Effect of PSP Toxins in White Leg Shrimp *Litopenaeus vannamei* Boone, 1931

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ABSTRACT: Cultured shrimp are often exposed to different toxic products during rearing practices that may affect survival and quality of the product. An evaluation of the effects of paralytic shellfish toxins (PSP) from species of *Gymnodinium catenatum* in white leg shrimp (*Litopenaeus vannamei*) has been carried out in this study. Death was observed at doses > 5.0 MU (equivalent to 1.1 μg/g of STX), while lower doses provoked paralysis of pereiopods, disequilibrium, and abdominal spasms in the animals. Target organs such as the heart and brain were severely damaged, with cohesion loss and cell density reduction evidenced by histological analysis. Hence, pond productivity and quality of the harvested organisms may be affected by PSP toxins. This is the 1st report on the effect of PSP toxins from *G. catenatum* in white eg shrimp.

Keywords: food safety, *Gymnodinium catenatum*, *Litopenaeus vannamei*, paralytic shellfish poisoning, shrimp aquaculture

Introduction

The shrimp aquaculture industry has grown considerably over the past 2 decades. The worldwide consumption of cultured shrimp has increased approximately 4-fold since 1984 (FAO 2006). Nevertheless, the impact of toxic phytoplankton blooms in culture systems has not been fully evaluated (Shumway 1990; Falconer 1993; Hallegraeff 1993; Karunasagar and others 1997; Scoging 1998). Outbreaks of toxic dinoflagellate and cyanobacteria in culture ponds have caused severe losses in shrimp farms in Mexico (Cortés-Altamirano and Núñez-Pastén 1992; Cortés-Altamirano and others 1997; Alonso-Rodríguez and Páez-Osuna 2003; Ochoa and others 2004) and yet the toxin effects in shrimp remain unknown. Deleterious effects of marine toxins in other cultured marine organisms include a reduced growth rate, loss of immunocompetence, abnormal behavior, and death, thus reducing overall production yield (Lightner and others 1978; Foxall and others 1979; Roberts and others 1979; Hwang and others 1990; Pérez-Linares and others 2003). On the other hand, evaluation of contaminated shrimp as a toxin vector in the food chain deserves attention since these organisms consume phytoplankton throughout their life as a source of fatty acids and other nutrients (Olivera and Alano-Cohelo 1997; Alonso-Rodríguez and others 2004). This study was undertaken to explore the effect of paralytic shellfish toxins (PSP) produced by *Gymnodinium catenatum* on white leg shrimp (*Litopenaeus vannamei* Boone, 1931) under an acute-exposure mode test.

Materials and Methods

The *G. catenatum* strain (GCCV-6) was obtained from the Collection of Marine Dinoflagellates of the CIBNOR and cultured in Fernbach flasks with f/2 medium (Guillard and Ryther 1962; Guillard 1975). The medium was prepared in seawater (33 psu), filtered with a 0.45-μ membrane, and sterilized at 121 °C, 15 lb, for 20 min (Band-Schmidt and others 2004). Culturing was carried out at 26 ± 1 °C, 12:12 h light/dark, and a light intensity of 150 μmol/m^2/s for 17 d, to allow its exponential phase. The biomass was harvested by centrifugation (6500 × *g*, 10 min, 4° C). Analysis of PSP toxins was carried out according to the AOAC official method for PSP (AOAC 1997) with conditions initially proposed by Lawrence and others (1995). Briefly, the dinoflagellate biomass was suspended and homogenized in 5 mL of 0.1 N HCl, mixed with glass beads (1 mL, 0.5 mm Ø), and shaken for 10 min in a Vortex for cell disruption. The mixture was then boiled for 5 min and the final extract filtered twice: first with a filter paper (particle retention ≥ 100 μm) and then through a 0.45-μm membrane. The filtered extract was stored at –20 °C. Mouse bioassay (AOAC 1997) was used to determine the toxicity of the extract (mouse units, MU/g body weight) which is expressed as micrograms of saxitoxin equivalents (μg STXeq/g of biomass) according to Oshima (1995). Several dilutions of extract solution were prepared to obtain different doses: 0.5 MU (0.11 μg/g STXeq), 1.0 MU (0.22 μg/g STXeq), 5.0 MU (1.1 μg/g STXeq), and 20 MU (4.4 μg/g STXeq). The composition and toxin profile of the original extract was determined by HPLC/FLD after solid phase extraction cleanup (SPE C18 and SPE-COOH cartridges, Bakerbond, J.T. Baker, Phillipsburg, N.J., U.S.A.). The oxidation of the toxins to obtain the correspondent fluorescent derivatives was carried out prior to chromatographic separation by treatment with periodate and hydrogen peroxide (Lawrence and others 2004). The oxidized derivatives were injected (20 μL) into a silica base reverse-phase column (C18, 4.6 × 250 mm; 5 μm, Phenomenex Luna, Torrance, Calif., U.S.A.) using 10 mM ammonium formiate (pH 6.0), and 10 mM ammonium formiate-acetonitrile (100:4, pH 6.0) in a gradient elution fashion with a flow rate of 1.5 mL/min. Fluorescent detection was set at an excitation wavelength of 340 nm and emission wavelength of 395 nm, in an HPLC-FLD system (Agilent® 1200 Series, Santa Clara, Calif., U.S.A.) with a quaternary pump and autoinjector. Toxin profile was compared against standard solutions of STX, NEO, dcSTX, GTX1,4, GTX2,3, and B1 provided by the Natl. Research Council of Canada (NRC, Halifax, Canada).

