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Effect of dissolved metabolites of the dinoflagellate *Gymnodinium catenatum* (Graham, 1943) on the white shrimp *Litopenaeus vannamei* (Boone, 1931): A histological study

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ABSTRACT

Harmful algae blooms (HABs) are a conspicuous phenomenon that affect the coastal zone worldwide. Aquaculture industry zones are not excluded from being affected by HAB that cause organism mortality and jeopardize their innocuity due to the contamination by phytotoxins with the concomitant economic losses. Direct ingestion of metabolites from HAB species or organisms contaminated with phycotoxins together with dermal absorption of dissolved metabolites (DM), including toxins, are the two main routes of poisoning. From these poisoning routes, the effect of DM, particularly paralytic shellfish toxins (PST), has been relatively understudied. This intoxication route can be conspicuous and could be involved in many significant mortalities of cultivated marine organisms. In this study, white shrimp juveniles (2.1 g wet weight) of *Litopenaeus vannamei* were exposed to extracts of 10^4, 10^5 and 10^6 cells/L of the dinoflagellate *Gymnodinium catenatum*, a PST producer. The experiment ended after 17 h of exposure when shrimps exposed to 10^6 cells/L extract started to die and the rest of the shrimps, from this and other treatments, did not respond to gentle physical stimulus and their swimming activity was low and erratic. Toxin concentrations were determined using high performance liquid chromatography while qualitative and quantitative histological damages were assessed on the tissues. In general, most toxins were accumulated in the hepatopancreas where more than 90% were found. Other tissues such as intestine, muscle, and gills contained less than 10% of toxins. Compared to the control, the main significative tissue damages were, loss of up to 80% of the nerve cord, 40% of the muscle coverage area, and reduction of the gill lamella width. Also, atrophy in hepatopancreas was observed, manifested by a decrease in the height of B cells, lumen degeneration and thinning of tubules. Some damages were more evident when shrimps were exposed to higher concentrated extracts of *G. catenatum*, however, not all damages were progressive and proportional to the extract concentration. These data confirm that PST dissolved enter the shrimp, possibly via the gills, and suggest that dissolved metabolites, including PST, may cause tissue damage. Other dissolved metabolites produced by *G. catenatum*, alone or in synergy, may also be involved. These

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results also pointed out the importance of dissolved molecules produced for this dinoflagellate and the potential effect on cultured shrimp.

1. Introduction

Aquatic micro-organisms produce a wide range of metabolites with various biological actions. One of the most known metabolites is the saxitoxin and its analogs, collectively named paralytic shellfish toxins (PST). This family of molecules blocks the sodium channel in humans and other organisms [1] causing the interruption of the nervous signal transmission. Analogs of PST are produced by many dinoflagellates and bacteria which are present worldwide and, in some areas, lead to harmful algae blooms (HABs). According to some hypothesis, PST molecules can function as allelopathic metabolites, pheromones, nitrogen storage, and participate in the chromosome structural organization [2]. Concerning fisheries and aquaculture, PST have been affecting these industries resulting in an implementation of monitoring programs for food safety [3,4]. In some areas, however, cost benefit analyses for management and exploitation of mollusks are not balanced, having low or null cost benefits and economically unable to be exploited [5].

World farmed shrimp production in 2019 reached more than 7.7 million tons, representing a value of over 33 billion US dollars, and was mainly produced in Asia and Latin America [6]. Pacific white shrimp (*Litopenaeus vannamei*) is the top shrimp species of commercial importance. The annual global yield of *L. vannamei* reached 4.4 million tons, accounting for 80% of the total cultured shrimp production [6]. Although there is no report on the global HAB incidence and problems in shrimp farms, according to the analysis of Mann [3] in shrimp cultures in Australia, episodes of significant stock losses on farms were potentially misdiagnosed and attributed to other causes, particularly diseases, when in fact disease was a secondary consequence to HAB blooms [3]. The presence of dissolved metabolites (DM) in the aquatic environment, such as those produced by HAB could also contribute to misdiagnose since, as the in case of PST and bioactive extracellular compounds [7], they are challenging to extract from the aqueous medium [8]. This problematic could be ubiquitous in many countries. Thus, dissolved HAB compounds can be found in nearby shrimp cultures or hatcheries and transported into the water source, despite the use of filtration systems. Exudates or cells rupture in the water supply and filter systems also contribute to increase the dissolved fraction facilitating the entrance towards the facilities. Further, the ineffective control of water quality in the input system of aquaculture ponds may also contribute to the intake of dissolved metabolites [9]. This suggests that besides nocive algae species, DM are conspicuous in the aquatic environment, and that in aquaculture ponds during a HAB, they can increase their presence during the bloom decadence due to phytoplankton mortality. It is known that waterborne toxins like okadaic acid and dinophysistoxin-1, can be assimilated by shellfish thru passive absorption (reviewed in Ref. [10]) and in the case of PST, affect locomotion and cause tissue damage impairing their function and, in some cases, causing mortality [11–14].

The relationship between the presence of HAB events and shrimp mortality in Mexican coasts has been reported [15]. The dinoflagellate *G. catenatum* is one of the main recurrent bloom forming species in the Mexican Pacific coast [16,17] suggesting that its PST or other metabolites could be involved in shrimp mortalities. To the best of our knowledge, this is the first study to present quantitative results of histological damages of dissolved extracts of the PST producer *G. catenatum* on juveniles of the white shrimp *L. vannamei* (Boone, 1931). The main goal of this study was to determine if waterborne PST enter and cause damages to the shrimp tissues. The objectives were to (1) determine the PST uptake and their concentration in diverse organs, (2) evaluate shrimp behavioral impacts to the DM exposure, and (3) assess quantitatively the histological damages of DM exposure on shrimp organs. Results show significant histological damages of DM in shrimp organs.

2. Material and methods

2.1. Dissolved metabolites

Gymnodinium catenatum strain LC62, isolated from coastal water of Lázaro Cardenas, Michoacán (Mexico) by M.C. Rodríguez Palacio, was cultured in three 2 L flasks containing 1.5 L of modified GSe medium, under culture conditions described previously [18]. Cells in mid-exponential growth phase were collected in 50 mL centrifuge tubes and concentrated by centrifugation at 5000 rpm for 15 min in a normal centrifuge; the humid cell pellet was sonicated (35 kHz) for 5 min in an ice bath and clarified by centrifugation (3000 rpm) for 5 min. The above procedures were repeated for each flask until all the culture extract was obtained. Extracts were stored in a freezer (−20 °C) until experimentation. To determine the number of cells of each extract, a 2 mL aliquot (duplicate) was taken prior to the extract procedure; cells were fixed with Lugol solution and counted in a 1 mL Sedgewick-Rafter chamber. Also, a 10 mL sample of each flask was filtered through a GF/F filter to analysis the *G. catenatum* PST toxin content (described below). Once obtained the *G. catenatum* cell extract was kept in a 50 mL flask and stored −40 °C.

2.2. Paralytic shellfish toxins

The toxin profile of the strain, was determined by high performance liquid chromatography (HPLC) with fluorescence detection according to Refs. [19,20], and was composed by N-sulfocarbamoyl analogs B1/2 and C1/2 as the most abundant analogs, with an average content in molar basis of 70% followed by decarbamoyl analogs (dcNEO, dcGTX2/3, and dcSTX) with 29%, and minor percentages of GTX2/3 (0.31% molar basis) (Fig. S1). Cell toxin content was 218.3 pg and average cell toxicity, based on toxin analogs contents and the toxicity equivalent factors [21] was 30 ± 7.14 pg STXeq.

