 × 10³ cells scallop⁻¹ day⁻¹) with *I. galbana* (8 × 10³ cells scallop⁻¹ day⁻¹), they showed an active filtration state without producing pseudofeces. Researchers have observed that bivalves show good feeding responses to mixtures of toxic and nontoxic microalgae (Bricelj et al. 1991, 1996a; Li et al. 2001). However, feeding only a toxic alga brings a contrary response (Bardouil et al. 1993; Bricelj et al. 1996b; Matsuyama et al. 1999).

In pectinids, there does not appear to be adverse effects from exposure to PSP producers. For example, *Pecten maximus* fed *A. tamarense* for 15 days showed no adverse effects (Lassus et al. 1992). In our study, during the long-term feeding trials, scallops fed the non-toxic microalgae mixture during the acclimation period and scallops fed the mixture of *G. catenatum* with *I. galbana* showed production of byssus. Control specimens and trial specimens during depuration fed exclusively *I. galbana* did not show production of byssus. This process, while not experimentally tested, was observed every day. This physiological response could provide a useful index of the physiological state of the animal. Several authors claim that byssus production is a normal physiological response to toxic dinoflagellates (Shumway and Cucci 1987; Gainey and Shumway 1988). Our results support the interpretation that scallops fed only *I. galbana* were not in optimum conditions; that is, they did not produce byssus. *I. galbana* is an adequate diet ingredient for bivalves because it provides high energy, but it needs to be supplemented with species that improve alimentary quality (Blanco-Pérez et al. 1997). Several authors recommend that, under controlled conditions, a mixture of microalgae best satisfies the nutritional requirements of bivalves (Díaz and Martínez 1992; Lora-Vilchis and Doktor 2001). Other microalgae provide good quality fatty acids, known to be essential for marine bivalves (Trider and Castell 1979; Robinson 1992; Berntsson et al. 1997). Dinoflagellates, including *G. catenatum*, are a good source of essential fatty acids, but how this stimulates filtration activity is still unknown (Napolitano and Ackman 1993; Mansour et al. 1999, 1999b, 2003).

Toxin uptake, compartmentalization, and depuration HPLC with online fluorescent detection (HPLC-FLD) samples for toxin profiles, providing extra information, such as metabolic transformations of toxins with modification of PSP profiles (Oshima 1995b; Lagos 1998). More than 20 structurally-related PSP derivatives have so far been identified in toxigenic dinoflagellates and the bivalves that consume them. The most commonly encountered toxins are saxitoxin (STX), neosaxitoxin (neoSTX), gonyautoxins I, II, III, IV (GTX 1–4), B1 (GTX5), B2 (GTX6), and C1-C4 (Bricelj and Shumway 1998). In this study, the toxins present in *G. catenatum* belong to the gonyautoxins group (GTXs). STX and neoSTX were not present in our cultures. A large proportion of gonyautoxins was observed in cultures from Japan, Spain, and Tasmania (Oshima 1993). Toxin composition in dinoflagellate strains can vary with geographical location and can be influenced by environmental factors or experimental conditions (Anderson et al. 1990). PSP profiles found in *N. subnodosus* are mainly a reflection of the toxin profile of the dinoflagellate, with the GTXs group in all tissues, plus some traces of STX detected in the labial palps and digestive gland. GTXs are the most abundant PSP in shellfish extracts, and in most cases, GTXs account for high toxicity of shellfish worldwide (Lagos 1998, Lagos 2003). Evidence from Japan and Canada, where PSP profiles obtained from a shellfish samples show the same GTXs found in water samples collected during the peak of algal blooms (Bricelj et al. 1991). However, most shellfish contain a mixture of several PSPs, and may therefore have different toxin profiles from those of the dinoflagellate to which they were exposed (Cembella et al. 1994). The presence of STX in some samples could occur when less toxic forms change to more toxic forms, and are reflected as different profiles (Sullivan 1988; Lehane 2000). STX is the final detectable degradation products of the various C-toxins and gonyautoxins (GTXs) that undergo chemical and enzymatically mediated reductive cleavage, (desulfation and dehydroxylation), and hydrolysis, (decarbamoylation and desulfation) (Lehane 2000). Changes in the toxin profile of shellfish tissues may arise from selective
retention or elimination of individual toxins, epimerization, or from a variety of transformation processes (Bricelj and Shumway 1998). It indicates that labial palp and digestive gland in N. subnodosus play an important role in biotransformation of toxins once toxic cells are ingested, as occurs in other bivalves.