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For shrimp bioassays, juvenile shrimps (3.8 ± 0.3 g) were obtained from the CIBNOR and reproductive adults (41.7 ± 4.4 g) from Acuacultura Mahr, S.A. de C.V. Seven groups of 5 individuals each were maintained in plastic aquariums (40 L) with filtered seawater (35%) and constant aeration. Feeding was provided with commercial pellets (40% protein) according to recommended doses (Villalón 1991). The animals were injected with various toxin doses as follows: juveniles, 100 μL of 0.5, 1.0, and 5.0 MU; adults, 1.0 mL of 5.0, and 20.0 MU. The control assay consisted of a set of animals handled under similar conditions injected with 100 μL or 1.0 mL of the vehicle (0.1 N HCl). The animal responses, behavior, and tissue damage were registered for all doses. Eventually, time of death was recorded and surviving animals sacrificed after 10 to 15 min. All organisms were fixed with Davidson solution (Bell and Lightner 1988) and dyed with hematoxylin–eosin (H–E) stain for histology analysis. Three to 5-μm-thick tissue samples of gastric gland, muscle, heart, and supraesofagal ganglion were obtained using a microtome and analyzed with an optical microscope equipped with a camera. Image processing software was used for tissue analysis. Total area and average density of tissues were compared and analyzed by the 1-way analysis of variance (ANOVA) statistical test. Chi-square and Kolmogorov–Smirnov analysis was carried out to corroborate the homogeneity variance and normal distribution of data in order to apply a Student's *t*-test to determine the differences in area and density of the heart tissue.

Results and Discussion

HPLC-FLD analysis of GCCV-6 extract revealed the presence of 6 different toxins belonging to the carbamoyl group (STX, NEO, GTX2,3, and GTX1,4). In addition, 1 decarbamoyl toxin (dcSTX) was detected but no sulfocarbamoyl toxins were found (Figure 1). These results are partially consistent with the previous reports of Band-Schmidt and others (2005) and Gárate-Lizárraga and others (2005) in other GCCV strains. They observed a toxic profile dominated by decarbamoyl toxins (dcSTX, dcGTX) and sulfocarbamoyl toxins (B1, B2, and C2). On the other hand, this study also shows the presence of GTX1-4 and STX in the GCCV-6 strain. The different conditions used for sample pretreatment (extraction and cleanup) and for the HPLC analysis carried out could also explain some of these differences.

The shrimp showed abnormal behavior when extracts of PSP toxins were injected. The main symptoms observed were paralysis of antennae and pereiopods (walking legs) in all doses applied, and disequilibrium, atypical swimming, and resting on their flanks on the bottom of the aquaria when the organisms were injected with ≥ 1.0 MU. Slow and irregular movements of gills, pleopods (swimming legs), and maxillipeds were also observed during the experiment in both shrimp stages and all doses. Internal organ activity was observed despite paralysis. All doses of the toxic extract were lethal to the individuals (juvenile and adults), presenting convulsion, muscular spasms, and abdominal contractions before dying (Table 1). The control group did not show any abnormal behavior and/or swimming activity and all survived.