2.3. Shrimp-dinoflagellates bioassays

Juveniles of *L. vannamei* (weight: 2.64 ± 0.44 g; length: 7.81 ± 0.44 cm) were obtained from a local shrimp farm (Blue Genetic, Mexico) and were placed in a 50 L tank at laboratory conditions (temperature of 23 °C, oxygen supplied with bubble stones, and food supply) for 24 h. From these shrimps, 10 organisms were placed in a 5 L plastic bottle with 3.33 L of seawater (3 org/L), and an extract equivalent to 10^6 cells/L of *G. catenatum*/L (10^6 hereafter) was added. Before the experiment, the pooled extract was thawed at ambient temperature, and resuspended in distilled water to obtain an extract with a density of 10^6 cell/mL. As a control, organisms without the dinoflagellate extract were used. A second and third exposure bioassay were simultaneously done in another set of bottles with the addition of extracts equivalent to 10^4 and 10^5 cells/L of *G. catenatum*, respectively (10^4 and 10^5 hereafter). All 10^4, 10^5, and 10^6 bioassays were done in triplicate. According to the estimated cell toxicity of *G. catenatum* (30 pg STXeq/cell), these extracts contained 0.3, 3.0, and 30.0 μg STXeq/L for the extracts of 10^4, 10^5, and 10^6, respectively. The experiment ended after 17 h of exposure when two shrimps exposed to the highest *G. catenatum* extract died and the remaining shrimps did not respond to gentle physical stimulus (pipette touch), and their swimming activity was slow and erratic. During the experiment no food was supplied.

Seven organisms from each treatment were dissected to separate gills, hepatopancreas, stomach, and muscle. This procedure was done on a cold steel plate to avoid tissue warming. In a previously weighted Eppendorf tube, each tissue was pooled, except the muscle, to have enough material for PST analyses. In the case of the muscle, only a section of the tail tissue of each organism was pooled to obtain ~2.0 g of tissue. Each pooled tissue was weighted and used for PST analysis. The remaining three organisms were injected with Davidson solution and kept in a flask containing Davidson solution for 24 h, according to Ref. [22]. These organisms were used for histological analysis (see below). Dissection and pooled tissues samples of the exposed shrimps was also done for the control organisms.

2.4. PST extraction and analysis

PST were extracted by adding a solution of 2000 μl of 0.05 M acetic acid to each tissue sample, disrupted with a Microson XL ultrasonic cell disruptor (Misonix, Farmingdale, NY, USA) for 60 s on an ice bath. Extracts were centrifuged at 5000 rpm for 10 min and filtered with 13 mm diameter syringe filters (0.22 mm pore size PVDF Millex membrane) to remove particulate material. Only muscle and HP tissues that were hand macerated. To assure a better toxin extraction in tissues, additional 2.0 mL of acetic acid were added to each pooled tissue and the cleaning procedure was repeated. Both extracts were pooled. Extracts from all tissues were lyophilized.

Lyophilized tissues were resuspended in 400 μL of 0.03 M of acetic acid and analyzed by HPLC with fluorescence detection according to Refs. [19,20]. It is important to point out that this method has a drawback to resolve complex toxin profile particularly when new analogs are present in the sample or their standards are not available [23]. Also, dcNEO and NEO have the same retention time in this method and therefore are not separated. However, according to Ref. [23], this *G. catenatum* strain does not produce NEO, so the corresponding chromatogram peak in this study was assumed to be dcNEO. Detection and quantification limits, based on the chromatogram peak height, base and noise signals of each toxin standard are shown in Table 1. Total toxicity in each tissue, in μg STXeq/kg wet weight, was calculated by using the toxicity equivalency factors [21] and the concentration of each analog.

2.5. Histological tissue treatment

2.5.1. Sampling

At the end of the experiment, three fixed shrimp were taken from each container and treatment. From each organism, the transverse dissection of the middle region of the cephalothorax was performed to obtain a longitudinal section that included the hepatopancreas [22]. The cephalothorax was placed on histocassettes.

2.5.2. Tissue processing

Tissues were dehydrated in ethanol, cleared in xylene, infiltrated and embedded in paraffin, sectioned (4.0 μm), and stained with Harris hematoxylin-eosin [24] for histological analysis. The histological slides were mounted in permanent medium of synthetic resin

Table 1
Limit of detection and quantification in the equipment used.

PST analog	LOD	LOQ
STX	6.6	22.3
dcSTX	14.1	47.0
NEO	1119.1	3730.5
GTX 2	37.1	123.8
GTX 3	42.0	140.1
dcGTX 2	48.2	160.9
dcGTX 3	45.8	152.9
dcNeo	37.1	123.8

STX, saxitoxin; dcSTX, decarbamoil STX, NeoSTX, neosaxitoxin; GTX, gonyautoxin; dcGTX, decarbamoil gonyautoxin; dcNeo, decarbamoil neosaxitoxin. LOD, Limit of detection (ng/mL); LOQ, limit of quantification (ng/L).

Entellan®. The histological structures of each tissue were observed under a microscope OLYMPUS BX41 at magnifications of 10, 20, 40, and 100×.

2.5.3. Quantitative evaluation

Observation of slides for histological analysis was performed on an Olympus BX41 light-field microscope and images were digitized using a Nikon Digital Sight DS-Ri1 digital microscope camera®. Microphotographs of the tissues were analyzed and digitalized at magnifications of 10, 20 and 40× using the Image Pro Premier software (version 9.0) Olympus-Media Cybernetics®. The numerical values of the coverture (area in μm^2) of the muscle and nerve cord were obtained from the digitized images. Height of B cells were obtained from three sections of the hepatopancreas from digitized images at 40×, measuring the heights from the basal region (in contact with the basement membrane) to the apical region (free edge) where the lumen is found.

2.5.4. Statistical analysis

Data analyses were performed separately for each organ (hepatopancreas, intestine, gills, and muscle). The three treatments integrated the fixed factor, and the different plastic flasks used as a replica of the same treatment were considered a random factor. A priori Shapiro-Wilk and Barlett's tests were applied to confirm the normal frequency distribution and homogeneity of variances of the data. Factorial analyses of variance (ANOVA) were used to evaluate the effect of treatments. The values in area (μm^2) expressed in % were transformed to arcsine function (arcsine $\sqrt{P}$) before analysis, but data are reported untransformed, as mean ± standard error. In cases where significant differences were found a Tukey test (HSD) post hoc analysis means comparison was used [25]. All analyzes were performed using Statistica software 8.0 (StatSoft Inc., Tulsa, UK). In all comparative analysis among exposed data and exposed versus control data a $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Behavioral observations

Four distinct behavioral acts were observed at glance in the organisms exposed to the extracts of *G. catenatum* when compared with the control treatment: Erratic swimming, disorientation (inability to maintain an upright position in the water column), surface swimming, approach to air bubbles and air stones. In the first case, organisms were swimming in one direction and suddenly they would turn and swim in the opposite direction. Disorientation refers to shrimp swimming with their legs upwards or sideways. Surface swimming refers to shrimp swimming towards the surface, settling passively at the bottom and returning once again to the surface. It was also common to observe shrimp approaching and remaining close to or resting on the air stones. These behaviors were more evident with the highest *G. catenatum* extract. Towards the end of the experiment, particularly in the 10^6 treatment, several organisms were immobile resting on the bottom and did not respond to gentle disturbing with a pipette. No mortalities were observed in 10^4 and 10^5 treatments after 17 h. Similarly, no mortality was observed in the control treatment and no abnormal behavior was observed.

3.2. Toxin profile and toxicity in shrimp tissues

Eight toxin analogs were recorded in the tissues: Three from the decarbamoyl (decarbamoyl saxitoxin and decarbamoyl gonyautoxin 2/3), two from the carbamoyl (Gonyautoxin 2/3) and three from the less potent sulfocarbamoyl analogs B1 and C1/2 (Table S1). Concerning the HP, the analogs GTX2/3 were the most abundant (more than 36%), followed by decarbamoyl analogs dcGTX2/3 in the 10^4 and 10^5 treatments. In the 10^6 treatment, the C-toxin contributed to 39% of the total toxin content, followed by GTX and dcGTX toxins.