Most of the toxin body burden in this scallop was contained in the viscera (83%). As a result of continuous filtration, PSP is usually concentrated in the digestive gland, or in the case of another species, the butter clam Saxidomus giganteus, in the siphon (Halstead and Schantz 1984). The digestive gland contained the highest body burden of toxin, as in other bivalves, including Placopecten magellanicus and Patinopecten yessoensis (Shumway and Cembella 1993; Bricelj and Cembella 1995). In contrast to toxin levels in viscera, levels were relatively low in the gill, mantle, and adductor muscle, tissues that contribute substantially to the total weight of soft tissues, but make a disproportionately low contribution to the toxin body burden (Cembella et al. 1994), especially in adductor muscle, which had the lowest retention level. It is notable that the limited capacity of the adductor muscle to accumulate toxins also favors marketing of scallops to the United States and Canada, where traditionally, only this tissue is consumed. Toxicity of the adductor muscle is typically one to three orders of magnitude lower than in the digestive gland (Bricelj and Shumway 1998). As indicated above, the toxin burden is dependent on dinoflagellate cell density, specific toxicity, and length of exposure. However, there is substantial intraspecific variation in toxin levels related to extrinsic factors (microgeography of the site and random patchiness in the distribution of toxic cells) and intrinsic factors (differences in individual feeding rates, susceptibility to PSP, body size, and physiological condition) (Bricelj and Shumway 1998).

Nodoplecten subnodosus is not efficient in accumulating PSP over 12 days, compared to other bivalves studied under similar experimental conditions. Uninhibited ingestion of toxic G. catenatum cells resulted in rapid increase in toxins in scallop tissues after a few hours of exposure; with viscera exceeded commonly accepted regulatory levels of PSP (80 µg STX eq 100 g⁻¹). According to Bricelj et al. (1996a), toxin accumulation is strongly influenced by the presence of nontoxic cells in a mixed phytoplankton assemblage, which may stimulate ingestion of toxic species. Toxins present in feces could be an indicator of the toxin content in the digestive system, since it has high absorption efficiency. Here, we found that, while concentrations of toxin components in the tissues varied during the exposure period, the toxin profile in the scallops was similar to the profile in G. catenatum.

Rapid release of toxins from all tissues occurred within the first days of depuration, except from the viscera. This indicated that toxins were not tightly bound to other tissues. After 12 days of depuration, toxins were present in small amounts in most tissues, except the adductor muscle, where it was negligible. PSP is slowly eliminated (Shumway and Cembella 1993), but the underlying PSP detoxification mechanism operating in bivalves is poorly understood. The low level in adductor muscle indicates that this tissue is usually safe for human consumption. Rates of toxin accumulation and elimination in and from bivalves differ among species and geographical situation, toxicity of specific dinoflagellates, and sequestering of toxins in tissues (Landsberg 1996). Some authors suggest that rates of accumulation are frequently greater than elimination and several studies have shown that the digestive gland detoxifies at a faster rate than other tissues (Bricelj et al. 1991). Different bivalve species exposed to the same algae bloom vary markedly in their ability to detoxify accumulated PSP. The mussel Mytilus edulis, undergoes detoxification in 1–10 weeks, but P. magellanicus, P. yessoensis, and Chlamys farreri typically take several months to years (Andrinolo et al. 1999; Zou et al. 2001).

In summary, food concentration and bivalve size play important roles in ingestion of toxic dinoflagellates by the scallop N. subnodosus. Even at high concentrations of G. catenatum cells, no deaths occurred, indicating that this scallop does not show adverse effects from consumption. Evidence supports the nutrient value of a mixture of algal cells in controlled culture conditions, and a mixed feed enhances G. catenatum uptake. Several physiological effects were observed that could serve as biological markers in situ for effects of PSP, such as production of byssus, which also indicates that polyunsaturated fatty acids in G. catenatum have nutritional value. Additionally, scallops are less affected, compared to other bivalves. The toxicokinetics of PSP in N. subnodosus, as indicated by many studies, are that visceral tissues accumulate the largest quantity of PSP. In our study, the labial palps and digestive gland seem to play an important role in toxin biotransformation, and low levels of accumulation occur in some tissues, compared to other bivalves, but these toxins are slowly depurated, except for the adductor muscle, an advantage that enhances marketability and consumption.

Acknowledgments We thank personnel at the Laboratorio de Bioquímica de Membrana of the Universidad de Chile for analyzing toxins and M. de Jesus Romero for assistance with laboratory work at CIBNOR. Financial support was provided by Centro de Investigaciones Biológicas del Noroeste (CIBNOR grant AC 3.1).
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