A substantial correlation between the time of death and the amount of toxin was found (Table 1). Juvenile shrimp were more susceptible than adult animals. On the other hand, differences in abdominal muscle and gastric gland tissue structures were not observed in organisms injected with the toxin or in the control

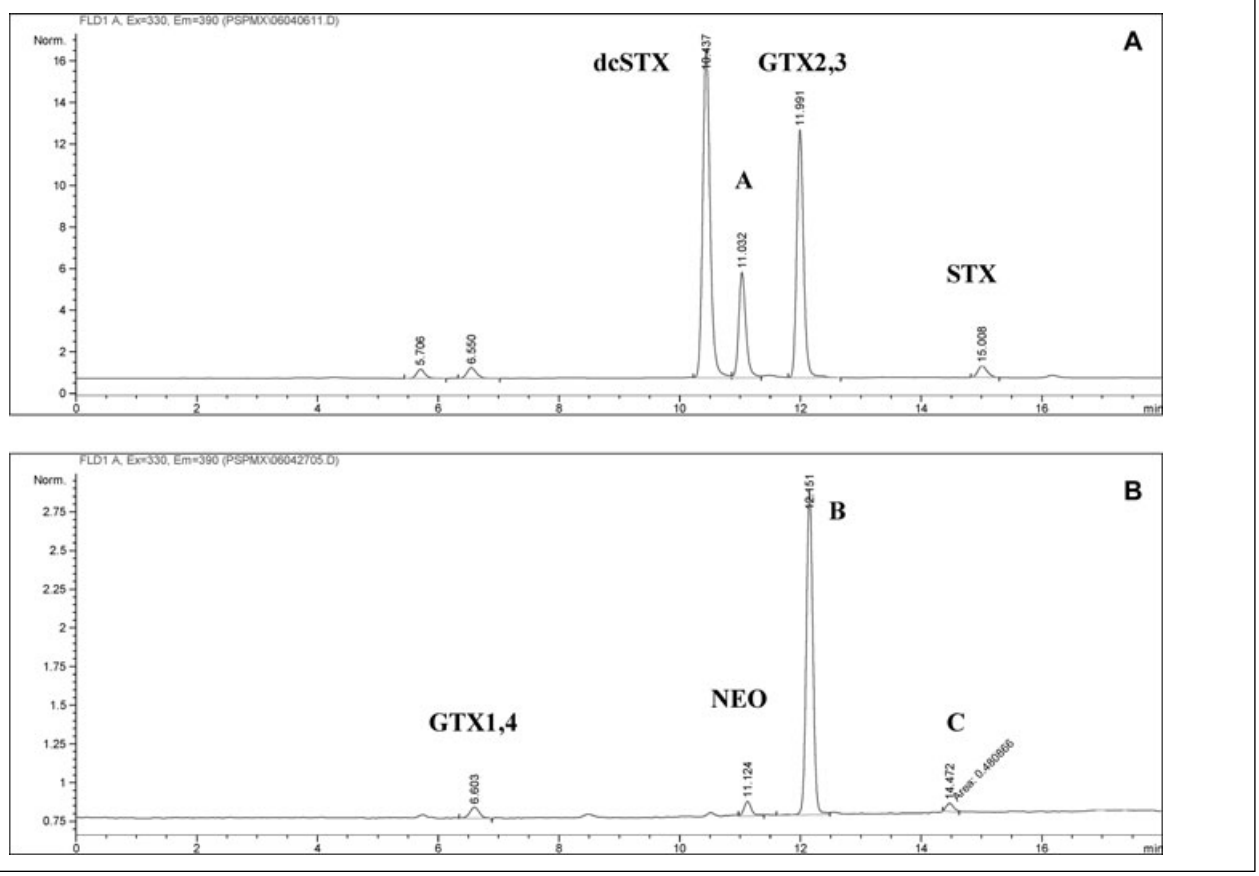

Figure 1 — Toxin profile of *G. catenatum* (GCCV-6) detected with HPLC-FLD and prechromatographic oxidation. The presence of the carbamoyl toxins STX, NEO, GTX2,3, GXT1,4, and of the decarbamoyl toxin dcSTX, becomes clear (A, B, and C are secondary oxidation products): (A) peroxide derivatization; (B) periodate derivatization.