The contents of the saxitoxin analogs found in muscle, dcGTX and GTX, are roughly similar to toxins found in the HP. In the case of the intestine, sulfocarbamoyl analogs were more abundant (49% in the 10^4 and 10^6 and 51% in the 10^5 treatment). In the gills the toxin analogs were also dominated by dc and GTX toxins in the 10^4 and 10^6 treatments. Interestingly, no toxins were detected in the 10^5 treatment.

When weight values are standardized by considering the molecular weight and expressed as molar percentage (% molar), contribution of B and C analogs were lower than 32% in the HP in all the treatments. In muscle, decarbamoyl and carbamoyl analogs represented more than 50%. In the intestine, B toxins were the only analogs detected in the 10^4 and 10^6 treatments; in the 10^5 treatment GTX2/3 was also detected and contributed with 63% to the total toxin content.

Toxin analogs were (in a tissue weight basis) mainly found in the hepatopancreas (HP) and represented 92.4%, 85.1%, and 80.7% of the total toxins, for the 10^4, 10^5, and 10^6 treatments, respectively. An increase of the toxin content in the HP with the increase of dissolved toxins exposed is also evident (from 961.6 to 1661.6 μg/kg), however this toxin increase was not statistically significant. In the gills, intestine, and muscle, there was no clear pattern in the toxin content with respect to extract concentration (Table S1).

Most toxins were accumulated in the hepatopancreas in all the treatments being the organ with the highest toxicity that ranged between 217.7 and 312.2 μg STXeq/kg (Table S1). Toxicity varies in other tissues and was less than 10% of the total toxic percentage except for the treatment of 10^6 where gills contained 37.3% and the muscle 12.2% of the total toxicity.

3.3. Histological damages

3.3.1. Hepatopancreas

All organisms exposed to the different concentrations of *G. catenatum* extracts showed alterations such as atrophy and delamination and thinning of tubules. These damages are more evident as dissolved cell content increases as noted in the HP exposed to the highest concentration (Fig. S2). Necrosis, indicated by the decrease in the height of B cell [26] in organisms exposed to *G. catenatum* extracts showed a progressive decrease of the height that is proportional to the cell concentration extract at which they were exposed. Cell height diminished in 38%, 53% and 62% in the 10^4, 10^5, and 10^6 treatments, respectively, when compared with the control (Fig. 1).

3.3.2. Muscle

In the concentrations of 10^5 and 10^6 cel/L extract treatments, shrimps presented edema, alterations in the cell cytoplasm, necrosis, hemocyte infiltration and atrophy (Fig. S3). A statistically significant decrease was observed in the area of muscle coverture between the control and those from the extract treatments (Fig. 2). However, treatments of 10^4 and 10^5 cel/L shown not significant differences in the area of muscle coverture.

3.3.3. Gills

Gill atrophy, detected as a decrease in the width of the lamellae and thinning of the gill (Fig. S4), was evidenced in all treatments, but they were only significative ($p < 0.05$) in the treatment with the highest cell extract concentration with close to 40% of width thinning in relation to the control (Fig. 3).

3.3.4. Nerve cord

Clearly, a progressive loss of the nerve cord was evident (Fig. S5). Quantitatively, the nerve cord area diminished by 37% in treatments of 10^4 and 10^5 (no statistical difference between them), and it decreased to 80% in the 10^6 treatment with a significative ($p < 0.05$) difference in relation to the control (Fig. 4).

4. Discussion

The effect of dissolved metabolites (DMs) of *G. catenatum*, in experimental conditions, on juveniles of the white shrimp *L. vannamei*, one of the main fisheries and main marine cultures in many countries [6] was tested, to our best knowledge, for the first time. The experiment simulated the presence of environmental *G. catenatum* cell concentrations that seem to be ubiquitous when HABs occur [8], particularly in the Pacific Mexican coast [16,17,27]. Results clearly show that DMs, including PST, from this dinoflagellate are uptaken, incorporated in tissues, and induced important behavioral changes and tissues damages even at the lowest concentration of DM (10^4 treatment). Also, these DM induced death of *L. vannamei* in a relatively short time (17 h) at the higher concentration (10^6 treatment). Although other metabolites can cause damages directly or in synergy with PST [28–33]. In the rest of the discussion, emphasis on the possible role of PST in the observed lesions will be considered since: a) PST have been demonstrated to affect other marine organisms [34–36,38] and cause partial immunity in bivalves [[38–40], reviewed in Ref. [41]], b) no measurement of other metabolites (Reactive Oxygen Species -ROS, poli-unsaturated fatty acids -PUFA, bioactive extracellular compounds) were done in this study, and, c) there are no reports of the production of these metabolites for *G. catenatum*, except for superoxide production [31,42] and PUFA [43,44]. With these details, let us then examine the possible role of dissolved PSP, without discarding the effects or interaction with other metabolites, to explain our results.

4.1. Behavioral observation

Paralysis symptoms such as immobility, erratic swimming, and disorientation, have been observed in experiments with shrimp

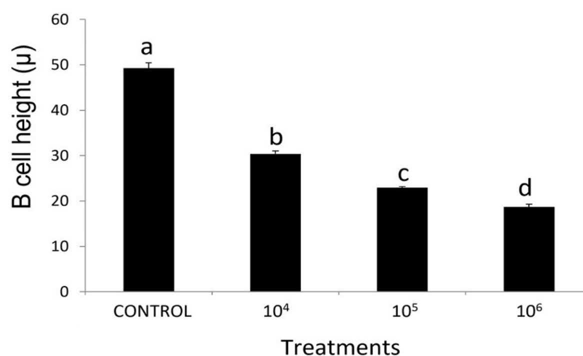

Fig. 1. Height (µm) of B cells of *Litopenaeus vannamei* hepatopancreas in the control and in 10^4, 10^5, and 10^6 treatments. Vertical bars: standard error. Different letters indicate statistical differences.

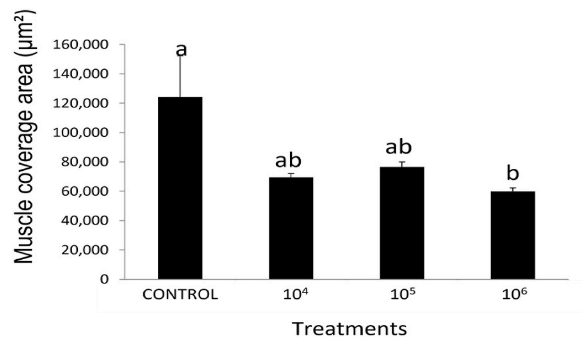

Fig. 2. Average area of muscle coverage (µm^2) from *Litopenaeus vannamei* in control and in the 10^4, 10^5, and 10^6 treatments. Vertical bars: standard error. Different letters indicate statistical differences.

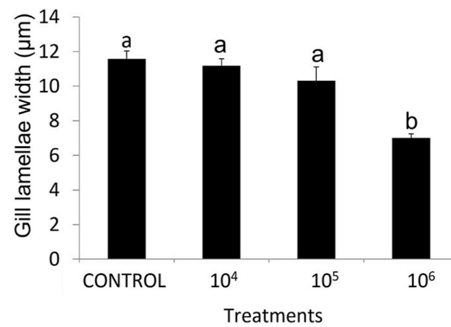

Fig. 3. Lamella width (µm) of shrimp hepatopancreas in control and in 10^4, 10^5, and 10^6 treatments. The distinct letters indicate significant statistical differences.