group. In contrast, exposed animals showed serious damage to the heart and brain tissues (Figure 2 and 3). The myocardial tissue is constituted of fiber packages thinner than the abdominal muscle (Bell and Lightner 1988; Lesson and others 1988), hence they are more susceptible to abrupt changes in their elastic capability (that is, paralysis). The myocardial fibers lost cohesion and were less dense and uniform in both juvenile and adult shrimp exposed to the toxins. Such effects were significantly different ($P < 0.05$) with respect to the controlled animals (ANOVA and Student's t-test). Additionally, the neuropile suffered visible damage in exposed shrimp showing lyses and/or loss of neurofiber density (Figure 3). Because the neuropile is the tissue where the synapses and stimulus processes take place, harm or inflammation can cause nervous system problems and behavioral abnormalities in organisms (Oliveira and others 2004).

The potential threat of PSP toxins in shrimps was evident through our experimental model. The negative effects on their tissues and their survival are clear based on the outcome of these trials (doses > 0.5 MU equivalent to 0.11 μg/g of STX, Table 1). These results are consistent with those of Hwang and others (1990) who found a lethal dose (LD_{99}) of 0.5 MU for 3 different shrimp species, and Foxall and others (1979) who reported an LD_{99} of

Table 1—Acute assay of injected paralytic toxins from *G. catenatum* (GCCV-6) to juvenile and adult of white leg shrimp *L. vannamei*.

Stage	Weight mean (g)	Length mean (cm)	Doses (MU)	Doses (eq.STX/g)	Time of death (mean)	Symptoms
Juvenile[a]	3.8 ± 0.3	8.3 ± 0.6	0.5	0.11	02'11"	Paralysis in pereiopods and antennae
			1.0	0.22	01'41"	Disequilibrium and atypical swim
			5.0	1.1	00'55"	Irregular movements of pleopods, gills and, maxillipeds
Adult[b]	41.7 ± 4.4	18.3 ± 0.6	5.0	1.1	06'36"	Convulsions, abdominal spasms, and death
			20.0	4.4	02'56"	

[a]100 μL injected.
[b]1.0 mL injected.