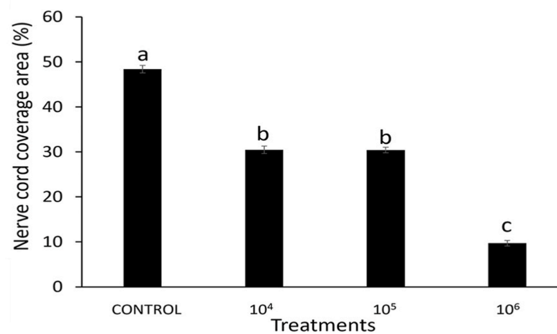

Fig. 4. Nerve cord coverage area (%), as the area of the tissue affected by edema/the total cord area x 100 (%), in control and in 10^4, 10^5, and 10^6 treatments. The distinct letters indicate significant statistical differences.

juveniles [45,46], brine shrimp [47], and in fish larvae [11–13] exposed to cells of *G. catenatum* or PST and agree with our observations. Paralysis seems to be a common feature when organisms are exposed to PST producer organisms [34,38,48] and could be a consequence of the PST pore-blocking in the sodium channel [1]. Also, PST injected intraperitoneally caused tissue damage [38,39,46] similar to our findings. Therefore, this study confirms that even dissolved PST and other metabolites produced by *G. catenatum* are uptaken, incorporated into the circulatory system and distributed to the different tissues of the shrimp *L. vannamei* causing several tissue damages and paralytic symptoms. In addition, this study adds the behavior of surface swimming, approach to air bubbles and air stones.

4.2. Paralytic shellfish toxins

Toxins often accumulate in bivalve tissues, mostly in the digestive gland [reviewed by Ref. [49]] and clearly shrimp also accumulate toxins in the hepatopancreas (Table S1). It is also clear in the PST data that a biotransformation process is occurring together

with a differential retention and elimination of specific toxins. For instance, dinoflagellate extracts contained mostly toxins from the sulfocarbamoyl group (around 70% in a mol basis of B and C-types) while in the shrimp's tissues these toxins had a minor contribution, and their contribution is tissue dependent without a clear pattern regarding the concentration of the extract. Processes such as epimerization, reduction, acid hydrolysis, and enzymatic hydrolysis are part of toxin metabolization in organisms [48,50,51]. The relevance of each process will depend on the tissue and the species [52]. Thus, for instance, some mollusks produce enzymes that acts on the carbamoyl group that lead to decarbamoyl analogs [48]. Strains of *G. catenatum* isolated from the Gulf of California are characterized by a high production of sulfocarbamoyl analogs, above 70% in a mol basis [23] therefore, the presence of more structurally complex analogs (B and C-types) also contribute to a high biotransformation as suggested by Ref. [48]. Another source of toxins involved in the biotransformation would be the benzoate analogs [53] since these analogs have been found in several *G. catenatum* strains isolated from Mexican waters [23]. Unfortunately, benzoyl analogs cannot be detected with the toxin detection method used in this study.

4.3. Overall effect

The effect of the cellular content on the shrimp tissues shows different quantitative damages suggesting diverse vulnerability or susceptibility. Thus, for instances HP shows a progressive damage, as shown by the decrease of the B cells height, from the lowest to the highest DM extract treatment; while in gills, nerve system and muscle, the damages were similar in both 10^4 and 10^5 treatments. Although saxitoxin and analogs exert their effect by binding directly to the voltage-dependent sodium channels in nerve and muscle cell membranes, they are relatively very stable [54]. Other molecules such as ROS have a lifetime in the scale of seconds to hours [55] and have a limited time to react with tissues. Therefore, damages are not dependent of the quantity of the extract because metabolites, such as ROS, are saturating the target tissue's receptors and they denature in a short time. It is known that the STX or dinoflagellates containing extracts injected or exposed to them, can induce oxidative stress in bivalves, fish, cladocerans and mammals [37,56–58]. Activation of the antioxidant system in shrimps exposed to the extracts of *G. catenatum* in our experiments could also be occurring [59] as a response to induction of ROS production.

4.4. Hepatopancreas

Hepatopancreas (HP) is one of the most important organs in shrimp and through the different epithelial cell types R-, F- and B-cells, is involved in many basic and critical functions for the digestion, detoxification of organic xenobiotics and some heavy metals, absorption and metabolize of nutrients, storage, and catabolism of energy reserves like lipid globules and glycogen [60,61]. B cells, an important component of the HP, are responsible for the production of digestive enzymes, nutrient accumulation, intracellular digestion, and transport of digested material [62]. In this study, damages in HP are evident as shown by the tubule's delamination, atrophy, and cell sloughing in HP, and decrease of the B cell height (Fig. 2). These damages are evident even at the lower extract exposition (10^4). Results agree with [59] that observed a critical unbalance between oxidants and antioxidants and that the defense mechanism of antioxidants was declined in the Chinese shrimp, *Fenneropenaeus chinensis* exposed to cells of *Alexandrium tamarense* resulting in peroxidation and apoptosis in the hepatopancreas. It is interesting to note that in Liang et al. experiments, no mortality was observed even at 96 h after exposure to 1000 cell of *A. tamarense*/mL. In this study, mortality initiated 17 h after exposure, which suggest that *G. catenatum* contains a higher concentration of toxic compounds. Although in our work the exposure to the dinoflagellate was as disrupted cells and it is known that the activity of the superoxide dismutase (SOD), an indication of the presence of superoxide, is higher in lysed cell when comparing them with intact cells [29]. Also, *G. catenatum* is a high producer of superoxide (59.7 ± 15.2 cellular chemiluminescence units) only a few units below of some extremely nocive raphidophyte species such as *Chattonella marina*, *C. antiqua*, and *C. globosa* [31,42].

4.5. Nerve cord

As part of the nerve system, the nerve cord coordinates neural signaling from the brain to the body and vice versa, integrating sensory input and locomotor output. Also, the nervous system is a major source of locally-released peptide paracrines and circulating peptide hormones [63] many of them involved in reproductive maturation, mating, spawning, larval and adult growth, as well as disease resistance [28]. Nerve cord damage was important in all treatments, but it is more evident in the 10^6 treatment. This tissue can also be affected (targeted) by many bacteria and virus [22,64] and as shown in this study, it is also affected by the cell extract of *G. catenatum*. Interestingly, and according to our experience, the severe loss of nerve cord found in this study, with a reduction of 37% in 10^4 and 10^5 and 87% in 10^6 exposure is not comparable as those induced by viral or bacterial infection [65,66]. Additionally, saxitoxin and analogs may interfere directly nerve functions by blocking the sodium channels [1]. Therefore, rupture of the nerve cord and the PSP blocking effects would result in shrimp sensor and locomotion failure as well as in an unbalance of hormonal associated actions. This severe nerve cord loss can be a distinctive damage of the *G. catenatum* extract.

4.6. Gills

Gills are a vital multifunctional organ specialized oxygen and ion exchange tissues that include high surface area lamella and extensive vasculature that permits the inhabiting of zones of variable parameters such as estuarine and fresh water [67]. Also, gills are involved in xenobiotic uptake/outtake and hypoxia physiological processes [68]. In this study, a decrease of lamella width was found

in all treatments with the highest lamellae thinning occurring in the 10^6 treatment. This gill change could decrease or impair its function. Behavioral acts such as surface swimming and approaching to air bubbles and air stones, may be a consequence of the loss of the gill functionality (impairment of oxygen transfer) and probably could lead to other organ dysfunctions such as heart failure. The possible role of PST in this failure cannot be neglected. Doyle and McMahon [69] showed that tetrodotoxin, a similar channel pore blocker toxin [70] as PST, at concentrations of 10^{-7} M and above, stopped both heart and ventilatory pumping in animals with a wet weight >5 mg of the sand shrimp *Metapenaeus ensis*. A similar scenario could be occurring in our experiments with shrimps suffering of low oxygen uptake which would trigger hyperventilation in gills and carbohydrate metabolism, to maintain the high energy demands needed for an increase in the ventilation rate [71]. Thus, *G. catentum* metabolites could cause gill damage, allowing the entrance of DM and, once inside the organism, PST may block heart sodium channels, resulting in the disruption of the process of respiratory gas exchange and its distribution to other organs. These effects could induce gill atrophy and the behavioral activities observed. Gill damages can also be involved in the entrance of dissolved metabolites into the organism (see below).

4.7. Muscle

Muscles are involved in shrimp's locomotion modes including walking, swimming, and tail-flipping; and are also involved in activities as cruising, migration, and foraging [70,72]. Tail-flipping, additionally, provides the highest speed and acceleration and is used for escaping predators [73]. A decrease of the area of muscle coverage can be interpreted as a loss of muscle and therefore with an impairment of the locomotion modes. In all treatments loss of muscle was comparable and varied between 44.7% and 48.7% with no significative differences. This muscle loss conduced to the low swimming capacity (lethargy) and lack of response to gentle disturbance observed. These data suggest that muscle is highly susceptible to damaging metabolites from the extract of *G. catenatum*, in this case metabolites targeting the muscle. Loss of almost half of the muscle coverage, in relation to the control, even in the lower extract concentration, supports this suggestion. Paralysis due to PST can also contribute to locomotion impairment.

4.8. Proposed uptake mechanism for PST and other metabolites in shrimp

In this study, uptake of DM, including PST, by shrimps could be via ingestion and/or through the permeable tissues such as gills and skin [7,30,67]. In crustacea, such as shrimp, most of their body is covered by an exoskeleton that is impermeable and composed mainly of chitin, as a major component, calcium carbonate, proteins and water that does not allow the flow of organic material [74]. The digestive route is also possible, however since the exposure of shrimps to metabolites in this study was by dissolving them in water it can be assumed that PST ingestion was not significant. A more plausible route is via gills as it has been suggested to occur in the Atlantic salmon (*Salmo salar*) exposed to waterborne PST [75].

Litopenaeus vannamei has highly branched gills [76] with a complex morphology [77]. To the best of our knowledge, it is not known if *L. vannamei* has a tight or leaky epithelia in the paracellular route between epithelial cells from the gills. However, *L. vannamei* grows naturally in salinities ranging from 1 to 40 g/L [78], using different osmotic regulation mechanisms such as hyper-osmotic regulation at low salinity and hypo-osmotic regulation at high salinities [79]. Therefore, it can be assumed that it osmoregulates with a leaky epithelium together with an active ion transport [67]. This leaky epithelium can facilitate the entrance of the dissolved toxins towards the circulatory system. Besides the "leaky" characteristics of gills, PST damages on gills could also be facilitating their uptake. According to Ref. [31] and experimenting with an epithelial cell line originating from the gill explant of adult rainbow trout *Oncorhynchus mykiss*, the toxins GTX1-4, B1-2, and STX affected gill cell viability in 30%, 20%, and 10%, respectively. Gill cell viability is also potentiated by other metabolites such as polyunsaturated fatty acids (PUFA), among many others [30,80]. However, these authors pointed out that these effects may not be the direct cause of gill cell viability changes, but it may trigger other physiological changes that alter their viability, such as ion exchange alteration, causing an increase in membrane permeability that may result in cell death through osmotic shock [30]. The dinoflagellate *G. catenatum* is a high superoxide a producer [31,42], thus, it is plausible to assume that waterborne exposition of these metabolites might damage shrimp's gill membrane and their viability and would favors their intake. Once inside the gills, the branchial efferent vessels transporting the oxygenated hemolymph, will carry them to the pericardial sinus surrounding the heart and then redistribute them through the hemolymph to other parts of the organism [81]. Thus, according to the above stated, PST and other harmful molecules might be uptaken mostly via gills and then transported into to the circulatory system, affecting internal organs such as the hearth, muscle, nervous system, and hepatopancreas, as shown in this study. In this last organ, PST is mostly accumulated.

5. Conclusion

Exposure of shrimps to DM derived from *G. catenatum*, even at a low concentration, triggers DM entry, possibly via gills, and induced tissues damage. These findings, suggest that DM of *G. catenatum* represent a potential danger for both, cultured and wild shrimp of *L. vannamei* populations. In culture conditions, DM can be introduced through the water inputs, into shrimp culture ponds as well as into the hatching laboratories causing nocive effects. For wild populations, exposure to DM would give them a higher vulnerability to opportunist predators impacting the survival and recruitment, as well as increasing the risk of transferring toxins to other organisms, including humans.

The limitation of the present study was that only tissue damages and behavioral changes were evaluated. However, in the natural marine environments, there are multifaceted stressors that affected the overall organism survival and health. Another limitation was that nocive metabolites such as reactive oxygen species, poli-unsaturated fatty acids or other bioactive extracellular compounds were

not identified and quantified. The study was limited to determine PST in the different tissues. Future studies must overcome these limitations. Also, experimentation with other genetically selected families of shrimps is encouraged. Surely, the generated information will help to generalize the role of DM on shrimp damages and mortality leading to a higher understanding of the impact of *G. catenatum* DM metabolites in shrimp.

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Author contribution statement

Jose Bustillos, Ph.D.: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Carmen Rodríguez-Jaramillo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Christine Band-Schmidt: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Francisco Hernández-Sandoval; Erick Núñez-Vázquez: Analyzed and interpreted the data.

Ana Flores-Chavarria: Performed the experiments.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17018.

References

[1] W.A. Catterall, C.S. Morrow, R.P. Hartshorne, Neurotoxin binding to receptor sites associated with voltage-sensitive sodium channels in intact, lysed, and detergent-solubilized brain membranes, J. Biol. Chem. 254 (1979) 11379–11387.

[2] K.D. Cisuck, G.S. Sayler, An overview on the marine neurotoxin, saxitoxin: genetics, molecular targets, methods of detection and ecological functions, Mar. Drugs 11 (2013) 991–1018, https://doi.org/10.3390/md11040991.

[3] D. Mann, Characterizing and Managing Harmful Blooms that Cause Production Loss on Australian Prawn Farms, Project Report, Department of Agriculture and Fisheries Queensland, Brisbane, 2017, 58 pp 2 appendix, https://era.daf.qld.gov.au/id/eprint/6101/.

[4] L. Liu, J. Chen, H. Xiuping, S. Hao, Z. Lian, B. Wang, First determination of extracellular paralytic shellfish poisoning toxins in the culture medium of toxigenic dinoflagellates by HILIC–HRMS, Ecotoxicol. Environ. Saf. 204 (2020), 111042, https://doi.org/10.1016/j.ecoenv.2020.111042.

[5] D.F. Boesch, D.A. Anderson, R.A. Horner, S.E. Shumway, P.A. Tester, T.E. Whitledge, Harmful algal blooms in coastal waters: options for prevention, control and mitigation, in: NOAA Coastal Ocean Program, Decision Analysis Series No. 10. Silver Springs, MD, 1996, p. 46. Available at: https://coastalscience.noaa.gov/data_reports/harmful-algal-blooms-in-coastal-waters-options-for-prevention-control-and-mitigation/ (accessed 10.04.22).

[6] FAO, The State of World Fisheries and Aquaculture 2020, in: Sustainability in Action. Rome, 2020, https://doi.org/10.4060/ca9229en.

[7] J. Castrec, P. Soudant, L. Payton, D. Tran, P. Miner, C. Lambert, N. Le Goïc, A. Huvet, V. Quillien, F. Boullot, Z. Amzil, H. Hégaret, C. Fabioux, Bioactive extracellular compounds produced by the dinoflagellate *Alexandrium minutum* are highly detrimental for oysters, Aquat. Toxicol. 199 (2018) 188–198, https://doi.org/10.1016/j.aquatox.2018.03.034.