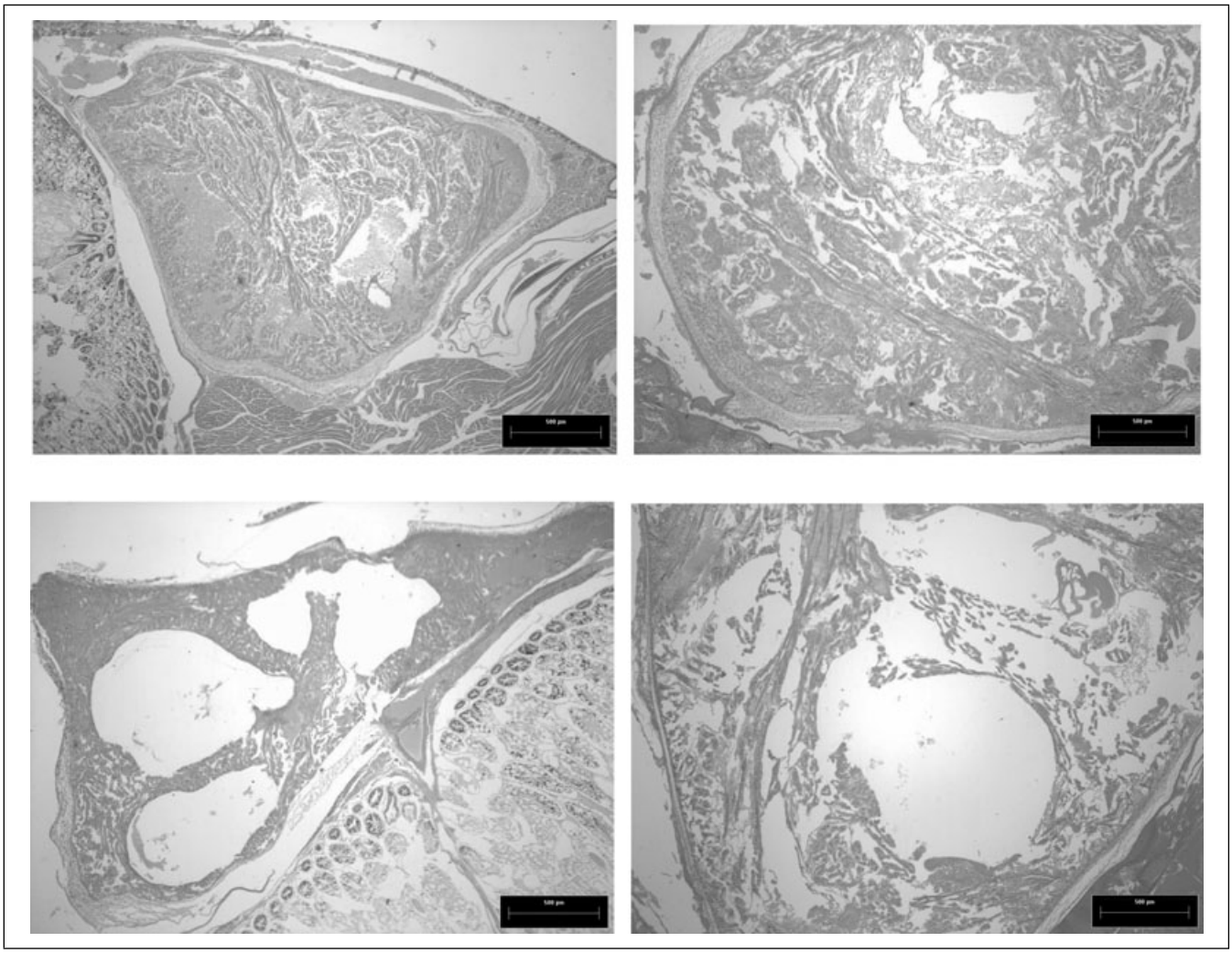

Figure 2—Cross-section of shrimp heart tissue exposed to PSP toxins. Upper figures correspond to control experiment; lower left figure corresponds to animals exposed to 5.0 MU (1.1 μg/g STXeq) toxin extract, and lower right figure to 20.0 MU (4.4 μg/g STXeq) toxins extract. Bar = 500 μm. Staining carried out with H–E.

0.01 μg PSP/g of body weight in *Cancer irroratus* exposed to toxins of *Gonyaulax tamarensis* with a time of death of 20 min. Also, the toxin profile must determine the response and time of death of exposed animals (FAO 2004). Accordingly, *G. catenatum* would appear to be more toxic than *G. tamarensis*. On the other hand, the lack of damage in muscle and hepatopancreas tissues indicates that these tissues are not affected by such toxins and/or are able to regenerate faster than other tissues. The effect of PSP toxins on the supraesofagal ganglion or brain tissue of shrimp, on the other hand, may explain the behavior observed (disequilibrium, abnormal swimming, paralysis of legs, abdominal spasms, and death). All these symptoms may result from obstruction of the brain stimuli process and/or interruption of connections within the appendages and muscle of the organism (Oliveira and others 2004). Studies in mammals have shown that high doses of PSP toxins affect contractile myocardium fibers, reducing blood pressure, and causing arrhythmia, followed by ventricular fibrillation and finally cardiac arrest (Andrinolo and others 1999; Lehane 2000), similar to our observations.

Conclusions

The toxin profile of *G. catenatum* strain (GCCV-6) includes some of the most toxic compounds found in the PSP toxin group (FAO 2004; Band-Schmidt and others 2005). This species of dinoflagellate is common in the Pacific coast of Mexico, where shrimp aquaculture has been developed for more than 10 y. Therefore, the blooms of *G. catenatum* in the area may jeopardize the production of this industry. Our study showed that *L. vannamei* is susceptible to paralytic toxins in acute exposures. Further investigations of toxin kinetics, accumulation, and possible depuration of PSP toxins in shrimp tissues under chronic exposures can lead us to consider these organisms as vectors of phycotoxins to the human population. This is the 1st report of *G. catenatum* toxin effect in white leg shrimp.