[8] K.A. Lefebvre, B.D. Bill, A. Erickson, K.A. Baugh, L. O'Rourke, P.R. Costa, S. Nance, V.L. Trainer, Characterization of intracellular and extracellular saxitoxin levels in both field and cultured *Alexandrium* spp. samples from Sequim Bay, Washington, Mar. Drugs 6 (2008) 103–116, https://doi.org/10.3390/md20080006.

[9] H.W. Walker, Harmful Algae Blooms in Drinking Water: Removal of Cyanobacterial Cells and Toxins, CRC Press, Boca Raton, London New York, 2015. Available at: https://books.google.com.mx/books?hl=es&lr=&id=G4uZBQAAQBAJ&oi=fnd&pg=PP1&dq=Walker,+H.W.+2015.+Harmful+Algae+Blooms+in+Drinking+Water:+Removal+of+Cyanobacterial+Cells+and+Toxins.+CRC+Press.+Boca+Raton,+FL.&ots=H6xd5VkP3p&sig=QSYPqy2zba0uKbaadLtnpZIYQM4#v=onepage&q&f=false (accessed 10.04.22).

[10] J. Blanco, Accumulation of *Dinophysis* toxins in bivalve mollusks, Toxins 10 (2018) 453, https://doi.org/10.3390/toxins10110453.

[11] S. Gosselin, L. Fortier, J.A. Gagné, Vulnerability of marine fish larvae to the toxic dinoflagellate *Protogonyaulax tamarensis*, Mar. Ecol.: Prog. Ser. 57 (1989) 1–10.

[12] K.A. Lefebvre, V.L. Trainer, N.L. Scholz, Morphological abnormalities and sensorimotor deficits in larval fish exposed to dissolved saxitoxin, Aquat. Toxicol. 66 (2004) 159–170, https://doi.org/10.1016/j.aquatox.2003.08.006.

[13] K.A. Lefebvre, N.E. Elder, P.K. Hershberger, V.L. Trainer, C.M. Stehr, N.L. Scholz, Dissolved saxitoxin causes transient inhibition of sensorimotor function in larval Pacific herring (*Clupea harengus pallasi*), Mar. Biol. 147 (2005) 1393–1402, https://doi.org/10.1007/s00227-005-0048-8.

[14] J. Han, Y. Park, H.H. Shin, A.-Y. Shin, H.-M. Kang, J. Lee, Y.-U. Choi, K.-W. Lee, Effects of dinoflagellate *Gymnodinium catenatum* on swimming behavior and expression of heat shock protein (hsp) genes in the brine shrimp *Artemia franciscana*, Harmful Algae 110 (2021), 102146, https://doi.org/10.1016/j.hal.2021.102146.
[15] E.J. Núñez-Vázquez, I. Gárate-Lizárraga, C.J. Band-Schmidt, A. Cordero-Tapia, D.J. López-Cortés, F.E. Hernández-Sandoval, A. Heredia-Tapia, J. Bustillos-Guzmán, Impact of harmful algal blooms on wild and cultured animals in the Gulf of California, J. Environ. Biol. 32 (2011) 413–423. PMID: 22315821.
[16] C.J. Band-Schmidt, J.J. Bustillos-Guzmán, D. López-Cortés, I. Gárate-Lizárraga, E.J. Núñez-Vázquez, F.J. Hernández-Sandoval, Ecological and physiological studies of *Gymnodinium catenatum* in the Mexican Pacific: a review, Mar. Drugs 8 (2010) 1935–1961, https://doi.org/10.3390/md8061935.
[17] C.J. Band-Schmidt, L.M. Durán-Riveroll, J.J. Bustillos-Guzmán, I. Leyva-Valencia, D.J. López-Cortés, E.J. Núñez-Vázquez, F.E. Hernández-Sandoval, D. V. Ramírez-Rodríguez, Paralytic toxin producing dinoflagellates in Latin America: ecology and Physiology, Front. Mar. Sci. 6 (2019) 1–39, https://doi.org/10.3389/fmars.2019.00042.
[18] C.J. Band-Schmidt, M.G. Zumaya-Higuera, D.J. López-Cortés, I. Leyva-Valencia, S.I. Quijano-Scheggia, C.J. Hernández- Guerrero, Allelopathic effects of *Margalefidinium polykrikoides* and *Gymnodinium impudicum* in the growth of *Gymnodinium catenatum*, Harmful Algae 96 (2020), 101846, https://doi.org/10.1016/j.hal.2020.101846.
[19] C. Hummert, M. Ritscher, K. Reinharde, B. Luckas, Analysis of the characteristic PSP profiles of *Pyrodinium bahamense* and several strains of *Alexandrium* by HPLC based on ion-pair chromatographic separation, post-column oxidation, and fluorescence detection, Chromatographia 45 (1997) 312–316, https://doi.org/10.1007/BF02505576.
[20] R.C. Yu, C. Hummert, B. Luckas, P.Y. Qian, M.J. Zhou, Modified HPLC method for analysis of PSP toxins in algae and shellfish from China, Chromatographia 48 (1998) 671–676.
[21] FAO/WHO, Technical Paper on Toxicity Equivalency Factors for Marine Biotoxins Associated with Bivalve Molluscs. Rome, 2016, p. 108.
[22] T.A. Bell, D.V. Lightner, A Handbook of Normal Penaeid Shrimp Histology, World Aquaculture Society, Baton Rouge, USA, 1988, p. 75.
[23] J.J. Bustillos-Guzmán, C.J. Band-Schmidt, L.M. Durán-Riveroll, F.E. Hernández-Sandoval, D.J. López-Cortés, E.J. Núñez-Vázquez, A. Cembella, B. Krock, Paralytic toxin profile of the marine dinoflagellate *Gymnodinium catenatum* from the Mexican Pacific as revealed by liquid chromatography coupled with tandem mass spectrometry, Food Addit. Contam. 32 (2015) 381–394, https://doi.org/10.1080/19440049.2014.1000978.
[24] G.L. Humanson, Animal Tissue Techniques, Freeman, San Francisco, CA, 1972, p. 641.
[25] J.H. Zar, Biostatistical Analysis, fifth ed., Prentice-Hall/Pearson, Upper Saddle River, xiii, 2010, p. 944.
[26] A.E. Velázquez-Lizárraga, J.L. Juárez-Morales, I.S. Racotta, H. Villarreal-Colmenares, O. Valdes López, A. Luna-González, C. Rodríguez-Jaramillo, N. Estrada, F. Ascencio, Transcriptomic analysis of Pacific white shrimp (*Litopenaeus vannamei*, Boone 1931) in response to acute hepatopancreatic necrosis disease caused by *Vibrio parahaemolyticus*, PLoS One 14 (2019), e0220993, https://doi.org/10.1371/journal.pone.0220993.
[27] M. Liu, H. Gua, B. Krock, Z. Luoa, Y. Zhangd, Toxic dinoflagellate blooms of *Gymnodinium catenatum* and their cysts in Taiwan Strait and their relationship to global populations, Harmful Algae 97 (2020), 101868, https://doi.org/10.1016/j.hal.2020.101868.
[28] H. Ma, B. Krock, U. Tillmann, U. Bickmeyer, M. Graeve, A. Cembella, Mode of action of membrane-disruptive lytic compounds from the marine dinoflagellate *Alexandrium tamarense*, Toxicon 58 (2011) 247–258.
[29] J.J. Dorantes-Aranda, A. Seger, J.I. Mardones, P.D. Nichols, G.M. Hallegraeff, Progress in understanding algal bloom-mediated fish kills: the role of superoxide radicals, phycotoxins and fatty acids, PLoS One 10 (7) (2015), e0133549. https://doi:10.1371/journal.pone.0133549.
[30] J.I. Mardones, J.J. Dorantes-Aranda, D. Peter, P.D. Nichols, G.M. Hallegraeff, Fish gill damage by the dinoflagellate *Alexandrium catenella* from Chilean fjords: synergistic action of ROS and PUFA, Harmful Algae 49 (2015) 40–49, https://doi.org/10.1016/j.hal.2015.09.001.
[31] J.A. Marshall, M. de Salas, T. Oda, G. Hallegraeff, Superoxide production by marine microalgae: I. Survey of 37 species from 6 classes, Mar. Biol. 147 (2005) 533–540, https://doi.org/10.1007/s00227-005-1596-7.
[32] U. Tillmann, T.L. Alpermann, R.C. Da Purificaçao, B. Krock, A. Cembella, Intra-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*, Harmful Algae 8 (2009) 759–769, https://doi.org/10.1016/j.hal.2009.03.005.
[33] H.S. Flores, G.H. Wikfors, H.G. Dam, Reactive oxygen species are linked to the toxicity of the dinoflagellate *Alexandrium* spp. to protists, Aquat. Microb. Ecol. 66 (2012) 199–209. https://doi: 10.3354/ame01570.
[34] V.M. Bricelj, L. Connell, K. Konoki, S.P. MacQuarrie, T. Scheuer, W.A. Catterall, V.L. Trainer, Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP, Nature 434 (2005) 763–767.
[35] S.P. Colin, H.G. Dam, Latitudinal differentiation in the effects of the toxic dinoflagellate *Alexandrium* spp. on the feeding and reproduction of populations of the copepod *Acartia hudsonica*, Harmful Algae 1 (2002) 113–125, https://doi.org/10.1016/S1568-9883(02)00007-0.
[36] S.P. Colin, H.G. Dam, Comparison of the functional and numerical responses of resistant versus non-resistant populations of the copepod *Acartia hudsonica* fed the toxic dinoflagellate *Alexandrium tamarense*, Harmful Algae 6 (2007) 875–882, https://doi.org/10.1016/j.hal.2007.05.003.
[37] N. Estrada, M.J. Romero, A. Campa-Córdova, A. Luna, F. Ascencio, Effects of the toxic dinoflagellate, *Gymnodinium catenatum* on hydrolytic and antioxidant enzymes, in tissues of the giant lions-paw scallop *Nodipecten subnodosus*, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 146 (2007) 502–510, https://doi.org/10.1016/j.cbpc.2007.06.003.
[38] N. Estrada, C. Rodríguez-Jaramillo, G. Contreras, F. Ascencio, Effects of induced paralysis on hemocytes and tissues of the giant lions-paw scallop by paralyzing shellfish poison, Mar. Biol. 157 (2010) 1401–1415, https://doi.org/10.1007/s00227-010-1418-4.
[39] N. Estrada, F. Ascencio, L. Shoshani, R.G. Contreras, Apoptosis of hemocytes from lion spaw scallop *Nodipecten subnodosus* induced with paralyzing shellfish poison from *Gymnodinium catenatum*, Immunobiology 219 (2014) 964–974.
[40] A. Astuya, C. Carrera, V. Ulloa, A. Aballay, G. Núñez-Acuña, H. Hégaret, C. Gallardo-Escárate, Saxitoxin modulates immunological parameters and gene transcription in *Mytilus chilensis* hemocytes, Int. J. Mol. Sci. 16 (2015) 15235–15250, https://doi.org/10.3390/ijms160715235.
[41] M. Lassudrie, H. Hegaret, G. Wikfors, P.M. da Silva, Effects of marine Harmful Algal Blooms on bivalve cellular immunity and infectious diseases: a review, Dev. Comp. Immunol. 108 (2020), 103660, https://doi.org/10.1016/j.dci.2020.103660.
[42] L.J. Fernández-Herrera, C.J. Band-Schmidt, T. Zenteno-Savín, I. Leyva-Valencia, C.J. Hernández-Guerrero, M. Muñoz-Ochoa, Cell death and metabolic stress in *Gymnodinium catenatum* induced by allelopathy, Toxins 13 (2021) 506, https://doi.org/10.3390/toxins13070506.
[43] G.M. Hallegraeff, P.D. Nichols, J.K. Volkman, S.I. Blackburn, Pigments, fatty acids, and sterols of the toxic dinoflagellate *Gymnodinium catenatum*, J. Phycol. 27 (1991) 591–599.
[44] M.F. Encinas-Yánez, Efecto de sustancias potencialmente alelopáticas de la rafidofícea *Chattonella marina* (Raphidophyceae) sobre el dinoflagelado *Gymnodinium catenatum* (Dinophyceae), M.Sc. Thesis, in: IPN-CICIMAR, 2023, p. 100 (In Spanish).
[45] R. Alonso-Rodríguez, I. Gárate-Lizárraga, B. Luckas, K. Reinhardt, J. Bustillos-Guzmán, Mortalidad de larvas de camarón en cultivo en Sinaloa, México, asociado a mareas rojas de *Gymnodinium catenatum*, in: Book of Abstracts XIII Reunión Nacional de la Sociedad Mexicana de Planctología (Nuevo Vallarta), 2004, pp. 54–55 (In Spanish).
[46] J. Pérez-Linares, J.L. Ochoa, A. Gago-Martínez, Effect of PSP toxins in whiteleg shrimp *Litopenaeus vannamei* Boone, 1931, J. Food Sci. 73 (2008) 69–73, https://doi.org/10.1111/j.1750-3841.2008.00710.x.
[47] J. Han, J.S. Park, Y. Park, J. Lee, H.H. Shin, K.W. Lee, Effects of paralytic shellfish poisoning toxin-producing dinoflagellate *Gymnodinium catenatum* on the marine copepod *Tigriopus japonicus*, Mar. Pollut. Bull. 163 (2021), 111937, https://doi.org/10.1016/j.marpolbul.2020.111937.
[48] V.M. Bricelj, S.E. Shumway, Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation, Rev. Fish. Sci. 6 (1998) 315–383. https://doi:10.1080/10641269891314294.
[49] J.H. Landsberg, The effects of harmful algal blooms on aquatic organisms, Rev. Fish. Sci. 10 (2002) 113–390, https://doi.org/10.1080/20026491051695.
[50] A. Cembella, S. Shumway, R. Larocque, Sequestering and putative biotransformation of paralytic shellfish toxins by the sea scallop *Placopecten magellanicus*: seasonal and spatial scales in natural populations, J. Exp. Mar. Biol. Ecol. 180 (1994) 1–22, https://doi.org/10.1016/0022-0981(94)90075-2.
[51] Y. Oshima, in: P. Lassus, G. Arzul, P. Gentien, C. Marcaillou (Eds.), Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms, Harmful Marine Algal Blooms, Lavoisier Publishers, Paris, France, 1995, pp. 475–480.

[52] M.I.C. Raposo, M.T.S.R. Gomes, M.J. Botelho, A. Rudnitskaya, Paralytic shellfish toxins (PST)-Transforming enzymes: a review, Toxins 12 (2020) 344. https://doi:10.3390/toxins12050344.

[53] P. Vale, Fate of Benzoate paralytic shellfish poisoning toxins from *Gymnodinium catenatum* in shellfish and fish detected by pre-column oxidation and liquid chromatography with fluorescence detection, J. Chromatogr., A 1190 (2008) 191–197, https://doi.org/10.1016/j.chroma.2008.03.009.

[54] W.M. Indrasena, T.A. Gill, Storage stability of paralytic shellfish poisoning toxins, Food Chem. 71 (2000) 71–77, https://doi.org/10.1016/S0308-8146(00)00143-6.