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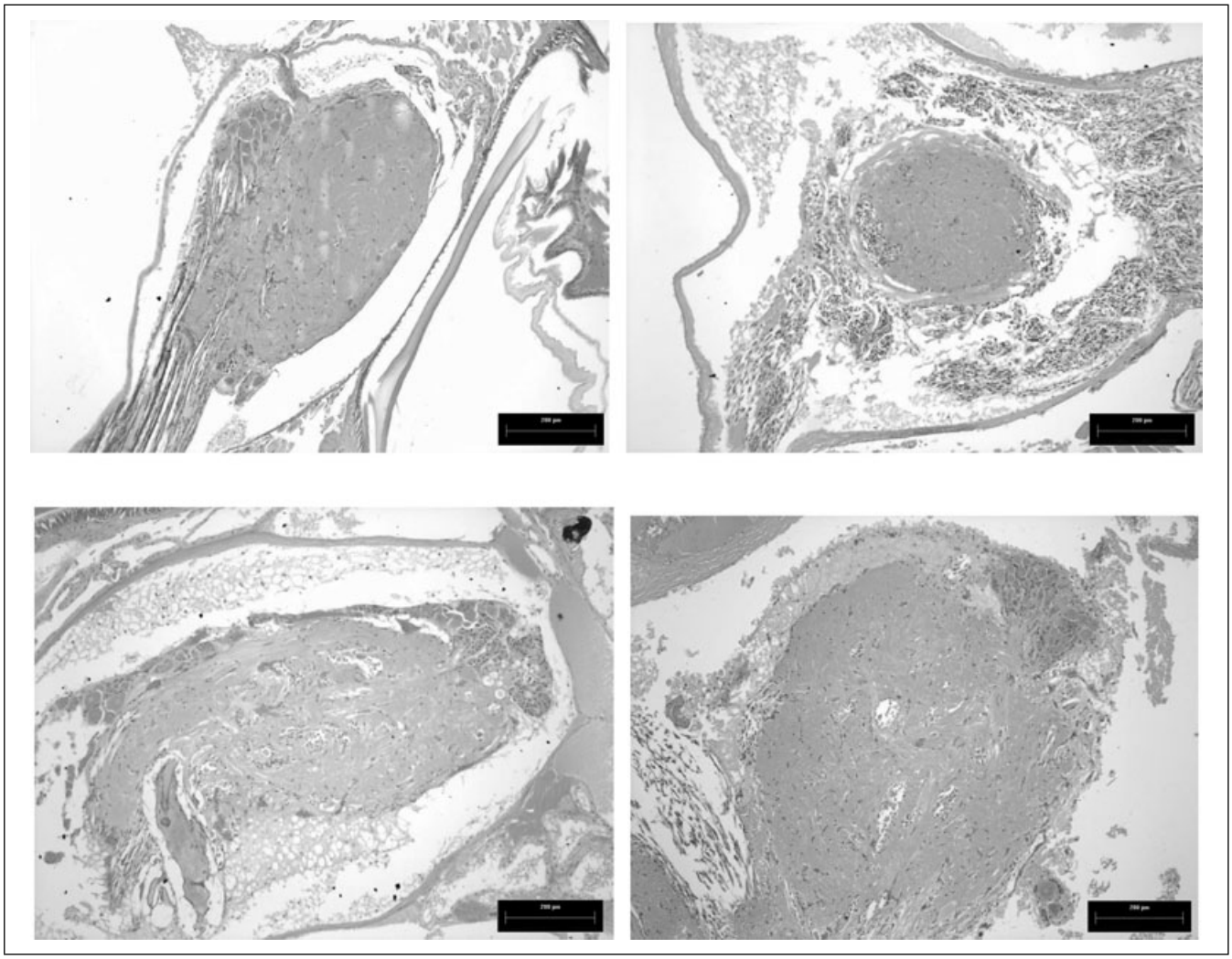

Figure 3—Cross-section of shrimp brain tissue showing normal neuropile ganglions (upper figures) and damaged neuropile ganglions (lower figures) after toxin exposure. Upper figures correspond to control experiment and lower figures to animals exposed to 5.0 MU (1.1 μg/g STXeq) of PSP toxins (left) and 20.0 MU (4.4 μg/g STXeq) of PSP toxins (right). Bar = 200 μm. Staining carried out with H–E.

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