[55] E.R. Zinser, The microbial contribution to reactive oxygen species dynamics in marine ecosystems, Environm. Microbiol. Rep. 10 (2018) 412–427, https://doi.org/10.1111/1758-2229.12626.

[56] M.M. Cestari, C.A.O. Ribeiro, V.A. Magalhães, V.L. Trudeau, H.C.S. de Assis, Saxitoxins induce cytotoxicity, genotoxicity and oxidative stress in teleost neurons *in vitro*, Toxicon 86 (2014) 8–15, https://doi.org/10.1016/j.toxicon.2014.04.016.

[57] C. Abi-Khalil, D.S. Finkelstein, G. Conejero, J. Du Bois, D. Destoumieux-Garzon, J.L. Rolland, The paralytic shellfish toxin, saxitoxin, enters the cytoplasm and induces apoptosis of oyster immune cells through a caspase-dependent pathway, Aquat. Toxicol. 190 (2017) 133–141, https://doi.org/10.1016/j.aquatox.2017.07.001.

[58] S. Liang, Z. Liang, X. Xiaogang, L. Jiarun, L. Moli, L. Xu, W. Shi, Z. Lingling, H. Xiaoli, B. Zhenmin, Genome-wide identification and characterization of SODs in Zhikong scallop reveals gene expansion and regulation divergence after toxic dinoflagellate exposure, Mar. Drugs 17 (2019) 700.

[59] Z. Liang, J. Li, J. Li, Z. Tan, H. Ren, F. Zhao, Toxic dinoflagellate *Alexandrium tamarense* induces oxidative stress and apoptosis in hepatopancreas of shrimp (*Fenneropenaeus chinensis*), J. Ocean Univ. China (Ocean. Coast. Sea Res.) 13 (2014) 1005–1011, https://doi.org/10.1007/s11802-014-2397-8.

[60] R. Gibson, P.L. Barker, The decapod hepatopancreas, Oceanogr. Mar. Biol. 77 (1979) 285–346.

[61] G. Vogt, Functional cytology of the hepatopancreas of decapod crustaceans, J. Morphol. 280 (2019) 1405–1444, https://doi.org/10.1002/jmor.21040.

[62] M.B. Bondad-Reantaso, L. Tran, D.T. Thanh Hue, What happens when hepatopancreas - shrimp's main organ for food absorption, digestion, and storage - becomes infected by a pathogen? FAO Aqua. Newsletter 51 (2013) 37–39.

[63] A.J. Kastin, Handbook of Biologically Active Peptides, Academic Press, Elsevier Inc, Burlington, MA, 2006, pp. 201–206.

[64] A. Prachumwat, P. Wechprasit, J. Srisala, R. Kriangsaksri, T.W. Flegel, S. Thitamadee, K. Sritunyalucksana, *Shewanella khirikhana* sp. nov. – a shrimp pathogen isolated from a cultivation pond exhibiting early mortality syndrome, Microb. Biotechnol. 13 (2020) 781–795, https://doi.org/10.1111/1751-7915.13538.

[65] M.S. Morales-Covarrubias, in: V. Morales, J. Cuéllar-Anjel (Eds.), Enfermedades bacterianas, Guía Técnica - Patología e Inmunología de Camarones Peneidos. Programa CYTED Red II-D Vannamei, Panamá, Rep. de Panamá, 2008, p. 270 (In Spanish).

[66] C. Pantoja, D.V. Lightner, in: V. Morales, J. Cuéllar-Anjel (Eds.), Enfermedades virales Pp. 55-114, Guía Técnica - Patología e Inmunología de Camarones Penaeidos. Programa CYTED Red II-D Vannamei, Panamá, Rep. De Panamá, 2008, p. 270 (In Spanish).

[67] R.P. Henry, Č. Lucu, H. Onken, H. Weihrauch, Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals, Front. Physiol. 3 (2012) 431, https://doi.org/10.3389/fphys.2012.00431.

[68] B. Aparicio-Simón, M. Piñón, R. Racotta, S.I. Racotta, Neuroendocrine and metabolic responses of Pacific whiteleg shrimp *Penaeus vannamei* exposed to hypoxia stress, Lat. Am. J. Aquat. Res. 46 (2018) 364–376, https://doi.org/10.3856/vol46-issue2-fulltext-12.

[69] J.E. Doyle, B.R. McMahon, Differential effects of tetrodotoxin (TTX) on cardiovascular and ventilatory function during development in the shrimp *Metapenaeus ensis*, Am. Zool. 37 (1997) 91A.

[70] F. Zhang, X. Xu, T. Li, Z. Liu, Shellfish toxins targeting voltage-gated sodium channels, Mar. Drugs 11 (2013) 4698–4723. https://doi:10.3390/md11124698.

[71] S.A. Ulaje, L. Rojo-Arreola, S.E. Lluch-Cota, F. Ascencio, P. Cruz-Hernández, M.T. Sicard, Gene expression and energetic metabolism changes in the whiteleg shrimp (*Litopenaeus vannamei*) in response to short term hypoxia, Aquat. Res. 50 (2019) 994–1004, https://doi.org/10.1111/are.13960.

[72] X. Yu, X. Zhang, Y. Duan, P. Zhang, Z. Miao, Effects of temperature, salinity, body length, and starvation on the critical swimming speed of white leg shrimp, *Litopenaeus vannamei*, Comp. Biochem. Physiol. Mol. Integr. Physiol. 157 (2010) 392–397.

[73] T. Amornpiyakrit, T. Arimoto, Muscle physiology in escape response of kuruma shrimp, Am. Fish. Soc. Symp. 49 (2008) 1321–1334.

[74] G. Gao, J. Wei, J. Yuan, X. Zhang, F. Li, J. Xiang, Transcriptome analysis on the exoskeleton formation in early developmental stages and reconstruction scenario in growth-moulting in *Litopenaeus vannamei*, Sci. Rep. 7 (2016) 1098. https://doi:10.1038/s41598-017-01220-6.

[75] M.J. Bakke, T.E. Horsberg, Kinetic properties of saxitoxin in Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*), Comp. Biochem. Physiol., C 152 (2010) 444–450. https://doi:10.1016/j.cbpc.2010.07.005.

[76] J.W. Martin, E.M. Liu, D. Strileya, Morphological observations on the gills of dendrobranchiate shrimps, Zool. Anz. 246 (2007) 115–125. https://doi:10.1016/j.jcz.2007.03.002.

[77] C.A. Hoster, H.D. Fonser, A morphological study on gills of the brown shrimp, *Panaeus aztecus*, Tissue Cell 10 (1978) 77–92, https://doi.org/10.1016/0040-8166(78)90008-3.

[78] A. Menz, B.F. Blake, Experiments on the growth of *Penaeus vannamei* boone, J. Exp. Mar. Biol. Ecol. 48 (1980) 99–111, https://doi.org/10.1016/0022-0981(80)90010-6.

[79] F.L. Castille, A.L. Lawrence, The effect of salinity on osmotic, sodium, and chloride concentrations in the hemolymph of euryhaline shrimp of the genus *Penaeus*, Comp. Biochem. Physiol. 68 (1981) 75–80. https://doi:10.1016/0300-9629(81)90320-0.

[80] Z. Zhou, X. Tang, H. Chen, Y. Wang, Comparative studies of saxitoxin (STX) -induced cytotoxicity in Neuro-2a and RTG-2 cell lines: an explanation with respect to changes in ROS, Chemosphere 192 (2018) 66–74, https://doi.org/10.1016/j.chemosphere.2017.10.083.

[81] H. Dugassa, D.G. Gaétan, Biology of white leg shrimp, *Penaeus vannamei*: review, World J. Fish Mar. Sci. 10 (2018) 5–17, https://doi.org/10.5829/idosi.wjfms.2018.05.17.